1   # Virus mediated transfer of nitrogen from heterotrophic bacteria to

2   # phytoplankton

3   Emma J. Shelford[1] and Curtis A. Suttle[1,2,3,4,5]

4   [1]Department of Earth, Ocean and Atmospheric Sciences, University of British Columbia,

5   Vancouver, Canada

6   [2]Department of Botany, University of British Columbia, Vancouver, Canada

7   [3]Department of Microbiology and Immunology, University of British Columbia, Vancouver,

8   Canada

9   [4]Institute of Oceans and Fisheries, University of British Columbia, Vancouver, Canada

10   [5]Canadian Institute for Advanced Research, Toronto, Canada.

11

12   Correspondence to: C. A. Suttle (suttle@science.ubc.ca)

13

14

1 Abstract

2

3   Lytic infection of bacteria by viruses releases nutrients during cell lysis and stimulates the

4   growth of primary producers, but the path by which these nutrients flow from lysates to primary

5   producers has not been traced. This study examines the remineralisation of nitrogen (N) from

6   bacterial lysates by heterotrophic bacteria and its transfer to primary producers. In laboratory

7   trials, *Vibrio* sp. strain PWH3a was infected with a lytic virus (PWH3a-P1) and the resulting 36.0

8   µM of dissolved organic N (DON) in the lysate was added to cultures containing cyanobacteria

9   (*Synechococcus* sp. strain DC2), and a natural bacterial assemblage. Based on the increase in

10   cyanobacteria, 74% (26.5 µM N) of the DON in the lysate was remineralised and taken up by

11   *Synechococcus* sp. strain DC2 cells. Lysate from *Vibrio* sp. strain PWH3a labeled with $^{15}NH_4^+$

12   was also added to seawater containing natural microbial communities, and in four field

13   experiments, stable isotope analysis indicated that the uptake of labeled N was 0.09 to 0.70 µmol

14   N per µg of chlorophyll *a*. The results from these experiments demonstrate that DON from

15   bacterial lysate can be efficiently remineralised and transferred to phytoplankton, and provides

16   further evidence that the viral shunt is an important link in nitrogen recycling in aquatic systems.

17

18 **1   Introduction**

19   Viruses are significant mortality agents of bacteria and phytoplankton in the ocean, consequently

20   affecting pathways and rates of nutrient cycling (Fuhrman, 1999; Suttle, 2005, 2007; Wilhelm

21   and Suttle, 1999) when particulate and dissolved organic matter (DOM) is released into the water

22   during cell lysis (Gobler et al., 1997; Middelboe and Jørgensen, 2006). Major constituents of the

1   released material are free and combined amino acids (Middelboe and Jørgensen, 2006), which

2   are taken up and metabolised by bacteria (Middelboe et al., 1996, 2003). When the C:N of DOM

3   is low relative to the nutritional requirements of heterotrophic bacteria, ammonium may be

4   released (Goldman et al., 1987; Hollibaugh, 1978), which evidence suggests can support

5   phytoplankton growth (Haaber and Middelboe, 2009; Shelford et al., 2012; Weinbauer et al.,

6   2011).

7   High rates of bacterial mortality from viral lysis imply a continuous and substantial flux of DOM

8   from lysates into seawater. Weinbauer et al. (2011) provided evidence of the importance of this

9   flux by showing that reducing viral abundance decreased the growth of *Synechococcus*, the

10 dominant primary producer during their experiments in the Gulf of Mexico and Mediterranean

11 Sea. It was postulated that *Synechococcus* growth may have been directly stimulated by uptake

12 of dissolved organic nutrients released by lysis, or indirectly through the incorporation of these

13 organics by uninfected bacteria and subsequent remineralisation of inorganic nutrients. Evidence

14 that mineralization of DOM and release of ammonium by uninfected bacteria stimulates

15 phytoplankton growth was shown by Shelford et al. (2012).

16 The present contribution demonstrates, in the laboratory and field, that uninfected bacteria

17 metabolise dissolved organic N (DON) released as the result of viral lysis of bacteria and release

18 ammonium that supports the growth of phytoplankton.

19

2 Methods

2.1 Laboratory cultures

A semi-continuous culture of *Synechococcus* sp. strain DC2 (Bigelow, CCMP #1334; WH7803) was grown on artificial seawater (Berges et al., 2001), modified by adding 5 M bicine (Healey and Hendzel, 1979), 124 μM NH_4Cl instead of nitrate, and 13 μM K_2HPO_4. Cultures were maintained at 19°C and continuous light (42 μmol quanta $m^{-2} s^{-1}$). Experiments were started when cultures entered nutrient limitation near the end of exponential growth, as determined by epifluorescence microscopy counts.

The gram negative marine bacterium *Vibrio* sp. strain PWH3a, also known as *Vibrio natriegens* strain PWH3a (Suttle and Chen, 1992; Weinbauer et al., 1997) and *Vibrio alginolyticus* strain PWH3a (Poorvin et al. 2011) was grown on artificial seawater with 5 mM bicine, 500 μM NH_4Cl, 100 μM K_2HPO_4, and 1 mM glucose as a carbon source for a C:N:P ratio of 60:5:1. The cultures were grown at 25°C and continuously mixed at 100 rpm.

Bacteriophage PWH3a-P1 was added in eight-fold excess abundance (multiplicity of infection of 8:1) to cultures of *Vibrio* sp. strain PWH3a at the end of exponential growth, as determined by absorbance at 660 nm (Ultrospec spectrophotometer, Biochrom, United Kingdom). The culture was incubated with the virus until absorbance decreased to 20% of the initial (~7 h). The lysate (Lys) was filtered through a 0.22 μm pore-size Durapore membrane (Millipore, Billerica, MA) and kept at 4°C for approximately 20 h. The number of cells lysed prior to filtration was determined by flow cytometry as described below (section 2.4.1). The amount of DON released was determined by the number of cells lysed multiplied by the measured cellular N quota for

1   *Vibrio* sp. strain PWH3a, 2.54 fmol cell^{-1} as described below (section 2.4.4). The result is the

2   amount of total N released by lysis of *Vibrio* sp. strain PWH3a.

3   Lysates for field experiments were prepared as above, except that *Vibrio* sp. strain PWH3a was

4   grown on $^{15}NH_4Cl$ instead of $^{14}NH_4Cl$ (90+ atom % ^{15}N, Isotec, Miamisburg, OH), and the

5   filtered lysate was kept at 4°C for 2 to 5 d until the experiments were initiated.

2.2   Growth of *Synechococcus* sp. strain DC2 on lysate from *Vibrio* sp. strain

7   PWH3a

8   Water was collected from Queen Charlotte Sound (51° 02.37N, 127° 52.38W) on 2 Oct 2011.

9   Temperature and salinity were 11°C and 32, respectively. Nitrate and phosphate concentrations

10   were 21.3 and 1.9 μM, respectively. The water was ultrafiltered using a 30 kDa molecular weight

11   cut-off tangential flow cartridge (Prep/Scale, Millipore, Billerica, MA) after pre-filtration

12   through a 0.45 μm pore-size polyvinylidene fluoride (PVDF) Durapore membrane (Millipore,

13   Billerica, MA) as outlined in Suttle et al. (1991). The ultrafiltrate was stored in the dark at 4°C

14   for a year until used in experiments. Bacteria in the ultrafiltrate grew to a density of 4.18×10^5

15   cells ml^{-1}, as determined by flow cytometry (described in section 2.4.1), and were used for the

16   regeneration experiment described below.

17   The experiment used *Synechococcus* sp. strain DC2, lysate from *Vibrio* sp. strain

18   PWH3a, and the bacterial assemblage (Bac) in ultrafiltrate from Queen Charlotte Sound in the

19   following six combinations (Figure 1): 1) DC2+Bac+lysate was the experimental treatment with

20   DON from lysate; 2) DC2+Bac was a control for *Synechococcus* sp. strain DC2 growth in the

21   presence of the bacterial assemblage without a DON source from lysate; 3) DC2+lysate was a

22   control for bacterial remineralisation in the *Synechococcus* sp. strain DC2 culture; 4) Bac+lysate

1 was a control to quantify ammonium remineralisation by the bacterial assemblage with the

2 addition of lysate; 5) Bac only was a control to determine the ammonium concentration of the

3 bacterial assemblage by itself; 6) DC2 only was a control to determine the ammonium

4 concentration and increase in cell number activity of *Synechococcus* sp. strain DC2 by itself. All

5 treatments were in triplicate in 1L polycarbonate Erlenmeyer flasks (Corning, New York). To

6 each appropriate treatment was added 10 ml of *Synechococcus* sp. strain DC2 culture, 100 ml of

7 bacterial assemblage, and/or 10 ml of lysate. The experimental treatment volume was 200 ml,

8 and volumes of control treatments were topped up to 200 ml by adding nitrate- and phosphate-

9 free artificial seawater to control treatments.

10 Treatments were incubated in the laboratory at 19°C under continuous light (42 μmol

11 quanta m^{-2} s^{-1} of photosynthetically active radiation) and sampled daily for cell abundance and

12 ammonium concentration.

13 ## 2.3 Uptake of ^{15}N from lysates in the field

14 Water was collected from the surface at Saanich Inlet (SI), the Fraser River Plume (FRP),

15 Semiahoo Bay (SB) and Jericho Pier (JP) in southern coastal British Columbia (Table 1). SI and

16 FRP samples were collected using Go-Flo bottles mounted on a rosette, which held a SBE 25

17 CTD (Sea-Bird, Bellevue, WA) for measuring temperature and salinity. SB and JP samples were

18 collected by hand using a 20 l carboy rinsed with 10% HCl and ultrapure water, and temperature

19 and salinity were measured with a hand-held thermometer and refractometer. Samples for

20 phytoplankton identification were collected from the whole water and preserved with Lugol's

21 acidic iodine solution (Edler and Elbrächter, 2010). The water was filtered through 118-μm

22 mesh-size Nitex screening to remove large particulates.

1   For each experiment, 0.22-μm filtered lysate from *Vibrio* sp. strain PWH3a was added to

2   Nitex-filtered seawater (SW+lysate) and compared to a control containing 0.22-μm filtered

3   seawater. For SB and JP, a third treatment included 0.22-μm filtered seawater and 0.9 μM

4    $^{15}NH_4Cl$(SW+N), to confirm that N stimulated production and that another factor was not

5   limiting phytoplankton growth, and to mirror the estimated N contributed by the lysate in the

6   SW+lysate treatment. Treatments were in triplicate. SI and FRP experiments were done in an on-

7   deck flow-through incubator with neutral density screening to reduce sunlight to approximate *in*

8   *situ* irradiance. For SB and JP, experiments were done at 19°C and 42 μmol quanta m^{-2} s^{-1}

9   continuous irradiance. Samples for cell abundance and ammonium concentration were collected

10   every 6 to 9 h for 1 to 2 d; samples for chlorophyll *a* were collected at the initial and final time

11   points, and samples for PO^{15}N were collected at the final time point.

12   **2.4   Sample Analysis**

13   2.4.1 Cell and virus counts

14   One ml samples were fixed with a final concentration of 0.5% gluteraldehyde for bacteria and

15   viruses, and 2% formaldehyde for phytoplankton, and flash frozen in liquid nitrogen and stored

16   at -80°C. Bacteria and viruses were stained with SYBR Green I (Sigma-Aldrich, St. Louis, MO),

17   while picophytoplankton were left unstained, immediately before counting by flow cytometry

18   (FACSCalibur, Becton Dickinson, Franklin Lakes, NJ) following the procedures of Brussaard

19   (2004), Gasol and Del Giorgio (2000), and Olson et al. (1993). Larger phytoplankton were

20   preserved with Lugol's solution, and the dominant taxa identified using a settling chamber (Edler

21   and Elbrächter, 2010) and light microscope (Axiovert 10, Zeiss, Canada).

2.4.2 Nutrient and chlorophyll *a* collection and analysis

Samples of 25 ml were filtered through acid-rinsed syringes fitted with MilliQ-soaked 0.45-µm

pore size, 25-mm diameter, cellulose-nitrate filters (Whatman, United Kingdom) in a Swinnex

filter holder (Millipore, Billerica, MA). The first 15 ml were used to rinse the 15 ml acid-rinsed

polypropylene screw-cap collection tubes (Sarstedt, Germany), before collecting and freezing the

final 10 ml at -20°C for subsequent nutrient analysis. The filters were folded, placed in

aluminum foil packages, and frozen dessicated at -20°C until chlorophyll *a* was extracted using

90% acetone and sonication, and analysed fluorometrically (Turner Designs, 10AU fluorometer,

Sunnyvale, CA) following Parsons et al. (1984).

Nitrate+nitrite and phosphate concentrations were analyzed on a Bran & Luebbe

AutoAnalyzer 3 using air-segmented continuous-flow analysis (Technicon, Oakland, CA), while

ammonium concentrations were determined fluorometrically (Holmes et al., 1999) using a TD-

700 fluorometer (Turner Designs, Sunnyvale, CA).

2.4.3 PO^{15}N size-fractionation, collection and determination of ^{15}N enrichment

For the field experiments, the ^{15}N incorporated was determined by collecting the particulate

material onto combusted (450°C for 4 h) glass-fibre (GF) filters (nominal pore size of 0.7 µm,

Whatman, United Kingdom), which were subsequently dried at 50°C for 2 d. Prior to collection

onto the GF filters, the SI and FRP samples were poured into a Sterifil (Millipore, Germany)

filtration tower fitted with a 1-µm pore-size polycarbonate filter (AMD Manufacturing, Canada).

While the sample was being gently filtered, the phytoplankton were kept in suspension and

rinsed, while the volume was maintained by adding 200 ml of ultrafiltrate. In this way, cells

1   captured by the 1-µm filter were retained for analysis of isotopic enrichment, while smaller cells

2   passed through. Samples for cell counts were taken before and after washing to determine the

3   proportion of cells lost by this process. Samples that were not rinsed with ultrafiltrate were also

4   filtered onto combusted GF filters to estimate the amount of ^{15}N uptake that was due to bacteria

5   that passed through the 1-µm pore size membrane. The SW+N treatment (SB and JP) was

6   filtered directly onto GF filters without rinsing. The $\delta\,^{15}$N-PON and total PON on the filters was

7   determined at the Stable Isotope Laboratory at Boston University on a GV Instruments IsoPrime

8   isotope-ratio mass spectrometer and a Eurovector elemental analyzer, calibrated against

9   atmospheric N_2 and IAEA standards N-1, N-2, and N-3 (replicate analysis within $\pm\,0.2$‰). Due

10   to instrument error, the stable-isotope data for SI and FRP are not replicated; whereas, duplicates

11   for JP and triplicates for SB were measured.

12   ## 2.4.4 Particulate C and N analysis

13   N cell quotas of *Synechococcus* sp. strain DC2 and *Vibrio* sp. strain PWH3a were determined

14   from cultures grown using the same media and conditions as described in section 2.1. Cultures

15   entering stationary phase for *Synechococcus* sp. strain DC2 as determined by epifluorescence

16   microscopy counts, and mid-exponential phase for *Vibrio* sp. strain PWH3a as determined by

17   absorbance, were filtered onto combusted GF filters, dried at 50°C for two days, and placed in a

18   desiccator until packaged into aluminum foil and analyzed on a CHN Elemental Analyser (Carlo

19   Erba NA-1500).

2.4.5 Calculations and statistical analysis

Total uptake rates of ^{15}N in the field experiments were calculated by dividing the total particulate ^{15}N on the filters by the volume filtered. Normalised uptake rates were calculated by dividing the total uptake rate by the initial chlorophyll a concentration for each incubation. The percent contribution of cells >1 μm to total ^{15}N uptake was calculated by dividing the total particulate ^{15}N on the filters of the 'washed' samples (adjusted for decrease in phytoplankton abundance) by the ^{15}N on the unwashed samples.

The differences between initial and final time points for *Synechococcus* abundances and ammonium concentrations were normally distributed and with equal variances according to Shapiro-Wilk and Levene's tests, respectively; hence, the significance of the results was analysed using one-way analysis of variance (ANOVA). The differences between initial and final time points for bacterial abundances in the laboratory experiment, while normally distributed, had unequal variances, and therefore a Kruskal-Wallis test was performed to test for significant differences.

The differences between initial and final time points for ammonium and chlorophyll a concentrations and bacteria and picophytoplankton abundances were normally distributed (except for the picophytoplankton data for treatment SW in SI, which had only two samples), and with equal variances (except the bacteria data for SI and JP). Significant differences between treatments were determined using two-tailed Student's t tests. Data which did not meet the assumptions for the Student's t test were analysed for significant differences using the non-parametric Mann-Whitney U test.

1   Because the final time point of the bacterial data for the SW+lysate treatment in SI was

2   approximately six-fold lower than the previous time point, concurrent with a significant increase

3   in viral abundance (data not shown), significance between the two treatments was calculated for

4   the differences between the initial and the fourth time points. The data were normally distributed

5   and had equal variances (Shapiro-Wilk and Levene's tests), and so a Student's t test was run.

6

3   RESULTS

8

3.1   Response of N-limited *Synechococcus* sp. strain DC2 to the addition of lysate

11   There was a greater increase in the abundance of N-limited *Synechococcus* sp. strain DC2 cells

12   exposed to lysate and a remineralising bacterial assemblage than when grown with each

13   component separately; the greatest increase occurred in the treatment DC2+Bac+lysate (Figure

14   2A), with final abundances significantly different among all treatments ($p<0.05$). Ammonium

15   decreased from ~1.0 μM to less than 0.2 μM except in the Bac+lysate treatment, in which it

16   increased to 8.24 ± 0.04 μM (Figure 2B), significantly higher than in the other treatments

17   ($p<0.05$). Bacterial abundance increased in all treatments, but the greatest increases occurred in

18   treatments with lysate addition (Figure 2C).

19   The proportion of N released by lysis of cultures of *Vibrio* sp. strain PWH3a that was taken up

20   by *Synechococcus* sp. strain DC2 cells was calculated using the N cell quotas for *Vibrio* sp. strain

21   PWH3a of 2.54 fmol cell^{-1}, and for *Synechococcus* sp. strain DC2 of 7.83 fmol cell^{-1}. Based on

1   the decrease in *Vibrio* sp. strain PWH3a cells from viral infection, multiplied by the N cell quota

2   of *Vibrio* sp. strain PWH3a, approximately 36.0 μM N was added to each incubation from

3   bacterial lysis (Table 2). The mean percent of N from lysate that was taken up by *Synechococcus*

4   sp. strain DC2 cells via remineralisation by bacteria was 74%.

3.2   Uptake of N from bacterial lysate by primary producers in field studies

6   Lysate from ^{15}N-labelled *Vibrio* sp. strain PWH3a cultures was added to seawater from Saanich

7   Inlet (SI), the Fraser River Plume (FRP), Semiahoo Bay (SB), and Jericho Pier (JP; Table 1).

8   Changes were followed in ammonium concentrations and in abundances of bacteria (Figure 3).

9   Bacterial abundances in SW+lysate treatments were significantly higher than in samples without

10   lysate (SW) by the final time point for FRP, SB, and JP ($p < 0.05$). In the SI sample, bacterial

11   abundances in the treatment with lysate added (SW+lysate) increased almost tenfold before

12   decreasing to below initial values by the final time point, concurrent with an increase in viral

13   abundance (data not shown). There were no significant differences between experimental and

14   control treatments for picophytoplankton abundances or for chlorophyll *a* concentrations in the

15   other stations (data not shown). Ammonium concentrations in SI, SB, and JP decreased to less

16   than 0.2 μM. In FRP, the SW+lysate treatment decreased to less than 0.04 μM before climbing

17   again to 0.25 μM, correlated with a spike in bacterial abundance. There was significantly greater

18   drawdown of ammonium in the SW+lysate treatment in every station except SB ($p < 0.05$). The

19   increase of bacteria and greater ammonium uptake at most stations indicates that bacterial

20   growth and overall uptake of ammonium was enhanced in these samples by the addition of

21   bacterial lysate.

1   There was uptake of lysate-derived N by cells >1 μm, which would be comprised primarily of

2   phytoplankton. The addition of lysate to the SW+lysate treatment for each station contributed

3   approximately 67.7 μM N for SI and FRP, and 0.44 μM N for SB and JP (Table 2). In order to

4   add a more reasonable concentration of N to the samples, the concentration of lysate N added to

5   SB and JP was greatly reduced compared to the other two previous experiments. Stable isotope

6   data collected from particulate organic material collected on 0.7-μm pore-size glass-fibre filters

7   indicated uptake of lysate by the particulate fraction (Table 3; Figure 4). From the ^{15}N data, the

8   calculated contribution to the total uptake by cells in the >1 μm size fraction was 46.3% (SI),

9   47.6% (FRP), and 100% (SB and JP). The large (>50%) contribution of the bacterial size fraction

10   to ^{15}N uptake in SI and FRP (Figure 4) corresponded to an increase in bacterial abundances

11   (Figure 3).

12   There was confirmed uptake of $^{15}NH_4$ in the treatment with ^{15}N addition (SW+N), with 0.61 ±

13   0.20 μM N (SB) and 0.44 ± 0.26 μM N (JP) taken up into the particulate fraction (corrected for

14   90 atom % ^{15}N; data not shown). Any ammonium produced by remineralisation of lysate in the

15   SW+Lys treatment, therefore, could be used by the microbes at these two stations.

16

4   DISCUSSION

18

19   Experiments in the laboratory and with natural systems show that N in viral lysates can be

20   remineralised by bacterial communities to fuel primary production. Studies have shown that lysis

21   by viruses produces bioavailable dissolved organic matter (e.g. Middelboe et al. 2003, Poorvin et

22   al. 2004), and that phytoplankton lysate can be remineralised (Gobler et al., 1997). Other studies

1   have provided evidence that ammonium from remineralisation may stimulate primary production

2   (Haaber and Middelboe, 2009; Shelford et al., 2012; Weinbauer et al., 2011); yet, data are

3   lacking on the mechanism involved in the transfer of N to primary producers from bacterial

4   lysates produced by viral infection. This study shows that phytoplankton in culture and in

5   environmental samples take up N from bacterial lysates, and that ammonium produced through

6   bacterial remineralisation of DON enables that uptake.

4.1   Remineralisation of nitrogen in bacterial lysates

8   The increase in bacterial abundance in all experiments, along with the production of ammonium

9   in the laboratory experiment, establishes that bacterial lysates produced through viral infection

10   are available for bacterial growth and potential ammonium remineralisation. In the laboratory

11   experiment, every treatment with added lysate had a greater increase of bacteria than treatments

12   without lysate, indicating that the bacteria used the added lysate for growth. For all field studies,

13   bacterial abundances increased significantly in the treatments with lysate over the controls

14   ($p<0.05$; Figure 3), demonstrating uptake of lysis products by the bacterial communities.

15   Ammonium concentration increased eightfold in laboratory treatments with lysate added to

16   bacteria (Bac+lysate; Figure 2B) from remineralisation of N in the lysate by the bacterial

17   community. In the other treatments, either lysate was not added as a source of DON (treatment

18   Bac), or *Synechococcus* sp. strain DC2 was present and consumed the ammonium that was

19   produced (the remainder of the treatments). There was no measured ammonium production in the

20   field studies except in the Fraser River Plume (FRP; Figure 3). The increase in ammonium at the

21   final time point in FRP may be due to the concurrent sharp increase in bacteria, likely increasing

1 ammonium remineralisation. Viral lysates can be used by the bacterial community for growth,

2 and excess N remineralised to produce ammonium.

4.2 Phytoplankton uptake of remineralised nitrogen

4 This study shows that remineralised N from virus lysis of bacteria can fuel the growth of primary

5 producers. Observations of increased ammonium production in the presence of viral lysis

6 (Haaber and Middelboe, 2009; Shelford et al., 2012), are extended by this study which

7 demonstrates that bacteria remineralise the organic N in lysates and produce ammonium, which

8 is then taken up by phytoplankton. The use of a cultivated bacterium for lysate production limits

9 generalisation of quantitative data to environmental systems; however, the observation that 74%

10 of the N in bacterial lysates was taken up by cultures of *Synechococcus* sp. strain DC2 provides

11 strong evidence that N from lysates may provide a large portion of the N taken up by

12 phytoplankton.

13 DON from bacterial lysates was remineralised by the bacterial assemblage into ammonium and

14 used to fuel primary production. In the laboratory experiment, the increase in ammonium in the

15 Bac+lysate treatment did not occur in the treatment with *Synechococcus* sp. strain DC2

16 (DC2+Bac+lysate; Figure 2B), indicating that the remineralised ammonium was taken up by

17 *Synechococcus* sp. strain DC2; this is consistent with the concurrent increase in *Synechococcus*

18 sp. strain DC2 cells (Figure 2A). The uptake of ^{15}N in seawater samples by organisms >1 μm

19 (Figure 4) also demonstrates that remineralised N in bacterial lysate supports primary

20 production, as suggested previously (Shelford et al., 2012; Weinbauer et al., 2011).

21 All four field studies showed that ^{15}N from labeled bacterial lysate was taken up by the >1-μm

22 fraction (Figure 4). This is likely largely the result of uptake by phytoplankton which dominate

1   this size fraction. As well, these rates represent minimum estimates, as ^{15}N released through

2   bacterial remineralisation or phytoplankton exudation (Bronk and Ward, 2000) is not included.

3   Although the field experiments do not provide quantitative estimates of the amount of N in lysate

4   that can be taken up, the results corroborate those from the laboratory experiment. An anomalous

5   result for the SB experiment was that the estimated amount of N taken up (0.67 ± 0.02 μM N;

6   corrected for 90 atom % ^{15}N; Figure 4) was higher than the calculated amount of N in the lysate

7   addition (0.44 μM N; Table 2); however, the lysate contribution is an estimate calculated from

8   the cellular N quota and the reduction in cell number of Vibrio sp. strain PWH3a, and some error

9   may derive from these calculations and from a lack of replicates in the cell number data.

10 Nonetheless, it is reasonable to conclude that most of the lysate added in the SB and JP

11 experiments was incorporated by primary producers, since at both stations 100% of the estimated

12 ^{15}N uptake was into the >1 μm fraction.

13 Nonetheless, not all of the N in lysates was taken up by primary producers. Although bacteria

14 can remineralise lysate (Bac+lysate; Figure 2B), and Synechococcus sp. strain DC2 can use

15 remineralised N from co-occurring bacteria (DC2+lysate; Figure 2A), in the laboratory only 74%

16 of the N in the lysate contributed to an increase in Synechococcus sp. strain DC2 abundance. As

17 well, in the FRP and SI samples, only about half of the ^{15}N uptake was into the >1 μm fraction.

18 Discrepancies between N uptake and phytoplankton growth may in part be because

19 phytoplankton have been observed to take up almost 74% more N than the net amount

20 incorporated (Bronk et al., 1994); thus, in our experiments more N may have been taken up than

21 was measured, because of DON release. Bronk et al. (1994) also measured DON turnover times

22 in seawater samples of 4 to 18 d, much longer than in our incubations; however, the DON in

23 seawater would also include phytoplankton exudates and refractory DON that would have a

1   different composition than bacterial lysates. Hence, the incubation times in our study may not

2   have been long enough to allow for uptake of more recalcitrant DON, which might have been

3   incorporated with time (Middelboe and Jørgensen, 2006). For SI and FRP, the lysate was added

4   at a relatively high concentrations (Table 2), which may also have contributed to incomplete

5   incorporation. In contrast, the lysate was added at a much lower concentration in the SB and JP

6   experiments, and the ^{15}N in the lysate was completely recovered in the particulate fraction,

7   indicating that the DON in the lysate was incorporated when added at more natural

8   concentrations. Finally, the incomplete transfer of N from lysate of *Vibrio* sp. strain PWH3a to

9   *Synechococcus* sp. strain DC2 in the laboratory, relative to the complete uptake of ^{15}N at SB and

10   JP, may indicate that complete remineralisation of the DON depends on the make-up of the

11   bacterial assemblage, or that some phytoplankton in natural waters may take up DON directly

12   (Bronk et al., 2007).

13   The increase in *Synechococcus* sp. strain DC2 abundance in the laboratory experiment (Figure

14   2A) can be explained by the different sources of N present in the experimental treatment

15   (DC2+Bac+lysate). A portion of the increase can be attributed to the 21.3 μM of nitrate present

16   in the Bac ultrafiltrate, a portion to the remineralisation of ammonium by the added bacterial

17   assemblage, and another portion to uptake of ammonium from remineralisation by contaminating

18   bacteria in the non-axenic *Synechococcus* sp. strain DC2 culture. Despite evidence for uptake of

19   DON directly by phytoplankton in field experiments (see Bronk et al. 2007 for a review),

20   preliminary studies to the current one showed no evidence for uptake of *Vibrio* sp. strain PWH3a

21   lysate uptake by axenic *Synechococcus* sp. strain DC2. If considered together, the increase of

22   *Synechococcus* sp. strain DC2 in the experimental treatment (DC2+Bac+lysate) can be

23   accounted for by the effects from each of these control treatments.

4.3 Ecological implications and future directions

Some studies have focused on the influence of phytoplankton lysates on fueling bacterial production (Gobler et al., 1997; Haaber and Middelboe, 2009). Phytoplankton lysates can be very important in certain situations, such as during a bloom termination (Brussaard et al., 2005), but bacterial lysates are produced constantly throughout the ocean. Suttle (1994) estimated that 10 to 20% of heterotrophic bacteria are lysed every day by viruses, which can vary widely dependent on location and conditions (e.g. Wilhelm et al. 1998, 2002). With an estimated 26-70 Pg C yr^{-1} of bacterial production in the euphotic zone (Ducklow and Carlson, 1992), a bacterial C:N of approximately 5 (Goldman et al., 1987), and loss rates of 10-20% from viral lysis, approximately 0.52-2.8 Pg N yr^{-1} is released from viral lysis of bacteria in the photic zone. Primary production C demand is estimated to be 49.3 Pg C yr^{-1} (Ducklow and Carlson, 1992), corresponding to 7.4 Pg N yr^{-1} according to the Redfield ratio (106C:16N). Therefore, an estimated 7-38% of global primary production can be supported from bacterial lysates from viral infection (Figure 5). This is a substantial source of recycled N available to fuel primary production, especially in regions that are N-limited.

The ability of primary producers to use N from bacterial remineralisation of DOM from viral lysis of bacteria indicates that viruses are not simply C sinks that disrupt trophic levels (Azam and Worden, 2004), but are important facilitators in N recycling pathways. Traditional food chain models state that C and other nutrients flow from primary producers to higher trophic levels. The introduction of the microbial loop (Azam et al., 1983) included bacterial dynamics, whereby DOM produced by the members of the traditional food chain is taken up by bacteria and reintroduced to the food web instead of being lost to the system. The viral shunt (Wilhelm and

1   Suttle, 1999) introduced viruses as a 'short-circuit,' removing particulate C from primary

2   producers, consumers, and bacteria to the pool of organic matter. This model emphasises viruses

3   as a loss mechanism of foodweb C; however, implicit in these models is that nutrients, as well as

4   C, must also be released by viral lysis, and that N and P are likely recycled with greater

5   efficiency than C (Suttle, 2007). The loss of C from the particulate pool is clear, but there is

6   evidence of increased productivity in the presence of viruses. In Fe-limited regions of the eastern

7   Pacific Ocean, viruses were shown to liberate Fe into the DOM pool, where it was available for

8   uptake by primary producers (Poorvin et al., 2004). Weinbauer et al. (2011) provided evidence

9   for this phenomenon when they removed the viral fraction from a cyanobacteria bloom and

10   primary production ceased. Shelford et al. (2012) confirmed that observation by demonstrating a

11   reduction in both ammonium remineralisation and phytoplankton abundance with removal of

12   viruses. The current study demonstrated in four separate field experiments that N is transferred

13   from bacterial lysates to phytoplankton biomass. Weinbauer et al. (2011) hypothesised that

14   primary production decreased in their experiments due to either lysates providing a direct source

15   of nutrients for the *Synechococcus* blooms, or through bacterial remineralisation providing

16   inorganic nutrients. This study supports the interpretation that remobilization of nutrients by viral

17   lysis of bacteria and subsequent remineralisation by uninfected bacteria supported the observed

18   growth of *Synechococcus*.

19

1 **Author contributions**

2 E. Shelford and C. A. Suttle designed the experiments. E. Shelford performed the experiments

3 and analysed the samples except for the stable isotope data which was analysed by the Stable

4 Isotope Facility at UC Davis, and the nitrogen cell content which was analysed by Maureen Soon

5 at UBC. E. Shelford prepared the manuscript with contributions from C. A. Suttle.

6

7 **Competing Interests**

8 The authors declare that they have no conflict of interest.

9

10 **Acknowledgements**

17

1 References

2 Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A. and Thingstad, F.: The

3 ecological role of water-column microbes in the sea, Mar. Ecol. Prog. Ser., 10, 257–263, 1983.

4 Azam, F. and Worden, A. Z.: Microbes, molecules, and marine ecosystems, Science, 303, 1622–

5 1624, 2004.

6 Berges, J. A., Franklin, D. J. and Harrison, P. J.: Evolution of an artificial seawter medium:

7 improvements in enriched seawater, artificial water over the last two decades, J. Phycol., 37,

8 1138–1145, 2001.

9 Bronk, D. A., Glibert, P. M. and Ward, B. B.: Nitrogen uptake, dissolved organic nitrogen

10 release, and new production., Science, 265, 1843–1846, doi:10.1126/science.265.5180.1843,

11 1994.

12 Bronk, D. A., See, J. H., Bradley, P. and Killberg, L.: DON as a source of bioavailable nitrogen

13 for phytoplankton, Biogeosciences, 4, 283–296, 2007.

14 Bronk, D. A. and Ward, B. B.: Magnitude of dissolved organic nitrogen release relative to gross

15 nitrogen uptake in marine systems, Limnol. Oceanogr., 45, 1879–1883, 2000.

16 Brussaard, C. P. D.: Optimization of procedures for counting viruses by flow cytometry, Appl.

17 Environ. Microbiol., 70, 1506–1513, doi:10.1128/AEM.70.3.1506, 2004.

18 Brussaard, C. P. D., Kuipers, B. and Veldhuis, M. J. W.: A mesocosm study of *Phaeocystis*

19 *globosa* population dynamics, Harmful Algae, 4, 859–874, doi:10.1016/j.hal.2004.12.015, 2005.

1   Ducklow, H. W. and Carlson, C. A.: Oceanic bacterial production, in advances in microbial

2   ecology, vol. 12, edited by K. Marshall, pp. 113–181, Plenum Press, New York, 1992.

3   Edler, L. and Elbrächter, M.: The Utermöhl method for quantitative phytoplankton analysis, in

4   Microscopic and molecular methods for quantitative phytoplankton analysis, edited by B.

5   Karlson, C. Cusack, and E. Bresnan, pp. 13–20, UNESCO, Paris, 2010.

6   Fuhrman, J. A.: Marine viruses and their biogeochemical and ecological effects, Nature, 399,

7   541–548, 1999.

8   Gasol, J. M. and Del Giorgio, P. A.: Using flow cytometry for counting natural planktonic

9   bacteria and understanding the structure of planktonic bacterial communities, Sci. Mar., 64, 197-

10   224, 2000.

11   Gobler, C. J., Hutchins, D. A., Fisher, N. S., Cosper, E. M. and Sañudo-Wilhelmy, S.: Release

12   and bioavailability of C, N, P Se, and Fe following viral lysis of a marine chrysophyte, Limnol.

13   Oceanogr., 42, 1492–1504, 1997.

14   Goldman, J. C., Caron, D. A. and Dennett, M. R.: Regulation of gross growth efficiency and

15   ammonium regeneration in bacteria by substrate C:N ratio, Limnol. Oceanogr., 32, 1239–1252,

16   1987.

17   Haaber, J. and Middelboe, M.: Viral lysis of *Phaeocystis pouchetii*: implications for algal

18   population dynamics and heterotrophic C, N and P cycling, ISME J., 3, 430–441,

19   doi:10.1038/ismej.2008.125, 2009.

1   Healey, F. P. and Hendzel, L. L.: Indicators of phosphorus and nitrogen deficiency in five algae

2   in culture, J. Fish. Res. Board Canada, 36, 1364–1369, 1979.

3   Hollibaugh, J. T.: Nitrogen regeneration during the degradation of several amino acids by

4   plankton communities collected near Halifax, Nova Scotia, Mar. Biol., 45, 191–201, 1978.

5   Holmes, R. M., Aminot, A., Kerouel, R., Hooker, B. A. and Peterso, B. J.: A simple and precise

6   method for measuring ammonium and marine and freshwater ecosystems, Can. J. Fish. Aquat.

7   Sci., 56, 1801–1808, 1999.

8   Middelboe, M. and Jørgensen, N. O. G.: Viral lysis of bacteria: an important source of dissolved

9   amino acids and cell wall compounds, J. Mar. Biol. Assoc. UK, 86, 605–612, 2006.

10 Middelboe, M., Jørgensen, N. O. G. and Kroer, N.: Effects of viruses on nutrient turnover and

11 growth efficiency of noninfected marine bacterioplankton, Appl. Environ. Microbiol., 62, 1991–

12 1997, 1996.

13 Middelboe, M., Riemann, L., Steward, G., Hansen, V. and Nybroe, O.: Virus-induced transfer of

14 organic carbon between marine bacteria in a model community, Aquat. Microb. Ecol., 33, 1–10,

15 2003.

16 Olson, R. J., Zettler, E. R. and D, D. M.: Phytoplankton analysis using flow cytometry, in

17 handbook of methods in aquatic microbial ecology, pp. 175–186, Lewis Publishers, Boca Raton.,

18 1993.

19 Parsons, T. R., Maita, Y. and Lalli, C. M.: Manual of chemical and biological methods for

20 seawater analysis, Pergamon Press., 1984.

1   Poorvin, L., Rinta-Kanto, J. M., Hutchins, D. A. and Wilhelm, S. W.: Viral release of iron and its

2   bioavailability to marine plankton, Limnol. Oceanogr., 49, 1734–1741, 2004.

3   Poorvin L., Sander S. G., Velasquez I., Ibisanmi E., LeCleir G. R., Wilhelm S.W.: A comparison

4   of Fe bioavailability and binding of a catecholate siderophore with virus-mediated lysates from

5   the marine bacterium Vibrio alginolyticus PWH3a. J. Exp. Mar. Biol. Ecol., 399, 43-47, 2011.

6   Shelford, E. J., Middelboe, M., Møller, E. F. and Suttle, C. A.: Virus-driven nitrogen cycling

7   enhances phytoplankton growth, Aquat. Microb. Ecol., 66, 41–46, doi:10.3354/ame01553, 2012.

8   Suttle, C. A.: The significance of viruses to mortality in aquatic microbial communities, Microb.

9   Ecol., 28, 237–243, doi:10.1007/BF00166813, 1994.

10   Suttle, C. A.: Viruses in the sea., Nature, 437, 356–361, doi:10.1038/nature04160, 2005.

11   Suttle, C. A.: Marine viruses--major players in the global ecosystem., Nat. Rev. Microbiol., 5,

12   801–812, doi:10.1038/nrmicro1750, 2007.

13   Suttle, C. A., Chan, A. M., and Cottrell, M. T. Use of ultrafiltration to isolate viruses from

14   seawater which are pathogens of marine phytoplankton. Appl. Environ. Microbiol. 57, 721-726,

15   1991.

16   Suttle, C. A. and Chen, F.: Mechanisms and rates of decay of marine viruses in seawater., Appl.

17   Environ. Microbiol., 58, 3721–3729, 1992.

18   Weinbauer, M. G., Bonilla-Findji, O., Chan, A. M., Dolan, J. R., Short, S. M., Šimek, K.,

19   Wilhelm, S. W. and Suttle, C. A.: *Synechococcus* growth in the ocean may depend on the lysis of

20   heterotrophic bacteria, J. Plankton Res., 33, 1465–1476, doi:10.1093/plankt/fbr041, 2011.

1   Weinbauer, M. G., Wilhelm, S. W., Suttle, C. A. and Garza, D. R.: Photoreactivation

2   compensates for UV damage and restores infectivity to natural marine virus communities., Appl.

3   Environ. Microbiol., 63, 2200–2205, 1997.

4   Wilhelm, S. W., Brigden, S. M. and Suttle, C. A.: A dilution technique for the direct

5   measurement of viral production: a comparison in stratified and tidally mixed coastal waters.,

6   Microb. Ecol., 43, 168–173, doi:10.1007/s00248-001-1021-9, 2002.

7   Wilhelm, S. W. and Suttle, C. A.: Viruses and nutrient cycles in the sea, Bioscience, 49, 781–

8   788, doi:10.2307/1313569, 1999.

9   Wilhelm, S. W., Weinbauer, M. G., Suttle, C. A. and Jeffrey, W. H.: The role of sunlight in the

10   removal and repair of viruses in the sea, Limnol. Oceanogr., 43, 586–592, 1998.

11

12

13

1   Table 1 Locations and environmental parameters for field sampling stations: Saanich Inlet (SI),
2   Fraser River Plume (FRP, Semiahoo Bay (SB), and Jericho Pier (JP). Environmental and
3   biological conditions measured include temperature (Temp), salinity (Sal), nitrate (NO_3^-),
4   phosphate (PO_4^{2-}), ammonium (NH_4^+), and the dominant phytoplankton.

Station	Location	Date	Temp (°C)	Sal	NO_3^- (μM)	PO_4^{2-} (μM)	NH_4^+ (μM)	Dominant phytoplankton
SI	48.592°N, -123.505°W	13 Sep 2012	14	27.9	1.24	0.75	0.54	Mixed assemblage
FRP	49.072°N, -123.402°W	13 Sep 2012	11	28.0	10.9	1.12	0.07	*Phaeocystis, Skeletonema, Leptocylindrus*
SB	49.013°N, -123.037°W	26 Aug 2013	20.2	29	0.00	2.40	0.08	Cyanobacteria Unknown ciliate
JP	49.277°N, -123.202°W	27 Aug 2013	19.5	21	0.00	0.20	0.11	Diatoms (mixed assemblage)

5

1   Table 2 *Vibrio* sp. strain PWH3a dynamics during the creation of lysates by viral infection
2   (Laboratory = experiments with cultured *Synechococcus* sp. strain DC2, SI = Saanich Inlet, FRP
3   = Fraser River Plume, SB = Semiahoo Bay, JP = Jericho Pier).

	Laboratory	SI and FRP	SB and JP
Vibrio sp. strain PWH3a abundance pre-virus (cell l^{-1})	3.96×10^{11}	7.87×10^{11}	7.89×10^{10}
Vibrio sp. strain PWH3a abundance post-virus incubation (cell l^{-1})	1.12×10^{11}	9.43×10^{10}	6.38×10^{10}
Multiplicity of infection (MOI)	1	0.41	5
Total N release (µM)	721	1760	38.4
N addition to experimental incubations (µM)	36.0	67.7	0.44

4

5

1 Table 3 Uptake by the particulate fraction of ^{15}N from bacterial lysate from four field stations,
2 and the same uptake normalised by initial chlorophyll a concentrations. Error measurements are
3 standard error of triplicate incubations for SB, and range of duplicate incubations for JP. SI and
4 FRP are singleton measurements.

	Total uptake of ^{15}N (μM)	Uptake of ^{15}N normalised by chlorophyll a (μmol μg^{-1})
Saanich Inlet (SI)	1.67	0.27
Fraser River Plume (FRP)	1.76	0.70
Semiahoo Bay (SB)	0.60 ± 0.02	0.70 ± 0.16
Jericho Pier (JP)	0.50 ± 0.1	0.09 ± 0.00

5

6

1
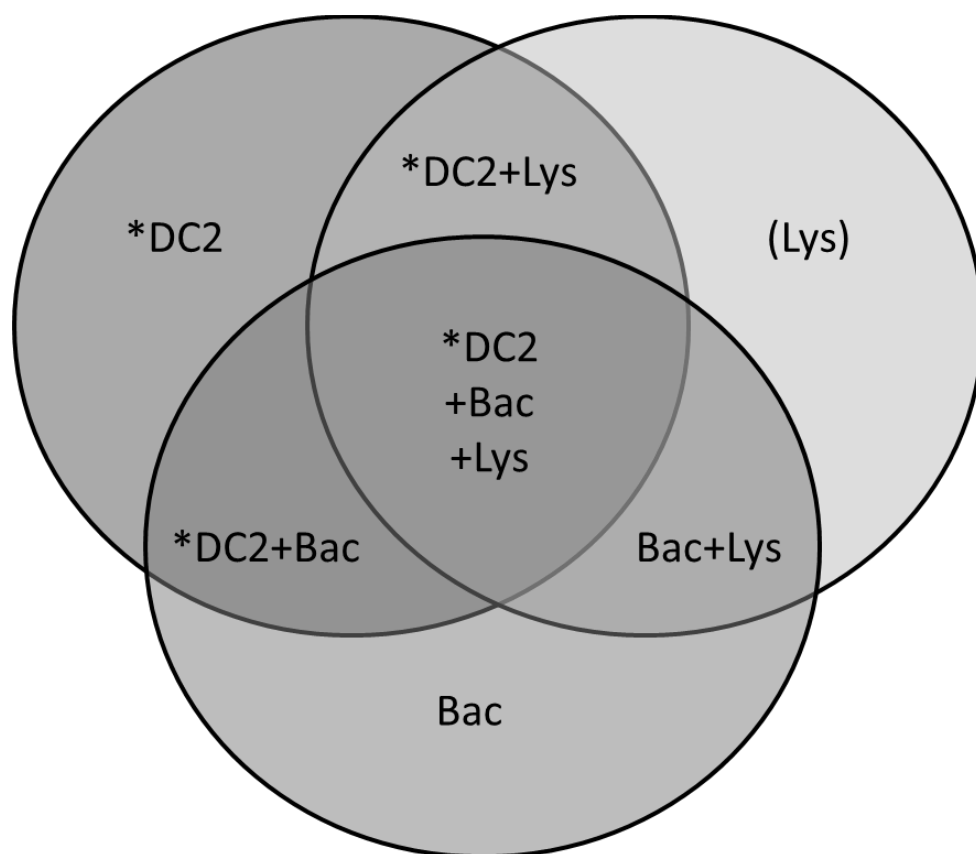
2
3 Figure 1 Diagram of treatments in the laboratory experiment examining increase in
4 *Synechococcus* sp. strain DC2 abundance, in the presence of bacterial lysate from *Vibrio* sp.
5 strain PWH3a (Lys) and an environmental bacterial assemblage (Bac). Lys, while a component
6 of some of the treatments, was not a treatment by itself (indicated by parentheses). The * is an
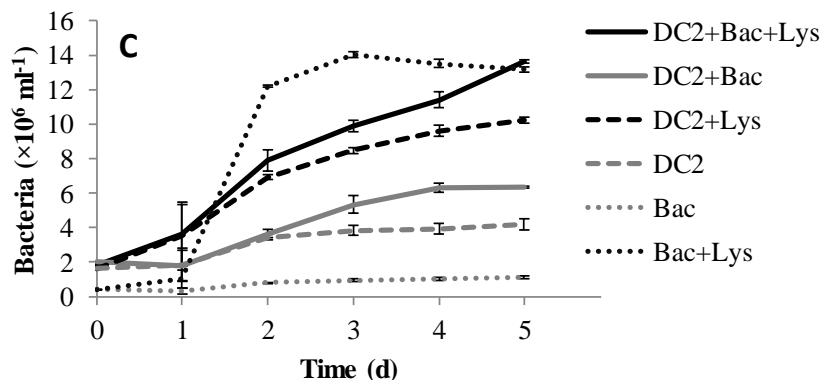7 indication that DC2 is not axenic, and therefore contains some heterotrophic bacteria.

8

9
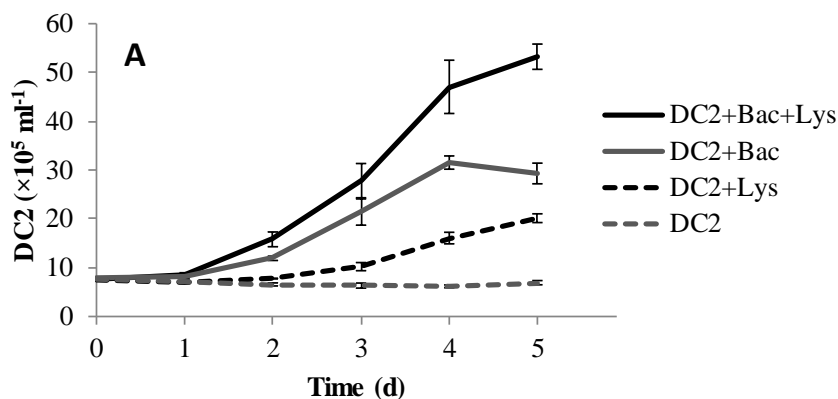
1
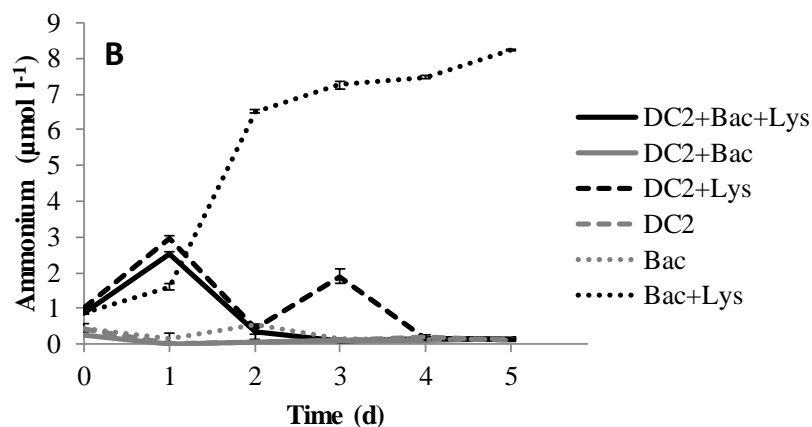
2

3

4 Figure 2 Time series of (A) *Synechococcus* sp. strain DC2 abundance, (B) ammonium
5 concentration, and (C) bacterial abundance in the laboratory experiment. Error bars are standard
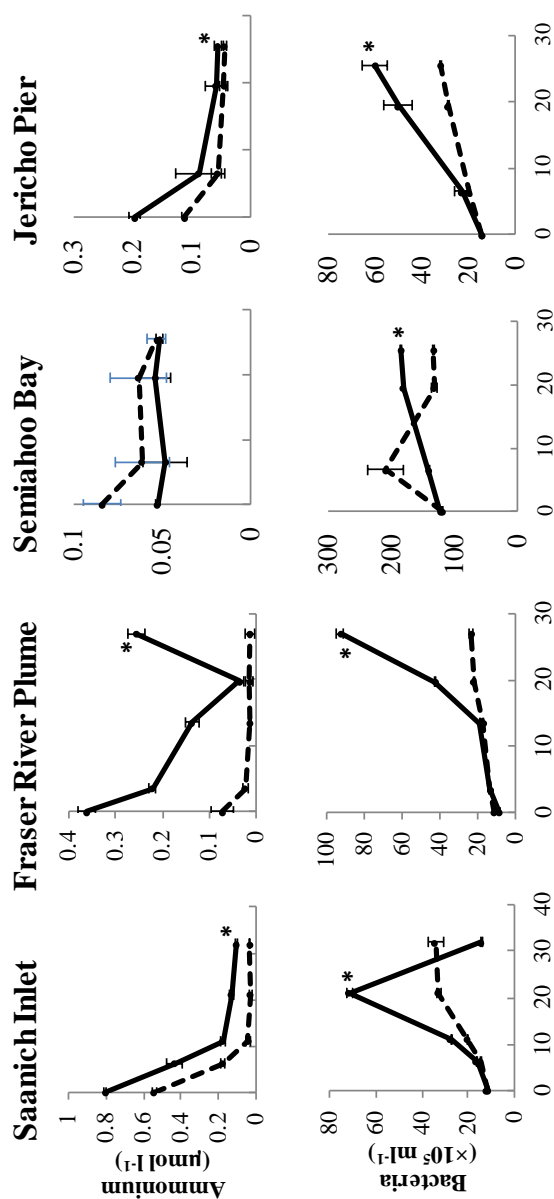6 error from triplicate incubations.

7

Figure 3 Time-course of ammonium concentrations and bacterial abundances. Solid and dashed lines indicate treatments with lysate addition (SW+lysate), or without (SW), respectively. Error bars are standard errors of triplicate incubations. Asterisks indicate significant differences between treatments. Note that the scales are different among plots.
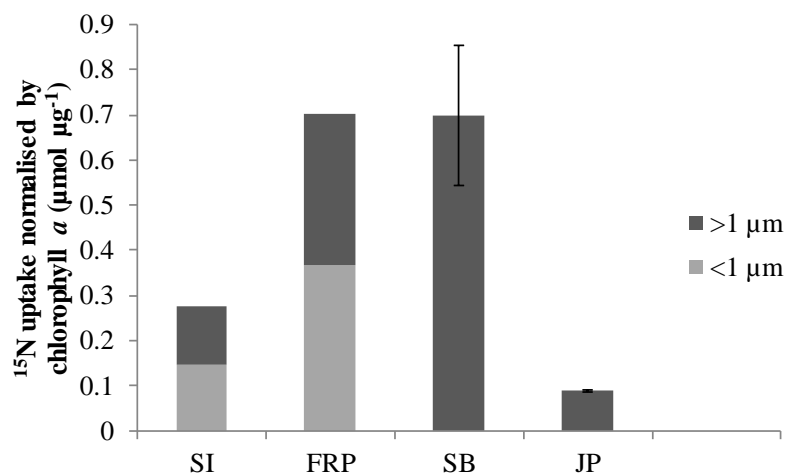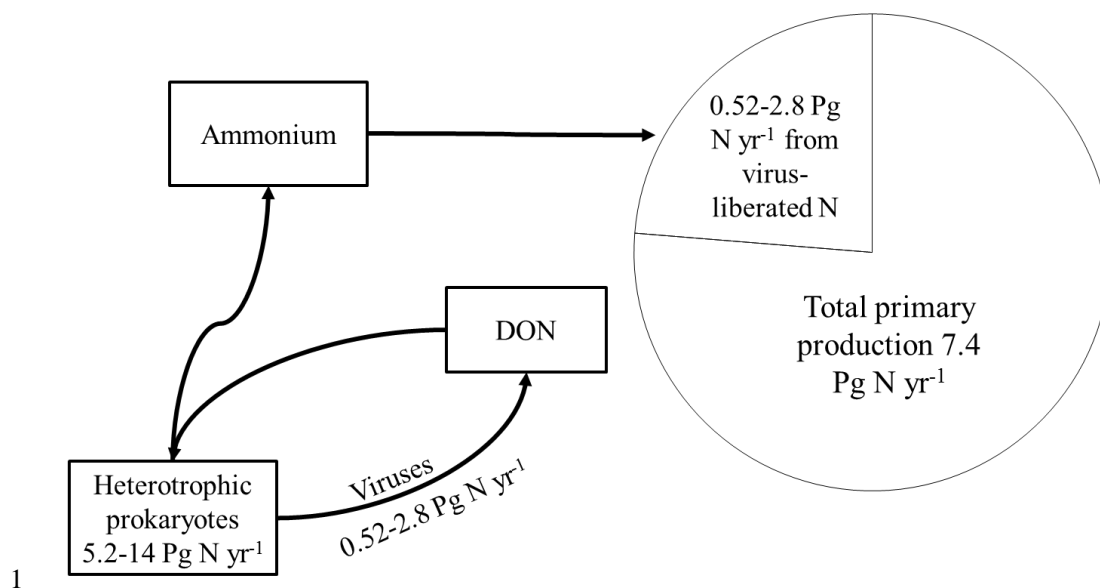
1

2
3 Figure 4 Uptake of ^{15}N normalised by initial chlorophyll *a* concentrations during the experiment
4 by the >1 μm and <1 μm fractions in treatments with lysate addition for Saanich Inlet (SI),
5 Fraser River Plume (FRP), Semiahoo Bay (SB), and Jericho Pier (JP). Error bars are standard
6 error of triplicate incubations for SB, and range of duplicate incubations for JP. SI and FRP are
7 singleton measurements.

8

9

1

Figure 5 Viruses release 0.52-2.8 Pg N yr^{-1} globally in the euphotic ocean from lysis of
heterotrophic prokaryotes. That dissolved organic N (DON) is available for remineralisation by
other prokaryotes into ammonium, which constitutes a large proportion of the total N demand of
primary producers.