Effects of acute ocean acidification on spatially-diverse polar pelagic foodwebs: insights from on-deck microcosms

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Abstract

The polar oceans are experiencing some of the largest levels of ocean acidification (OA) resulting from the uptake of anthropogenic carbon dioxide (CO₂). Our understanding of the impacts this is having on polar marine communities is mainly derived from studies of single species in laboratory conditions, while the consequences for food web interactions remain largely unknown. This study carried out experimental manipulations of natural pelagic communities at different high latitude sites in both the northern (Nordic Seas) and southern hemispheres (Scotia and Weddell Seas). The aim of this study was to identify more generic responses and greater experimental reproducibility through implementing a series of short term (4 day), multilevel (3 treatment) carbonate chemistry manipulation experiments on unfiltered natural surface ocean communities, including grazing copepods. The experiments were successfully executed at six different sites, covering a diverse range of environmental conditions and differing plankton community compositions. The study identified the interaction between copepods and dinoflagellate cell abundance to be significantly altered by elevated levels of dissolved CO₂ (pCO₂), with dinoflagellates decreasing relative to ambient conditions across all six experiments. A similar pattern was not observed in any other major

30 phytoplankton group. The patterns indicate that copepods show a stronger preference for
31 dinoflagellates when in elevated $p\text{CO}_2$ conditions, demonstrating that changes in food quality and
32 altered grazing selectivity may be a major consequence of ocean acidification. The study also found
33 that transparent exopolymeric particles (TEP) generally increased when $p\text{CO}_2$ levels were elevated,
34 but the response was dependent on the exact set of environmental conditions. Bacteria and
35 nanoplankton showed a neutral response to elevated $p\text{CO}_2$ and there was no significant
36 relationship between changes in bacterial or nanoplankton abundance and that of TEP
37 concentrations. Overall, the study illustrated that, although some similar responses exist, these
38 contrasting high latitude surface ocean communities are likely to show different responses to the
39 onset of elevated $p\text{CO}_2$.

40

41 **Key Words:** Arctic, Southern Ocean, copepod, phytoplankton, dinoflagellates, bacteria,
42 nanoplankton, transparent exopolymeric particles, $p\text{CO}_2$

1. Introduction

The on-going shift in seawater carbonate chemistry and related reduction of ocean pH (termed ocean acidification, OA) caused by increasing atmospheric carbon dioxide (CO₂) levels has the potential to affect a wide range of marine organisms (Fabry et al. 2009, Cooley et al. 2009). Taxa that inhabit oceanic surface layers are particularly at risk since that is where the greatest changes are being observed. Some taxa respond sensitively to elevated CO₂ levels, whereas the response in others is mixed and often highly species-specific (Engel et al. 2008, de Nooijer et al. 2009, Doney et al. 2012, Beaufort et al. 2011, Whiteley 2011). Our present understanding of OA impacts is largely limited to single species responses while the consequences for food web interactions remain relatively unknown (Rossoll et al. 2012). For instance, the response at the community level may dampen the overall impact to elevated CO₂ levels through altered physiological performance, prey switching and species replacement (Rossoll et al. 2013).

The solubility of CO₂ increases with decreasing temperatures, such that polar oceans have naturally higher CO₂ and correspondingly lower carbonate ion concentrations (Sabine et al. 2004). With a low buffer capacity and a relatively low starting baseline, polar oceans are particularly susceptible to ocean acidification and are expected to be the first to experience carbonate mineral undersaturation and large decreases in pH (McNeil and Matear 2008, Steinacher et al., 2009). As a result, the polar regions, and particularly the Arctic, have been a focus for studies into the effects of projected OA on the marine environment (Riebesell et al. 2013).

Most studies of the effect of OA on pelagic organisms have involved laboratory and mesocosm experiments. Laboratory monoculture experiments allow experimental conditions to be tightly controlled and are valuable at identifying possible mechanisms and impacts. However, the outcomes of these experiments cannot be fully realistic because species are studied in isolation from the natural ecosystem in which there may be shifts from more pH-sensitive to more pH-tolerant species.

67 Furthermore, the experimental conditions themselves (e.g. nutrient levels) may not be
68 representative of typical oceanic conditions. Some of these shortcomings are overcome in carrying
69 out mesocosm experiments, where large volumes (50 to 75 m³) of natural planktonic communities
70 are incubated *in situ* and manipulated with regards to artificially altering pH, carbonate chemistry and
71 nutrient levels (Riebesell et al. 2010). The logistical demands of such operations have, nevertheless,
72 restricted their deployment to inshore regions which may not adequately cover the range of
73 conditions and communities found in the open ocean.

74 To date, the main approach to considering planktonic community responses to OA in open-ocean
75 environments has been through short-term (1 to 14 d) incubations of natural communities on
76 scientific vessels using on-deck microcosms (1 to 50 L; Riebesell et al. 2000, Tortell et al. 2002,
77 Hutchins et al. 2007, Richier et al. 2014). Although the incubation volumes are relatively small
78 compared to mesocosms, this approach does allow identical experiments to be repeated across
79 natural gradients in carbonate chemistry, temperature and nutrients. The approach has the
80 advantage of examining a range of natural communities adapted to their particular environmental
81 ranges. Comparisons can be made on how each community responds to the same stressor (i.e.
82 changes in carbonate chemistry). The disadvantage of this approach is in the sudden induced change
83 in pH and carbonate chemistry to which the community is given little time to adapt. Nevertheless,
84 alternative longer-term, multigenerational approaches (Cripps et al. 2014) are best suited to studies
85 of single species, and over timescales that are unfeasible for spatially extensive, vessel-based
86 research. We used the short-term on-deck microcosm approach to consider the response of
87 planktonic foodwebs, from a range of polar and sub-polar environments, to acute OA perturbations.

88 OA may affect food-webs in a number of ways, each with their own feedbacks to other parts of the
89 system. Short-term acute-change experiments of this sort cannot characterise all such changes.
90 However, in performing identical experiments across a range of environmental starting conditions,
91 they can identify where there are common responses to acute change, or otherwise, how responses

92 vary according to the initial environmental conditions and community composition. In particular, we
93 investigate two separate, but not mutually exclusive, hypotheses:

94 i) that acute OA change will lead to the accumulation of transparent exopolymeric particles (TEP)
95 which will stimulate the microbial food web;

96 ii) that acute OA change will alter the nutritional quality of individual phytoplankton species and
97 lead to a change in zooplankton grazing preferences, so altering phytoplankton community
98 composition and potentially zooplankton body condition.

99 i) *Stimulation of TEP production* TEP are gel-like particles that form through coagulation of the
100 polysaccharide fraction of dissolved organic matter (Passow, 2002). It has been proposed that
101 increasing levels of $p\text{CO}_2$ in the surface ocean leads to more partitioning of the organic carbon fixed
102 by marine primary production into the dissolved rather than the particulate fraction. This enhances
103 the accumulation of dissolved organic carbon (DOC) in the surface ocean, and the concurrent
104 accumulation of TEP. Such an accumulation was first noted by Engel (2002) in on-board experiments
105 under increased $p\text{CO}_2$, although whether the accumulation was due to increased production or
106 modification of TEP structure remains uncertain (Weinbauer et al. 2011). Enhanced TEP has also
107 been associated with nutrient stress (Engel 2002, Engel et al. 2004, Bellerby et al. 2008). Increased
108 TEP aggregation can result in higher abundances and production of bacteria by attracting cells to a
109 microbial hotspot (Simon et al. 2002). Nevertheless, contrasting relationships between TEP and
110 bacteria have been found across diverse ocean areas (Corzo et al. 2005, Passow et al. 2001, Hung et
111 al. 2003, Santschi et al. 2003, Passow and Alldredge 1994, Baskar and Bhosle 2006). We investigated
112 if TEP production is affected by acute pH change and whether there is evidence of an enhancement
113 of the microbial community as a result. Furthermore, we also considered whether any such patterns
114 are altered by an additional trophic level (i.e. copepod grazers), given that copepods can directly
115 feed on clusters of TEP (Passow and Alldredge 1999, Ling and Alldredge 2003).

ii) *Changes to grazing preferences* There is growing evidence that enhanced $p\text{CO}_2$ levels alter the nutritional quality of individual phytoplankton species available to grazers via species specific changes in their biochemistry (Riebesell and Tortell 2011). One major factor is taxonomic differences in the kinetic properties of carbon acquisition. Diatoms, for instance, have a much higher enzymatic specificity for carbon than dinoflagellates (Tortell 2000), which affects the rate of carbon enrichment per unit $p\text{CO}_2$. $p\text{CO}_2$ increase may also alter carbohydrate and fatty acid content in certain phytoplankton (Wynn-Edwards et al. 2014). Zooplankton have specific stoichiometric dietary requirements (Nobili et al. 2013) and an ability to select the prey species that suit their overall needs (Kleppel 1993). Therefore, this study will consider how zooplankton grazing preferences may alter between different acute OA treatments, and whether zooplankton body condition is affected.

We conducted short-term bioassay CO_2 perturbation experiments using natural seawater collected from the surface ocean and brought into a controlled laboratory on the deck of the research ship. This natural unfiltered seawater was manipulated to raise $p\text{CO}_2$ to future predicted levels, if at an acute rate. Locally-caught herbivorous copepods were subsequently introduced into certain incubation chambers to simulate natural food-web scenarios. The procedure was repeated in a number of Arctic and Southern Ocean oceanic settings, with contrasting environmental characteristics. In so doing, the study examines both short-term physiological responses, through acute exposure to raised $p\text{CO}_2$ levels, alongside adaptive community variability, given that the communities being incubated were extracted directly from their natural setting. This means that responses will be dependent on initial community structure alongside community changes resulting from competition between the multiple genotypes present within the incubation. Overall, this study examines the effects of acute changes to CO_2 levels on real-world planktonic food-webs found in both polar oceans.

2. Methods

2.1 Sampling stations

This study was undertaken using on-board incubations of unfiltered seawater with and without additions of net-caught zooplankton as part of two open-ocean research campaigns. The research cruises were carried out on board RRS *James Clark Ross* in June-July 2012 in the sub-Arctic and Arctic Ocean (Cruise JR271) and January to February 2013 in the Southern Ocean (Cruise JR274). As part of these cruises, successful experimental incubations were performed in a total of six high-latitude environments (Table 1) with a diverse set of starting conditions. Two of the incubations were performed in Arctic waters, one in the open Greenland Sea (E03 271) and the other at the Greenland sea-ice margin (E04 271). A further two incubations were performed in the Southern Ocean, the first towards its northern limits close to South Georgia (E03 274), and the other, further south in the Weddell Sea, close to the South Sandwich Islands (E04 274). The remaining two incubations were carried out outside of true polar waters, one in the North Sea (E01 271) and the other in the sub-Arctic Barents Sea (E05 271).

2.2 Water collection

The incubations were set up with unfiltered seawater taken from the upper-mixed layer at the station location. On the day of the experimental setup, vertical CTD profiles were performed to characterise water column variables (temperature, salinity, chlorophyll fluorescence) and determine water column structure. The depth of water collection for the experimental setup was then decided upon based on these initial profiles and was typically from the middle of the upper mixed layer. The unfiltered water was collected from dedicated CTD casts using a titanium frame CTD and acid-cleaned 24 x 10 L Rosette OTE (Ocean Test Equipment) bottles. On deck, the bottles were immediately transferred to a class-100 filtered air environment within a trace-metal clean container to avoid contamination. The unfiltered water was transferred using acid-cleaned silicon tubing to 1 L

acid-cleaned clear Duran bottles and the bottles checked for any extraneous copepods, which were removed before the bottles were sealed, pending carbonate chemistry manipulation and the addition of hand-picked live copepods from net-catches (see below). Subsamples were collected simultaneously for time-zero (T0) measurements of each of the variables to be measured over the subsequent time course (Table 2).

Further subsamples at time zero (T0) were taken directly from the CTD and immediately measured for total alkalinity (TA) and dissolved inorganic carbon (DIC) to characterise the carbonate chemistry system in seawater; details for the analytical methods can be found below. The remaining variables of the carbonate system were calculated with the CO2SYS programme (version 1.05, Lewis and Wallace, 1998; van Heuven et al., 2011), using the constants of Mehrbach et al. (1973) refitted by Dickson and Millero (1987). Carbonate chemistry in the experimental bottles was subsequently manipulated using equimolar additions of strong acid (HCl, 1 mol L⁻¹) and HCO₃⁻ (1 mol L⁻¹), as recommended by Gattuso et al. (2010) for increasing DIC at constant TA. The volumes of HCl and HCO₃⁻ required to adjust *p*CO₂ to the chosen target values (750 and 1000 µatm) were calculated from the measured ambient state of the carbonate system in seawater using CO2SYS.

2.3 Zooplankton collection

Immediately after the collection of water, a motion-compensated Bongo net (61 cm diameter, 100 µm and 200 µm mesh) was deployed between 0 and 200 m to capture live zooplankton. Subsamples of the net catches were immediately analysed to determine the approximate species composition and decide on the most appropriate calanoid species (and developmental stage) to use for the incubation experiments. The choice of species varied between experimental stations, with *Calanus finmarchicus* being used in the North Sea (E01 271), *C. hyperboreus* in the Arctic (E03 271 and E04 271) and *Calanoides acutus* in the Southern Ocean (E03 274 and E04 274). The exact species identity of the calanoid used in the Barents Sea was unclear and classified as *Calanus finmarchicus/glacialis*. The chosen developmental stage was mainly copepodite stage V (CV),

although, in E04 274, it was necessary to combine this stage with females because numbers were relatively low. Individual copepods were picked out from sub-samples analysed under a light microscope using titanium forceps (to minimise iron contamination) and acid washed pipettes and transferred to 10 mL glass vials containing filtered seawater and maintained at ambient sea-surface temperature. Each glass vial contained either 5 or 10 specimens, the number differing between stations to account for individual specimen size and in situ concentrations. A total of 18 glass vials were populated with copepods in readiness for their subsequent introduction into the incubation experimental bottles.

2.4 Set-up and running of experiments

In the trace-metal clean container, the 1 L Duran bottles containing the unfiltered sea-water were modified with varying aliquots of equimolar HCl and HCO_3^- to achieve nominal $p\text{CO}_2$ levels of either 750 μatm or 1000 μatm . Eight individual Duran bottles per $p\text{CO}_2$ level were prepared, while a further ten Duran bottles were left unmodified. This number allowed for two sets of triplicate bottles containing copepods in ambient conditions, 750 μatm $p\text{CO}_2$ and 1000 μatm $p\text{CO}_2$, as well as two sets of single bottles without copepods for each $p\text{CO}_2$ treatment and two sets of double bottles without copepods in ambient conditions. Accordingly, the copepods in the glass vials were gently poured into six of the eight modified bottles for each $p\text{CO}_2$ treatment, and six of the ten unmodified bottles. Each of the bottles was topped up to the rim and the lids sealed. The 26 bottles (i.e. 2 identical sets of 13 bottles) were placed on 3 plankton wheels within a controlled temperature container and maintained at ambient surface-water temperature (Fig. 2). Irradiance ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$) was provided by daylight simulation LED panels (Powerpax, UK) over a 24 h light cycle which approximated the ambient photoperiod and light intensity at 5 m (Richier et al. 2014).

The first set of 13 bottles was removed from the plankton wheels after 24 h and inspected to determine if there were any dead copepods in the incubation. The bottles were then immediately

213 subsampled for the concentration of nutrients (including ammonium), TA and DIC and transparent
214 exopolymeric particles (TEP) as well as the abundance of bacteria, nanoplankton, and major
215 phytoplankton taxa. It was not possible to make all measurements on all bottles because of the
216 volumes required for each analytical method. However, each measurement was made on at least
217 one bottle with copepods and one without copepods at the three different $p\text{CO}_2$ levels (ambient,
218 750 μatm and 1000 μatm). The copepods were filtered out of the remaining water, rinsed in
219 deionised water (Milli-Q, Millipore) and snap-frozen in liquid nitrogen for subsequent elemental
220 composition analysis.

221 The remaining set of 13 bottles were checked for copepod mortalities at 48, 72 and 96 h after the
222 start of the incubation. At the 96 h timepoint, the take-down procedure was repeated, with the
223 exception that samples for bacteria, nanoplankton and phytoplankton abundance were not taken,
224 given that they would most likely be subject to exponential growth and overgrazing effects
225 respectively after this length of time in volumes of 1L.

226 **2.5 Measurements of biological and chemical variables**

227 *Nutrients* Samples for macronutrients (nitrate (NO_3^-) + nitrite (NO_2^-)), silicic acid (dSi) and phosphate
228 (PO_4^{3-}) were collected directly from the incubation bottles into a 25 mL polystyrene container and
229 stored at 4°C pending analysis within 12 h. The samples were run on a Skalar San+Segmented Flow
230 Autoanalyzer using colorimetric techniques (Kirkwood, 1996) with the exception that the flow rate
231 of the sample through the phosphate channel was increased to improve reproducibility and peak
232 shape.

233 For ammonium analysis, 10 mL samples were collected in polypropylene vials and reagent added,
234 following Kerouel and Aminot (1997). Fluorometric analysis was performed 24 h after the addition of
235 reagent. Concentrations were estimated to an accuracy of nanomoles L^{-1} .

Carbonate chemistry Samples for dissolved inorganic carbon (DIC) and total alkalinity (TA) were collected from the incubation bottles in 250 mL borosilicate glass bottles. TA and DIC measurements were performed using either a VINDTA 3C system (Marianda, Germany), an Apollo SciTech DIC analyser (AS-C3) or an Apollo SciTech Alkalinity Titrator (AS-ALK2), depending on availability of the instruments during the cruises. On the VINDTA 3C system, DIC was measured by coulometric titration and TA by potentiometric titration and calculated using a modified Gran plot approach (Bradshaw et al., 1981). The Apollo uses a CO₂ infrared detector (LICOR 7000) to measure DIC and an open cell titration to measure TA. We have routinely used the different instruments in our lab and have found no systematic differences between them, therefore the data is inter-comparable with no significant difference between the methods. Certified Reference Materials (CRMs) (A.G. Dickson, Scripps Institution of Oceanography, batch 117 in the Arctic, batches 119 and 120 in the Southern Ocean) were used at the beginning, middle and end of each run on all instruments as a control for both DIC and TA. Precision was taken as the standard deviation of repeated analysis of CRMs. For the VINDTA 3C instrument, this was $\pm 2 \mu\text{mol kg}^{-1}$ for TA and $\pm 3 \mu\text{mol kg}^{-1}$ for DIC. For the Apollo instrument, TA was $\pm 1.5 \mu\text{mol kg}^{-1}$ and DIC $\pm 3 \mu\text{mol kg}^{-1}$. The remaining variables of the carbonate system were calculated with the CO2SYS program (MATLAB version 1.1; Lewis and Wallace, 1998; van Heuven et al., 2011) using the carbonate equilibrium constants of Mehrbach et al. (1973) refitted by Dickson and Millero (1987), the boric acid dissociation constants of Dickson (1990) and the ratio of total boron to salinity recommended by Lee et al. (2010).

TEP Samples of TEP were collected by filtration of 200 mL of seawater through 25 mm diameter polycarbonate filters (0.45 μm pore-size, Sterlitech) at constant vacuum (200 mBar). Three separate filtrations were carried out on each 1 L bottle that was analysed. The particles retained on the filters were stained with 500 μL of 0.02% aqueous solution of Alcian blue in 0.06% acetic acid (pH 2.5). The dye was pre-filtered using a polycarbonate filter (0.2 μm pore-size; Sterlitech) before use. Stained filters were rinsed once with deionised water (Milli-Q, Millipore) and then transferred into 15 mL polypropylene centrifuge tubes (Fisher Scientific) and stored at -20°C . Onshore, the amount of

262 Alcian Blue adsorbed onto the filters was determined following a soak in 6 mL of 80% sulphuric acid
263 for 2 h and determination of the absorbance of the resulting solution at 787 nm (absorption
264 maximum) using a spectrophotometer (U-1800, Hitachi). The amount of Alcian blue in the solution
265 was directly related to the weight of the polysaccharide that was retained on the filter (Passow and
266 Alldredge, 1995).

267 *Bacteria and nannoplankton* For each sample, 1.6 mL of seawater was removed and placed into a 2
268 mL polypropylene screw cap vial containing 80 μ L of paraformaldehyde (PFA, 1% final
269 concentration). The vials were then placed in a fridge and left for no longer than 12 hours before
270 being analysed by flow cytometry (BD FACSort instrument) to enumerate bacterioplankton and
271 protist abundance (Marie et al. 1997, Zubkov et al. 2007). Samples were stained with SYBR green I
272 nucleic acid dye for a minimum of 30 minutes, before being analysed. Samples were run at a low
273 flow rate of 0.02 mL min⁻¹ for 1 minute to determine bacterioplankton concentration and at a flow
274 rate of 0.2 mL min⁻¹ for 3 minutes to determine concentrations of phototrophic and heterotrophic
275 protists. For each sample, a known volume of bead solution was added as an internal standard. The
276 bead solution consisted of Fluoresbrite multicolour 0.5 μ m and 1.0 μ m beads dispersed in 400 mL of
277 sterile Milli-Q (Millipore) water. The bead concentration was calculated before use using the FACS
278 calibur flow cytometer and a syringe pump (Zubkov and Burkhill 2006).

279 *Phytoplankton composition and abundance* 100 mL of seawater was taken from the incubation
280 bottles and immediately preserved in a 2% final concentration of acidic Lugol's solution stored in 250
281 mL amber glass bottles. Phytoplankton (>10 μ m) was identified and enumerated in 50 mL Hydro-Bios
282 settling chambers on a SP-95-I inverted microscope, following Poulton et al. (2007).

283 *Elemental analysis of copepods* Total carbon, hydrogen and nitrogen of either single copepods or
284 batches of 5 or 10 copepods were determined using a CE-440 Elemental Analyser (Exeter Analytical,
285 Inc.). The copepods were either taken immediately from the net catch (T0 population) or post-
286 incubation, rinsed in deionised water and placed in tin capsules before snap freezing in liquid

287 nitrogen and maintained at -80°C for analysis on-shore. On-shore, the capsules were dried overnight
288 at 50°C and then combusted at 1800°C in the Elemental Analyser to determine total carbon,
289 hydrogen and nitrogen through analysis of the products of combustion by gas chromatography.
290 Elemental analysis of copepods from experiment E01 271 was not performed because of a
291 processing error.

292 **2.6 Statistical treatment**

293 Differences between $p\text{CO}_2$ treatments (*treatment effects*) were analysed using a 1-way ANOVA
294 provided that they first passed tests for normality (Kolmogorov-Smirnov test) and equal variance
295 (variability about group means), else they were analysed non-parametrically with a 1-way ANOVA on
296 ranks. Paired t-tests were used to determine the impact of the addition of copepods to incubations,
297 subject to the same tests for normality and equal variance. Attempts to fit linear regressions were
298 made to the relationship between the relative changes in TEP and either bacteria or nannoplankton,
299 using the Sigmaplot 12.5 package (Sysstat Software Inc.) using a least-squared fitting method. Fits
300 where the slope of the curve were not significantly different from 0 were otherwise considered as
301 trends of which the consistency was compared between treatments. The influence of copepods on
302 the abundance of bacteria or nannoplankton was assessed through comparison of 95% confidence
303 intervals between incubations with- and without copepods (Zar 1996).

3. Results

3.1 Starting conditions

Physical conditions Experiments at 4 of the 6 stations were carried out under true polar conditions where surface mixed-layer temperatures were between 2.2°C and -1.6°C (E03 271, E04 271, E03 274 and E04 274). Experiment E05 271 was initiated in the sub-Arctic Barents Sea, with surface mixed-layer temperatures at around 6.5°C. Experiment E01 271 was sited in the North Sea, where the temperature was 10.7°C (Table 2). Nutrient concentrations varied considerably, with phosphate and nitrate being at non-limiting concentrations in both of the Southern Ocean stations (E03 274 and E04 274) in contrast to the very low levels observed at the North Sea station (E01 271). This is reflected in the N:P atomic ratio, which was close to the Redfield ratio of 16 in the Southern Ocean, but as low as 0.6 in the North Sea. The Arctic and sub-Arctic stations showed varying degrees of nutrient limitation. Silicate was also abundant in the Southern Ocean stations compared to the other stations, with the lowest concentrations being observed in the North Sea and Barents Sea. pH was lowest at the South Georgia Southern Ocean station (E03 274) at 8.050, with a corresponding $p\text{CO}_2$ level of 384 μatm . All other stations had pH values ranging between 8.1 and 8.2 and $p\text{CO}_2$ levels in the region of 300 μatm .

Biological conditions Chl-a concentrations in the mixed layer reached as high as 4.2 $\mu\text{g L}^{-1}$ at the Weddell Sea station (E04 271), and 3.03 $\mu\text{g L}^{-1}$ at the Greenland sea-ice margin station (E04 271) but only 0.31 $\mu\text{g L}^{-1}$ in the North Sea (E01 271). There appeared to be little correspondence between levels of Chl-a and TEP across stations, with relatively high TEP levels occurring at stations where Chl-a was both low (E01 271) and moderately high (E05 271). TEP concentrations were comparatively low in both Southern Ocean stations (E03 274, E04 274). The highest abundance of bacteria was observed in the open Greenland Sea (E03 271) and the Barents Sea (E05 271), at above 1 million cells mL^{-1} , while the Greenland sea-ice margin station (E04 271) contained the lowest numbers, at less

than half of those levels of abundance. Nannoplankton abundance similarly was highest at stations E03 271 and E05 271, and were almost an order of magnitude lower at the Weddell Sea station (E04 274).

The phytoplankton community analysed from the Lugol's samples was numerically dominated by diatoms at stations in the North Sea (E01 271), the Greenland sea-ice margin station (E04 271) and in the Weddell Sea (E04 274, Fig. 2). Dinoflagellates were the dominant taxa in the Barents Sea (E05 271) while there was more of an even mix of phytoplankton taxa in the open Greenland Sea (E03 271) and near South Georgia (E03 274). Those stations dominated by diatoms had relatively high cell counts, while cell counts were low where dinoflagellates made up a large fraction of the phytoplankton community.

The choice of calanoid species for the incubations differed between stations, based on their relative availability in net-catch samples (Table 1). There was as much variability in the C:N mass ratio within as between species, indicating that body condition varied according to locality (Table 3, Fig. 2).

Higher C:N mass ratios are considered to reflect better body condition in that such individuals contain greater amount of body reserves. *Calanus hyperboreus* CV in the open Greenland Sea (E03 271) had a median C:N mass ratio that was almost 30% higher than the same species and stage found at the Greenland sea-ice margin (E04 274). Similarly, *Calanoides acutus* (CV and female combined) in the Weddell Sea had C:N mass ratios that were at least double of those collected at South Georgia (CV only, E03 274). Therefore, the physiological states of the populations chosen as grazers in each set of incubations were diverse, reflecting different life-history and feeding trajectories up to the point of capture.

3.2 Outcome of manipulations on water chemistry

The influence of the equimolar additions of acid and base on nutrient and silicic acid levels and the carbonate chemistry is fully detailed in Appendices 1, 2 and 3. The manipulations were successful in

increasing DIC levels whilst maintaining relatively constant levels of TA. However, in some instances, the $p\text{CO}_2$ target levels were not attained, with the measured levels of $p\text{CO}_2$ after the manipulations being below those intended. The effects of the additions on nutrient and silicic acid concentrations was negligible given the minute volumes that were added.

3.3 Response of TEP and the microbial community

TEP response After 96 hours, TEP concentrations increased in all of the control (ambient conditions, no copepods) incubations across all six sets of experiments (*bottle effects*, Table 4). The rate of increase varied between experiments, with the highest rate observed at the Barents Sea station (E05 271, $5.71 \mu\text{g xanthan equivalent L}^{-1} \text{ h}^{-1}$) where the corresponding N:P atomic ratio was relatively low (5.5). The lowest rates occurred in the Southern Ocean stations (E03 274, E04 274, 0.1 to $0.4 \mu\text{g xanthan equivalent L}^{-1} \text{ h}^{-1}$), where N:P was close to the Redfield ratio of 16 (Redfield 1958).

Relative to this tendency for TEP accumulation over time, the only experiment to show an increase above that otherwise expected after 24 h was E04 274 for the $1000 \mu\text{atm}$ treatment (*treatment effects*, Table 4). However, by 96 h, significant increases were also found in E01 271, E04 271 and E05 271. Only experiments E01 271 and E04 274 showed significant increases in both the $750 \mu\text{atm}$ and $1000 \mu\text{atm}$ treatments after 96 h, the others being limited to significant increases in the $1000 \mu\text{atm}$ treatments only.

The effects of the presence of copepods on TEP were mixed (*copepod effects*, Table 4). None of the experiments showed any significant increase or decrease in TEP above background increases by 24 h. By 96 h, four of the six experiments showed significant increases in TEP (E01 271, E03 271, E05 271 and E03 274), while one experiment showed a significant decrease (E04 271) and one no significant change (E04 274).

Bacterial response Compared to the variance in bacterial cell counts for the starting conditions (T0 samples), there was no significant change in the abundance of bacteria after 24 h either at ambient

376 conditions or in the two $p\text{CO}_2$ treatments (Fig. 3). The presence or absence of copepods in the
377 incubations had a significant impact on bacterial abundance in certain instances. In ambient
378 conditions in E04 271 and in the 750 μatm treatment in E04 274, the presence of copepods
379 significantly reduced the number of bacterial cells. However, bacterial numbers were significantly
380 enhanced by the presence of copepods in the 1000 μatm treatments in experiments E05 271 and
381 E03 274. For the majority of experiments however, the presence or absence of copepods had very
382 little impact on bacterial numbers under ambient or increased $p\text{CO}_2$ levels.

383 *Nannoplankton response* There was similarly little pattern in the response of the nannoplankton
384 community to incubation conditions (Fig. 4). No significant change in the abundance of
385 nannoplankton cells after 24 h was detected in either ambient conditions or the two $p\text{CO}_2$
386 treatments compared to the variance observed in the T0 samples. The presence or absence of
387 copepods in the incubations did not make a significant impact in the majority of instances. However
388 in the 1000 μatm treatment of E01 271, and at ambient conditions in E04 274, there was a
389 significant enhancement in nannoplankton abundance when copepods were present.

390 *Relationship between TEP and microbial abundance* In the control incubations (ambient conditions
391 without the presence of copepods), there was a general negative trend between relative changes in
392 TEP concentration and bacterial cell abundance, although the relationship was not significant
393 (ANOVA, $F = 4.237$, $P = 0.109$, 5 df, Fig. 5). This trend was mainly driven by the two Southern Ocean
394 experiments (E03 274, E04 274), where there was a relatively large increase in TEP in E03 274
395 associated with a fall in bacterial abundance and a similarly large decrease in TEP in E04 274, where
396 bacterial abundance increased. The relative changes in TEP were less marked in incubations that
397 contained copepods. Overall, the relationship between TEP and bacteria was weak but potentially
398 negative, with neither increases in $p\text{CO}_2$ levels nor the presence of copepods having a strong impact
399 on the relationship.

400 A similar pattern was observed with regards the relationship between the relative change in TEP
401 concentration and nannoplankton abundance (Fig. 6). As with the bacteria, some of the highest rates
402 of change in nannoplankton were observed in the E04 274 experiment, where numbers increased 2
403 fold when incubated in the control incubations and 1.5 fold in all incubations containing copepods.
404 Correspondingly, there was a drop in TEP concentrations by almost 50% over 24 h. By contrast, in
405 E03 274, nannoplankton abundance almost halved in the control incubations where there was a
406 concomitant doubling in TEP concentration. These two extremities were the main drivers of a
407 negative relationship between TEP concentration and nannoplankton abundance that was
408 particularly evident in the control incubations. Nevertheless, the trend did not prove to be significant
409 ($F = 6.346$, $P = 0.065$, 5 df). This trend was weaker in incubations containing copepods either in
410 ambient conditions or at raised $p\text{CO}_2$ levels.

411 *Phytoplankton community response* In the majority of control incubations (i.e. ambient conditions,
412 no copepods), the abundance of phytoplankton cells increased over 24 h, many by between 1 and 5
413 fold (*Bottle effects*, Table 5). These increases were observed across all four of the major
414 phytoplankton taxa counted (diatoms, dinoflagellates, ciliates and flagellates). The exception was
415 experiment E04 274, where cell abundance of diatoms, dinoflagellates and ciliates in control
416 incubations decreased.

417 The impact of copepods on phytoplankton cell abundance was mixed, with cell numbers increasing
418 in some instances and decreasing in others (*Copepod effects*, Table 5). No major phytoplankton
419 taxon either universally increased or decreased across all experiments when incubated in ambient
420 conditions with copepods, rather, responses varied according to taxon in each incubation. For
421 example, in experiment E01 271, all taxa continued to increase in cell numbers when copepods were
422 present while, in E05 271, all but the flagellates decreased.

423 Similarly mixed responses in phytoplankton cell abundance were observed in the $p\text{CO}_2$ treatments
424 (*Treatment effects*, Table 5, Fig. 7). Compared to the responses observed in ambient conditions,

425 some taxa responded negatively and other positively to increased $p\text{CO}_2$ within the same experiment
426 (e.g. E03 271, E03 274). However, in two experiments (E01 271 and E04 274), all 4 major
427 phytoplankton taxa responded negatively to both the 750 μatm and 1000 μatm treatments when
428 copepods were present. Of particular interest however was the response of dinoflagellates, in which
429 there was a negative response within the incubations containing copepods at both 750 μatm and
430 1000 μatm $p\text{CO}_2$ across all experiments while, in the corresponding incubations without copepods,
431 the response was almost wholly positive. The difference in the dinoflagellate response between
432 incubations containing copepods to those without copepods was significant in both the 750 μatm
433 and 1000 μatm treatments (paired t-test: 750 μatm $t = -3.38$, $P = 0.02$, 5 df; 1000 μatm $t = -3.38$, $P =$
434 0.02). Such a consistent response to the presence or absence of copepods at raised $p\text{CO}_2$ levels was
435 not observed in the other major phytoplankton taxa.

436 *Copepod response* Compared to the variability observed in animals that were frozen immediately
437 after capture (T0 population), there was no significant increase in the carbon content of copepods
438 incubated for 24 h or 96 h in any of the treatments (Table 4). Over the course of the experiments
439 therefore, the incubated copepods did not appear to increase or decrease in body mass to a
440 detectable level.

441 C:N mass ratios of incubated copepods also did not appear to differ significantly from the T0
442 populations in the majority of instances (Fig. 8), indicating that copepods neither added to nor used
443 up body reserves during the course of the experiments. However, significant differences were found
444 in experiment E05 271, where the C:N mass ratio increased relative to the T0 population in all but
445 the 24 h 1000 μatm incubation (Kruskall-Wallis 1-way ANOVA, $H = 55.45$, $P < 0.001$, 6 df).

446 Furthermore, there was a significant increase in the C:N mass ratio of E03 271 copepods incubated
447 for 96 h in ambient conditions (Kruskall-Wallis 1-way ANOVA, $H = 18.07$, $P = 0.006$, 6 df).

448 Nevertheless, on the whole, neither the length of the incubation nor the addition of $p\text{CO}_2$ had an
449 acute influence on copepod body mass or body condition.

4. Discussion

The present study carried out short-term (up to 4 d) incubation experiments of natural open-ocean communities with and without the presence of grazing copepods in microcosms in which $p\text{CO}_2$ levels had been manipulated. The major advantage of the approach was in the repetition of the same experimental technique on a diverse range of polar and sub-polar natural communities. The addition of a mesozooplankton grazer to these microcosms added to the complexity of the experimental system and made the interpretation of results more challenging. Nevertheless, we found that this approach revealed responses to acute OA change. In particular, the impact of $p\text{CO}_2$ treatments on dinoflagellate cell numbers was consistently and significantly affected by the presence or absence of copepods in the microcosms across each of the six diverse environments in which the experiments were performed. By comparison, the other phytoplankton taxa did not show any consistent response between treatments or experiments.

This study also set out to determine whether the widely reported observation of increased TEP as a result of CO_2 perturbation was prominent within a multi-trophic level experimental setup, and whether this had any impact on the microbial community. We found that TEP increased naturally at varying rates within control microcosms (i.e. ambient conditions without copepods). However, elevated $p\text{CO}_2$ treatments resulted in significant increases in TEP concentrations in some instances, but no change or significant decreases in others. Furthermore, there was no significant relationship between any observed change in TEP concentrations and the abundance of either bacteria or nanoplankton. Indeed, although weak, the general trend was that both sets of microorganisms were less abundant in those experiments where TEP was increasing most rapidly, which is contrary to the expectation that these organisms thrive on increased TEP production (Bellerby et al. 2008).

4.1 Sources of error

One of the main methodological errors was in the under achievement of $p\text{CO}_2$ targets in certain experiments. Although the manipulations were successful in elevating DIC levels while maintaining a constant TA, the measured $p\text{CO}_2$ were sometimes several hundred μatm below their target levels. Discrepancies between measured $p\text{CO}_2$ values and those calculated from DIC and TA have been previously identified, particularly at high $p\text{CO}_2$ levels (Hoppe et al. 2010, Hoppe et al. 2012). Amongst the sources of error are uncertainties in calculation of the carbonate system, miscalculations of the perturbation amounts, and handling errors when adding the chemicals to the incubation bottles. There is also the potential for measurement error, which we estimated to be up to 60 μatm $p\text{CO}_2$ when also including errors propagated in the calculation of the carbonate system. $p\text{CO}_2$ levels within the bottles were also subject to drift during the period of incubation, by an average of 36 μatm after 24 h and 60 μatm after 96 h. For this reason, the following discussion does not emphasise the precise differences in the response to the two different $p\text{CO}_2$ treatments but focuses more on the consistency of responses between all incubations where $p\text{CO}_2$ was manipulated.

4.2 Dinoflagellate response to acute $p\text{CO}_2$ elevation

Our study found a significant difference in the response of dinoflagellates to acute $p\text{CO}_2$ elevation, depending on the presence or absence of grazing copepods. Across all six experiments, there was a decline in dinoflagellate cell numbers in raised $p\text{CO}_2$ levels when copepods were present, whereas dinoflagellate numbers increased when copepods were absent. No significant change was observed in any of the other major phytoplankton taxa. One explanation for this pattern is the direct impact of copepods on dinoflagellates in that the feeding behaviour and preferences of the copepods alter under enhanced $p\text{CO}_2$ conditions, resulting in them selecting dinoflagellates to a greater extent than under ambient conditions. Alternatively, the presence of copepods may have had an indirect negative effect on dinoflagellate abundance when $p\text{CO}_2$ levels were raised.

Support for the direct impact of copepods on dinoflagellates assumes that dinoflagellates become an increasingly suitable food item under elevated $p\text{CO}_2$. Kleppel et al. (1991) was one of the first to

498 argue that dinoflagellates are more nutritious than diatoms to zooplankton grazers. Dinoflagellates
499 contain 1.1-3.5 times more carbohydrate and 1.8-6 times more lipid than diatoms of equivalent
500 volume (Hitchcock 1982). Laboratory experiments have also shown that diets containing certain
501 dinoflagellates enhance copepod egg production rates (Stoecker and Egloff 1987) whereas diatoms
502 could suppress these rates (Miralto et al. 1999, Ianora et al. 2004, although see Irigoien et al. 2002).

503 Nevertheless, to explain the pattern in the present study, dinoflagellates must not only be the prey
504 item of choice, but become increasingly preferred when $p\text{CO}_2$ is raised. Under high $p\text{CO}_2$ conditions,
505 increasing carbon availability has the potential to change the stoichiometry of nutrients to primary
506 producers. Carbon to nutrient ratios, which are already comparatively high in diatoms, will increase
507 under such scenarios, making them of inferior quality to herbivorous consumers (Sterner and Elser
508 2002, Boersma et al. 2008, Malzahn et al. 2007, Schoo et al. 2013, Nobili et al. 2013). The lower
509 enzymatic specificity for CO_2 in dinoflagellates makes them less responsive to CO_2 enrichment and
510 their carbon to nutrient ratios are less affected than diatoms (Tortell 2000), and they retain their
511 quality as food items. Consumers, such as calanoid copepods, are more constrained with respect to
512 their body nutrient composition than primary producers and thus have to cope with excess amounts
513 of carbon relative to essential nutrients since they aim to maintain homeostasis (Sterner and Elser
514 2002). Selection of the most stoichiometrically suitable food items is likely to play a major role in
515 maintaining this homeostasis. Nevertheless, this selectivity may alter during different life-stages,
516 and carbon-rich foods may be preferable during phases when the copepods are actively storing
517 reserves as fatty acids, particularly long chain polyunsaturated fatty acids. Such fatty acids may also
518 be altered to varying extents in different phytoplankton taxa under enhanced $p\text{CO}_2$ (Wynn-Edwards
519 et al. 2014).

520 Furthermore, a direct impact of copepods on dinoflagellates means that these grazers must have the
521 capacity to select between different prey items (Kleppel 1993). There has been a long history of
522 study and debate on the capacity of copepods to feed selectively, dating back to Esterly (1916).

523 Copepods are generally thought of as filtration feeders, meaning that they principally capture
524 particles no smaller than the inter-setal distance on their feeding appendages. However, Koehl and
525 Strickler (1981) and Paffenhöfer et al. (1982) demonstrated that, in the low Reynolds number
526 environments in which they exist, copepods are suspension feeders where the second maxillae push
527 parcels of water containing particles towards the mouth rather than filtering particles from the
528 water. There is also abundant evidence that copepods can select based on food quality. For instance,
529 Mullin (1963) demonstrated that *Calanus helgolandicus* ingests more exponentially growing than
530 senescent diatoms per unit time. Cowles et al. (1988) showed that *Acartia tonsa* distinguishes
531 nutritional quality in mixtures of nutritionally rich and poor phytoplankton species. The same finding
532 was made by Kiørboe (1989) who further found a relationship between the quality of ingested food
533 and egg production rates. In summary, direct impacts of copepods on dinoflagellates at acutely
534 enhanced $p\text{CO}_2$ levels is supported by other findings that enhanced $p\text{CO}_2$ is likely to decrease the
535 nutritional value of dinoflagellates less than diatoms, and that copepods are capable of selecting
536 phytoplankton cells based on their quality as food items.

537 It is more difficult to find supporting evidence for the possibility that copepods have an indirect
538 negative impact on dinoflagellate abundance under enhanced $p\text{CO}_2$ conditions. One means in which
539 this could occur is that the copepods may alter the chemical environment, through respiration and
540 excretion, making it less suited to dinoflagellate growth when $p\text{CO}_2$ is raised. Flynn et al. (2015)
541 found that altering nutrient conditions changes the competitive balance between different
542 phytoplankton species exposed to OA. However, there was little evidence that nutrient levels were
543 significantly altered after 24 h and CO_2 becomes more, rather than less, available as a result of the
544 experimental treatments and should be non-limiting to cell division. Another possibility is that the
545 copepods grazing on other prey items may cause a trophic cascade, releasing predatory pressure on
546 other dinoflagellates grazers. However, dinoflagellates are relatively large cells and are unlikely to be
547 major prey items of any other heterotrophic organisms within the incubations other than copepods.

548 Therefore, the direct impact of copepods on dinoflagellates through selective grazing is the most
549 likely explanation for the observed pattern.

550 **4.3 Copepod body condition**

551 We found there to be no detectable change in copepod body condition in CV and females stages of
552 *Calanus finmarchicus/glacialis*, *C. hyperboreus*, and *Calanoides acutus*, assessed in terms of the C:N
553 mass ratio, as a result of raised $p\text{CO}_2$ treatments either after 24 h or 96 h of incubation. Neither was
554 there a concomitant rise or fall in the mass of carbon and nitrogen, which would indicate a change in
555 body mass over the incubation period. The exception was experiment E05 271, where the C:N mass
556 ratio significantly increased. However, the same level of increase was observed in both ambient and
557 raised $p\text{CO}_2$ conditions, indicating that this pattern was more likely to be a bottle effect than a
558 treatment effect.

559 The fact that there was little response to $p\text{CO}_2$ treatments in any of the copepod species is
560 consistent with the majority of other studies on the sensitivity of copepods to raised CO_2 . In terms of
561 direct physiological responses, most studies have found no direct effects on copepods at CO_2 levels
562 within the range expected by the end of this century (Kurihara et al. 2004; Watanabe et al. 2006,
563 Kurihara and Ishimatsu 2008, Zhang et al. 2011, Vehmaa et al. 2012, Weydmann et al., 2012,
564 McConville et al. 2013, Niehoff et al. 2013). It is to be noted that the majority of these studies
565 focussed on adult females whereas only a few have concentrated on other life-history stages which
566 are likely to show greater sensitivity to elevated $p\text{CO}_2$ (Kurihara et al. 2004, Fitzer et al. 2012, Lewis
567 et al. 2013, Cripps et al. 2014).

568 There has been less attention on how copepod body condition responds to changes in the quality of
569 their food as a result of increased levels of $p\text{CO}_2$. For instance, Urabe et al. (2003) found lower
570 growth rates of the freshwater species *Daphnia pulicaria* at high $p\text{CO}_2$ due to altered algal C:P ratios.
571 Schoo et al. (2013) found that the copepod *Acartia tonsa* showed decreased development rate when

fed the algae *Rhodomonas salina* grown under increased $p\text{CO}_2$ levels. In a culture containing the diatom *Thalassiosira pseudonana* and the copepod *Acartia tonsa*, Rossoll et al. (2012) found that exposure to $p\text{CO}_2$ levels of 750 μatm led to a decrease in polyunsaturated fatty acids in the diatoms and a corresponding decrease in copepod egg production (Rossoll et al. 2012). Therefore, even though it is likely that copepods have the ability to select food items based on their quality, their stoichiometric homeostasis and growth potential will be affected when there is a lack of good quality food items available. The diversity of food items in the incubation chambers in the present study may have allowed the copepods in the present study to prevent decreases in body condition, at least for the short term duration of the incubations.

4.4 Net change in TEP

TEP increased in all of the control incubations (i.e. ambient conditions, without copepods) to varying degrees, with some of the highest rates of increase occurring in the most nutrient stressed sites (North Sea, Barents Sea). These increases took some days to be detectable, with no significant increases being found after 24 h. When compared to the changes documented in the control incubation, 4 out of the 6 experiments showed significant increases in the 1000 μatm $p\text{CO}_2$ treatment, with the remaining 2 experiments either showing a neutral or negative change. Only 2 of the experiments showed a significant increase in the 750 μatm $p\text{CO}_2$ treatment, the remaining 4 not showing any significant change. The impact of copepods on TEP concentrations was mixed, leading to increased concentrations in some experiments but neutral or negative changes in others.

Initial studies on the influence of raised $p\text{CO}_2$ on TEP formation found a relatively consistent pattern of greater TEP formation when $p\text{CO}_2$ levels were raised acutely (Bellerby et al. 2008, Weinbauer et al. 2011). The prevailing hypothesis is that, under high $p\text{CO}_2$ conditions, more of the organic carbon fixed by photosynthesis is channelled into the dissolved fraction and released from the cells. This leads to a greater standing stock of TEPs as the released matter coagulates into particulates. Higher

596 concentration of TEP in surface waters is therefore believed to be one of the most predictable
597 consequences of OA (Weinbauer et al. 2011).

598 Nevertheless, there has recently been some doubt regarding the universality of the response of TEP
599 to increased $p\text{CO}_2$ levels. In rolling tank experiments, where TEP was either in equilibrium or non-
600 equilibrium with its precursors, Passow (2012) found that OA as expected in the future oceans had no
601 impact on the process of TEP formation from its precursors. In field observations, MacGilchrist et al.
602 (2014) found a significant but highly variable effect of acute $p\text{CO}_2$ increase on TEP production, with
603 increased TEP production being closely linked to phytoplankton growth in some instances and as a
604 result of greater amounts of TEP being produced per cell in others. The MacGilchrist et al. (2014)
605 study used similar incubation techniques to the present study but was carried out in a variety of
606 environmental settings in European shelf seas. Their findings match those of the present study in
607 that the TEP response to raised $p\text{CO}_2$ levels is more likely to be dependent on the specific
608 environmental setting and cannot be assumed to be universal.

609 As well as being conducted in high latitude environments, the present study differed from that of
610 MacGilchrist et al. (2014) in introducing a further trophic level through the addition of grazing
611 copepods. Zooplankton have been found to feed effectively on clusters of TEP (Passow and
612 Alldredge 1999, Ling and Alldredge 2003). Nevertheless, there was no consistent impact of copepods
613 on TEP in the present study, suggesting that it is unlikely to be a preferred food item. This may be
614 particularly the case given that TEP is carbon rich (Passow 2012) and of low quality relative to
615 nutrient rich phytoplankton taxa such as dinoflagellates (Kleppel 1993).

616 **4.5 Response of bacteria and nanoplankton**

617 We found that there was no significant change in the abundance of either bacteria or
618 nanoplankton in response to raised $p\text{CO}_2$ levels. This is consistent with the results of mesocosm
619 studies where no or only a small effect of elevated $p\text{CO}_2$ levels was found on bacterial abundance

620 (Rochelle-Newall et al. 2004, Allgaier et al. 2008, Paulino et al 2008). Nevertheless, according to
621 Weinbauer et al. (2011), no study has shown a clear negative effect of elevated $p\text{CO}_2$ on prokaryotic
622 production at pH levels relevant in the context of OA. Thus, our study supports the wider body of
623 data so far collected that suggests a neutral or positive effect of OA on the growth rate of
624 microorganism populations.

625 The relationship between the relative change in TEP concentrations and bacterial abundance in the
626 present study was not significant, although there was a trend of bacterial numbers decreasing as TEP
627 increased. The relationship between TEP concentration and nanoplankton abundance showed a
628 similar trend. Corzo et al. (2005), Passow et al. (2001), Hung et al. (2003) and Santschi et al. (2003)
629 have reported positive relationships between TEP and bacteria, whereas Passow and Alldredge
630 (1994) and Bhaskar and Bhosle (2006) found negative or no correlation at all. In the Southern Ocean,
631 Ortega-Retuerta et al. (2009) found a positive relationship between TEP and bacterial abundance
632 within the upper mixed layer, although the slope was relatively shallow. There was a much stronger
633 relationship below the mixed layer. It was proposed that the relationship was mediated by
634 phytoplankton and may not be a direct relationship between the two components. The present
635 study only considered the upper mixed layer community which may have constrained our ability to
636 identify any such relationship between TEP and microorganisms. Alternatively, it is possible that the
637 increase in TEP we consistently observed over the course of the incubation experiments was the
638 result of bottle effects to which the bacterial- and nanoplankton communities could not respond. In
639 summary, we found no evidence to support the view that the microbial community is stimulated by
640 the greater levels of TEP production that may result from acute increases in $p\text{CO}_2$.

5. Concluding remarks

This study completed six controlled microcosm experiments on natural communities extracted from a diverse set of high latitude open-ocean sites and exposed to acute increases in $p\text{CO}_2$. The spatial diversity of the experiments allowed the determination of whether different communities showed similar or differing responses to a common acute perturbation in $p\text{CO}_2$. Although the short-term and acute nature of the perturbation was not a true simulation of the changes likely to take place over the coming decades in the natural environment, it did provide an insight into whether these differing communities are equally placed in adapting to the predicted environmental changes. Across a range of high latitude environments, we identified a similar trophic response in copepods to their phytoplankton prey, which was induced by elevated $p\text{CO}_2$. We also demonstrated that the response of TEP to acute $p\text{CO}_2$ perturbation depends on the precise set of environmental conditions and cannot be assumed to be highly predictable. Trophic impacts on the body condition of the grazing copepod community were not resolved and are better suited to longer-term experimental approaches.

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Tables

Bioassay	Date	Location	Latitude (°)	Longitude (°)	Water depth (m)	Mixed-layer depth (m)	Incubated taxa
E01 271	3/6/12	North Sea	56.26664	2.63319	74	13.600 (2.408)	<i>Calanus finmarchicus</i> CV
E03 271	13/6/12	Arctic	76.17524	2.54953	3758	29.667 (7.506)	<i>Calanus hyperboreus</i> CV
E04 271	18/6/12	Arctic	78.28063	4.28433	2257	11.000 (1.414)	<i>Calanus hyperboreus</i> CV
E05 271	24/6/12	Arctic	72.89080	26.00273	362	44.000 (4.301)	<i>Calanus finmarchicus/glacialis</i> CV
E03 274	25/1/13	Southern Ocean	-52.68930	-36.62303	2445	62.600 (10.569)	<i>Calanoides acutus</i> CV
E04 274	1/2/13	Southern Ocean	-58.08701	-25.92901	2899	35.828 (5.824)	<i>Calanoides acutus</i> CV and female

Table 1: Locations and attributes of the six experimental stations, including the species and stage of copepod added to the experimental incubation bottles

Bioassay	Mixed layer T (°C)	Mixed layer salinity	NO ₃ +NO ₂ μmol L ⁻¹	Si μM L ⁻¹	PO ₄ μmol L ⁻¹	[NH ₄ ⁺] nmol L ⁻¹	N:P (atomic ratio)	TA (μmol kg ⁻¹)	DIC (μmol kg ⁻¹)	pH	pCO ₂ (μatm)	TEP (μg L ⁻¹ xanthan equiv.)	Total bacteria (cell ml ⁻¹)	Total nanoplankt. (cells mL ⁻¹)
E01 271	10.700 (0.071)	35.100 (0.000)	0.043 (0.006)	1.351 (0.006)	0.077 (0.012)	2.974 (1.030)	0.604	2325.300 (0.436)	2082.267 (0.503)	8.153 (0.000)	300.452 (0.365)	176.570 (16.717)	806803.712 (41963.039)	5444.164 (341.646)
E03 271	1.880 (0.795)	34.920 (0.045)	9.253 (0.051)	5.821 (0.025)	0.650 (0.000)	111.437 (134.775)	14.407	2305.267 (6.529)	2130.000 (3.205)	8.159 (0.014)	289.287 (9.705)	99.037 (10.046)	1173950.914 (14221.856)	41275.690 (2242.764)
E04 271	-1.575 (0.050)	32.625 (0.050)	4.200 (0.156)	12.214 (0.023)	0.790 (0.000)	145.211	5.500	2234.567 (2.901)	2106.400 (5.671)	8.129 (0.009)	304.682 (7.219)	90.973 (6.804)	460466.414 (85179.903)	3839.254 (629.680)
E05 271	6.450 (0.058)	35.000 (0.000)	5.380 (0.544)	3.867 (0.151)	0.437 (0.025)	162.527 (162.836)	12.693	2314.050 (4.031)	2108.700 (2.022)	8.146 (0.003)	304.295 (2.154)	111.855 (11.460)	1135333.889 (152872.784)	12071.861 (1273.412)
E03 274	2.224 (0.021)	33.932 (0.002)	24.080 (0.252)	19.393 (0.095)	1.597 (0.006)	812.623 (70.177)	15.590	2287.800	2152.342 (6.112)	8.050	384	3.293 (1.678)	653401.452 (26549.817)	4358.355 (623.205)
E04 274	0.400 (0.037)	33.706 (0.005)	18.537 (0.605)	71.310 (0.473)	1.190 (0.046)	519.415 (308.750)	16.014	2293.000	2129.500 (4.355)	8.167	283	65.473 (2.887)	804153.919 (79884.942)	1476.402 (404.310)

Table 2: Starting conditions for experimental incubations. Values represent averages, while (SD) is included where more than 3 measurements were made.

Experiment/incubation		C (µg)	H (µg)	N (µg)	C:N (mass ratio)
E03 271					
T0 population		2228.53 (1033.07)	343.91 (154.36)	290.24 (97.60)	7.38 (1.44)
Ambient	24 h	2370.11 (718.46)	378.04 (104.09)	284.68 (50.08)	8.19 (1.51)
	96h	2944.47 (603.86)	464.80 (121.29)	330.63 (54.75)	8.86 (0.56)
750 µatm	24 h	2460.75 (775.92)	358.65 (123.53)	296.81 (60.40)	8.15 (1.06)
	96 h	2563.23 (595.72)	425.49 (99.41)	287.04 (51.66)	8.86 (0.59)
1000 µatm	24 h	1814.57 (623.17)	268.11 (62.74)	259.23 (88.34)	7.07 (1.40)
	96 h	2513.16 (738.55)	393.35 (120.84)	288.09 (63.67)	8.60 (0.85)
E04 271					
T0 population		1000.20 (520.57)	161.43 (83.04)	167.72 (49.15)	5.70 (1.47)
Ambient	24 h	1211.67 (346.37)	197.27 (55.64)	176.69 (30.51)	6.75 (1.04)
	96h	1454.91 (968.46)	190.75 (86.75)	195.59 (94.16)	7.06 (2.09)
750 µatm	24 h	908.51 (343.28)	149.38 (56.26)	161.69 (39.02)	5.55 (1.25)
	96 h	1330.19 (389.65)	216.62 (63.42)	191.71 (40.46)	6.85 (1.08)
1000 µatm	24 h	872.04 (237.25)	140.82 (38.32)	150.84 (32.41)	5.75 (0.90)
	96 h	1066.25 (882.90)	177.15 (144.76)	184.06 (73.86)	5.17 (2.05)
E05 271					
T0 population		74.06 (18.73)	10.83 (3.01)	17.92 (3.70)	4.15 (0.68)
Ambient	24 h	65.28 (22.50)	7.04 (3.14)	9.63 (5.01)	7.59 (2.07)
	96h	66.01 (26.04)	7.25 (3.74)	11.76 (5.33)	6.37 (2.77)
750 µatm	24 h	71.28 (35.02)	8.25 (4.41)	11.15 (4.74)	6.97 (3.13)
	96 h	68.60 (36.65)	7.40 (4.92)	12.31 (5.93)	5.57 (1.68)
1000 µatm	24 h	69.61 (35.78)	7.75 (4.68)	13.74 (5.14)	4.97 (1.21)
	96 h	66.98 (25.27)	7.30 (3.52)	11.92 (3.50)	5.66 (1.43)
E03 274					
T0 population		54.80 (12.20)	9.61 (2.21)	17.85 (3.26)	3.05 (0.21)
Ambient	24 h	66.75 (23.67)	9.76 (3.38)	18.32 (4.56)	3.57 (0.69)
	96h	50.75 (29.74)	6.90 (4.30)	14.56 (5.74)	3.32 (0.59)
750 µatm	24 h	45.33 (17.59)	6.63 (2.53)	14.59 (3.49)	3.03 (0.58)
	96 h	37.66 (14.32)	5.35 (2.14)	12.43 (4.48)	3.29 (1.58)
1000 µatm	24 h	52.16 (21.87)	7.33 (3.28)	15.55 (4.96)	3.30 (0.50)
	96 h	44.49 (21.68)	6.23 (3.04)	13.79 (4.76)	3.16 (0.69)
E04 274					
T0 population		256.57 (128.63)	46.51 (21.76)	45.02 (14.77)	5.45 (1.06)
Ambient	24 h	286.53 (188.66)	45.50 (30.36)	47.34 (20.72)	5.63 (1.63)
	96h	222.33 (158.99)	35.08 (28.00)	38.39 (18.41)	5.38 (1.61)
750 µatm	24 h	250.58 (152.42)	43.55 (29.73)	41.98 (18.13)	5.61 (1.59)
	96 h	218.71 (160.44)	34.64 (30.97)	35.56 (21.39)	6.09 (1.78)
1000 µatm	24 h	260.74 (153.65)	43.36 (30.60)	44.10 (16.74)	5.78 (1.81)
	96 h	218.09 (192.69)	37.14 (40.93)	38.43 (18.88)	4.98 (1.98)

Table 3: Average (SD) elemental composition of copepods either at time zero (T0) or after 24 h or 96 h of incubation at ambient or elevated $p\text{CO}_2$ conditions. All copepods were stage CV with the exception of E04 274 where a combination of CV and females were used.

Bioassay	T0 TEP (μg xanthan equiv. L^{-1}) [SD]	Bottle effects $\Delta\text{TEP h}^{-1}$	Treatment effects				Copepod effects	
			24 h (750)	24 h (1000)	96 h (750)	96 h (1000)	24 h	96 h
JR271 (E01)	176.6 [16.7]	0.6677	$\leftrightarrow$	$\leftrightarrow$	$\uparrow$	$\uparrow$	$\leftrightarrow$	$\uparrow$
JR271 (E03)	99.0 [10.0]	1.0553	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\uparrow$
JR271 (E04)	91.0 [6.98]	5.8465	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\uparrow$	$\leftrightarrow$	$\downarrow$
JR271 (E05)	111.9 [11.5]	1.6261	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\uparrow$	$\leftrightarrow$	$\uparrow$
JR274 (E03)	3.3 [1.7]	0.1004	$\leftrightarrow$	$\leftrightarrow$	$\leftrightarrow$	$\downarrow$	$\leftrightarrow$	$\uparrow$
JR274 (E04)	65.5 [2.9]	0.4496	$\leftrightarrow$	$\uparrow$	$\uparrow$	$\uparrow$	$\leftrightarrow$	$\leftrightarrow$

Table 4: Average concentrations [SD] of transparent exopolymeric particles (TEP) at the start of the incubation experiments (T0). *Bottle effects* were calculated as $(\text{TEP}_{96\text{h, ambient, non-copepod}} - \text{TEP}_{\text{T0}})/96$ and represent the rate of increase of TEP over the maximum duration of the experiments (96 h) in the control incubations (ambient, non-copepod). *Treatment effects* were determined through performing a 1-way ANOVA comparing concentrations of TEP in incubations with elevated levels of $p\text{CO}_2$ with those of ambient conditions. All of incubations in this test contained copepods. *Copepod effects* were determined through performing a t-test comparing concentrations of TEP in incubations containing copepods with those without copepods. $\downarrow$ denotes significantly lower concentrations of TEP ($P < 0.05$), $\uparrow$ significantly higher TEP ($P < 0.05$) and $\leftrightarrow$ no significant difference in TEP.

Bioassay	T0 Cells mL ⁻¹	Bottle effects	Copepod effects	Treatment effects			
Phytoplankton group (<i>Phy_x</i>)				With copepods		Without copepods	
				750	1000	750	1000
E01 271							
Diatoms	282.65	+	+	-	-	+	+
Dinoflagellates	7.37	+	+	-	-	+	+
Ciliates	0.35	+	+	-	-	+	+
Flagellates	0.27	+++	+	-	-	+	+
E03 271							
Diatoms	5.19	-	-	++	++	+	+
Dinoflagellates	13.01	+	+	-	-	+	+
Ciliates	3.84	-	+	+	-	-	+
Flagellates	0.84	+++	+	+	+	-	-
E04 271							
Diatoms	249.96	++	-	-	+	-	-
Dinoflagellates	9.54	++	+	-	-	+	0
Ciliates	1.35	0	-	-	-	+	+
Flagellates	0.68	++	+	-	-	+	+
E05 271							
Diatoms	0.77	++	-	-	-	0	0
Dinoflagellates	27.4	+	-	-	-	-	-
Ciliates	0.08	+	--	++	+	+	-
Flagellates	0	0	+	-	-	-	+
E03 274							
Diatoms	14.03	++	-	+	+	-	-
Dinoflagellates	4.41	-	+	-	-	+++	+++
Ciliates	0.85	++	+	+	+	-	+
Flagellates	0.22	+	+	-	-	-	+
E04 274							
Diatoms	1084.44	-	+	-	0	+	-
Dinoflagellates	8.22	-	-	-	-	0	+
Ciliates	28.25	-	+	-	-	+	-
Flagellates	1.64	+	--	-	-	-	-

Table 5: Concentrations (cells mL⁻¹) of the four major phytoplankton groups (*Phy_x*) in each of the experimental incubations. *Bottle effects* were calculated as $(Phy_{x,T0} - Phy_{x,ambient, non-copepods})/Phy_{x,T0}$, *Copepods effects* as $(Phy_{x,ambient, copepods} - Phy_{x,treatment, copepods})/Phy_{x,ambient, copepods}$ and *Treatment effects* as $(Phy_{x,copepods} - Phy_{x,non-copepods})/Phy_{x,copepods}$. '0' represents fold changes of between -0.05 to +0.05; '+' or '-', up to a 1 fold increase; '++' or '--', a 1 to 5 fold increase; '+++ or '---', a more than 5 fold increase.

Figure Legends

Fig. 1: Proportion and total cell concentration (cells mL⁻¹) of the four major phytoplankton taxa at the start of each of the six incubation experiments (T0).

Fig. 2: Carbon to nitrogen mass ratio (C:N) of a subset of the selected grazing copepods at the start of the incubation experiments (T0). The species/stages of the selected copepod grazers for each experiment are listed in [Table 1](#). Specimens from Station E01 271 were not analysed as a result of a processing error.

Fig. 3: Concentration of total bacterial cells (cell mL⁻¹) at the start of each incubation experiment (T0) and in each of the three treatments (ambient, 750 μ atm $p\text{CO}_2$ and 1000 μ atm $p\text{CO}_2$). Open circle denotes average concentration within incubations with copepods, filled circles, without copepods. Bars represent 95% confidence intervals of incubations containing copepods. Instances where the filled circles fell outside these confidence intervals indicate significant differences between concentrations in copepod versus non-copepod incubations.

Fig. 4: Concentration of total nanoplankton cells (cell mL⁻¹) at the start of each incubation experiment (T0) and in each of the three treatments (ambient, 750 μ atm $p\text{CO}_2$ and 1000 μ atm $p\text{CO}_2$). Open circle denotes average concentration of incubations with copepods, filled circles, without copepods. Bars represent 95% confidence intervals of incubations containing copepods. Instances where the filled circles fell outside of these confidence intervals indicate significant differences between concentrations in copepod versus non-copepod incubations.

Fig. 5: Relative change in bacterial cell concentrations in relation to the relative change in the concentration of transparent exopolymeric particles (TEP) across all six incubation experiments. Dashed lines represent trends fitted through least-squared regression. None of the trends were statistically significant.

Fig. 6: Relative change in nanoplankton cell concentrations in relation to the relative change in the concentration of transparent exopolymeric particles (TEP) across all six incubation experiments. Dashed lines represent trends fitted through least-squared regression. None of the trends were statistically significant.

Fig. 7: Relative response level of the four major phytoplankton taxa to treatments relative to that observed in incubations in ambient conditions. Response levels were graded as between 0 and 3 for each incubation experiment, as detailed in [Table 1](#). * indicates a significant difference between incubations with copepods and those without copepods (paired t-test, $P < 0.05$).

Fig. 8: Carbon to nitrogen mass ratio (C:N) of the selected copepod grazers either at the start of the incubation experiments (T0) or at their completion. The species/stages of the selected copepod grazers for each experiment are listed in [Table 1](#). Specimens from Station E01 271 were not analysed as a result of a processing error. * indicates a significant difference to the starting population (1-way ANOVA, $P < 0.05$).

Appendix 1: Parameter measurements on completion of the experimental incubations in ambient conditions. Starting conditions are given in Table 2.

Bioassay	Duration	Copepods	N:P (atomic ratio)	TA ($\mu\text{mol kg}^{-1}$)	DIC ($\mu\text{mol kg}^{-1}$)	pH	$p\text{CO}_2$ (μatm)	Average (SD) TEP ($\mu\text{g L}^{-1}$ xanthan equiv.)	Average (SD) total bacteria (cells mL^{-1})	Average (SD) total nanoflag. (cells mL^{-1})
E01 271	24	Yes	7.0	2322.5	2080.0	8.1	305.2	149.5 (7.0)	922122.7 (126373.0)	6054.3 (1548.3)
	24	No	1.215	2316.8	2078.1	8.1	309.8	143.3 (9.8)	827276.1	5912.2
	96	Yes	2.1	2309.1	2099.4	8.1	364.3	135.2 (10.6)		
	96	No	1.843	2306.1	2121.6	8.0	422.7	244.4 (37.0)		
E03 271	24	Yes	18.2	2299.9	2146.9	8.1	348.4	142.7 (11.2)	924927.7 (26989.6)	43954.0 (1680.8)
	24	No	14.403	2297.6	2132.2	8.1	322.0	128.4 (7.4)	872248.4	40346.4
	96	Yes	28.6	2299.4	2154.6	8.1	368.0	223.9 (11.2)		
	96	No	17.778	2301.4	2125.3	8.1	302.0	200.3 (2.8)		
E04 271	24	Yes	5.9	2229.3	2120.9	8.0	386.7	116.6 (19.9)	423270.3 (5039.0)	4470.2 (114.5)
	24	No	3.923	2225.5	2102.3	8.1	344.1	142.0 (15.8)	515806.7	4656.7
	96	Yes	4.6	2228.9	2112.3	8.1	363.6	192.9 (26.1)		
	96	No	0.236	2228.4	2028.8	8.3	195.2	651.3 (7.4)		
E05 271	24	Yes	12.5	2309.1	2121.0	8.1	348.7	145.1 (8.1)	1380493.1 (69973.4)	12950.0 (2351.8)
	24	No	12.689	2311.5	2123.9	8.1	352.9	160.0 (9.8)	1430876.3	14696.8
	96	Yes	10.1	2313.5	2096.0	8.2	296.9	145.1 (3.7)		
	96	No	8.582	2307.7	2088.0	8.2	292.8	268.0 (35.4)		
E03 274	24	Yes	15.4	2291.1	2142.7	8.1	342.0	5.7 (5.0)	673354.5 (232254.2)	4476.0 (217.1)
	24	No	15.300	2288.1	2138.1	8.1	337.0	7.6 (1.2)	361558.2	1527.6
	96	Yes	16.0	2262.5	2130.9	8.1	374.0	26.6 (1.9)		
	96	No	15.808	2286.7	2138.4	8.1	339.0	12.9 (3.6)		
E04 274	24	Yes	15.7	2291.9	2113.1	8.2	259.0	51.0 (11.8)	1043846.7 (28753.2)	3727.4 (470.1)
	24	No	15.256	2293.7	2119.3	8.2	267.0	37.9 (21.4)	1155703.6	4354.8
	96	Yes	15.9	2296.5	2085.2	8.3	215.0	82.1 (12.2)		
	96	No	16.494	NA	2164.3	NA	NA	108.6 (25.6)		

Appendix 2: Parameter measurements on completion of the experimental incubations in the 750 μatm $p\text{CO}_2$ treatments. Starting conditions are given in Table 2.

Bioassay	Duration	Copepods	N:P (atomic ratio)	TA (μmol kg^{-1})	DIC (μmol kg^{-1})	pH	$p\text{CO}_2$ (μatm)	Average (SD) TEP ($\mu\text{g L}^{-1}$ gum xanthan equiv.)	Average (SD) total bacteria (cells mL^{-1})	Average (SD) total nannoplankt. (cells mL^{-1})
E01 271	24	Yes	2.9	2322.0	2199.3	7.9	625.3	155.1 (8.4)	851978.2 (253396.5)	4011.9 (3242.0)
	24	No	2.0	2331.5	2207.0	7.9	620.6		756796.9	5064.8
	96	Yes	1.9	2333.5	2246.8	7.8	823.3	153.2 (7.5)		
	96	No	0.5	2328.8	2231.6	7.8	759.6			
E03 271	24	Yes	15.9	2314.0	2258.4	7.8	722.9	147.0 (6.7)	946909.8 (41416.8)	45213.7 (5527.9)
	24	No	15.4	2305.6	2235.6	7.8	640.9		950633.5	43088.2
	96	Yes	26.7	2308.1	2252.7	7.8	737.2	259.9 (20.7)		
	96	No	17.6	2306.2	2223.3	7.9	578.8			
E04 271	24	Yes	7.0	2231.7	2206.3	7.7	781.8	128.4 (20.1)	425599.1 (10102.0)	4869.8 (949.0)
	24	No	4.1	2232.1	2189.3	7.8	667.9		474527.5	5157.9
	96	Yes	5.5	2236.9	2197.9	7.8	706.5	183.6 (15.6)		
	96	No	0.1	NA	NA	NA	NA			
E05 271	24	Yes	12.4	2319.1	2226.3	7.8	664.2	140.2 (12.4)	1364566.6 (29206.0)	12495.6 (1920.1)
	24	No	12.7	2309.3	2215.1	7.8	653.1		1380145.0	15539.9
	96	Yes	9.9	2316.4	2188.5	7.9	531.4	170.0 (12.1)		
	96	No	9.1	2319.1	2195.6	7.9	535.8			
E03 274	24	Yes	15.2	2289.6	2180.9	8.0	451.0	3.3 (0.7)	836115.7 (19018.9)	2876.5 (1717.3)
	24	No	15.1	2291.8	2178.6	8.0	437.0		830448.8	3355.0
	96	Yes	15.9	2292.4	2174.3	8.0	420.0	27.5 (2.7)		
	96	No	15.8	2289.2	2177.6	8.0	440.0			
E04 274	24	Yes	15.4	2293.4	2178.3	8.0	398.0	49.0 (5.7)	1042692.9 (39800.6)	3492.6 (333.9)
	24	No	15.7	2296.0	2175.8	8.1	385.0		1248353.9	3576.1
	96	Yes	16.0	2298.0	2160.9	8.1	342.0	155.6 (9.5)		
	96	No	15.4	2296.4	2168.9	8.1	365.0	15.4		

Appendix 3: Parameter measurements on completion of the experimental incubations in the 1000 μatm $p\text{CO}_2$ treatments. Starting conditions are given in Table 2.

Bioassay	Duration	Copepods	N:P (atomic ratio)	TA (μmol kg^{-1})	DIC (μmol kg^{-1})	pH	$p\text{CO}_2$ (μatm)	Average (SD) TEP ($\mu\text{g L}^{-1}$ xanthan equiv.)	Average (SD) total bacteria (cells mL^{-1})	Average (SD) total nannoplankt. (cells mL^{-1})
E01 271	24	Yes	2.0	2334.3	2224.6	7.8	688.8	173.1 (21.0)	866416.6 (180754.5)	6313.0 (545.4)
	24	No	1.1	2342.3	2224.9	7.9	659.0		534542.3	3207.0
	96	Yes	2.8	2334.2	2263.9	7.7	943.8	173.7 (6.0)		
	96	No	1.0	2333.7	2253.1	7.7	866.5			
E03 271	24	Yes	17.2	2312.2	2272.8	7.7	827.1	130.9 (4.3)	950025.8 (47509.0)	47774.8 (2671.6)
	24	No	15.5	2310.8	2269.0	7.8	805.3		921361.4	40506.9
	96	Yes	23.0	2311.4	2299.2	7.7	1030.4	208.4 (19.7)		
	96	No	17.2	2314.9	2269.6	7.8	793.0			
E04 271	24	Yes	7.2	2240.1	2231.5	7.7	910.3	138.3 (7.5)	449130.9 (49422.8)	4965.5 (470.7)
	24	No	4.1	2233.4	2212.6	7.7	810.1		454930.6	4513.3
	96	Yes	3.0	2236.1	2211.4	7.8	774.9	377.7 (33.1)		
	96	No	0.1	2240.1	2148.4	8.0	417.8			
E05 271	24	Yes	12.1	2320.5	2261.9	7.7	870.0	151.3 (13.5)	1363161.4 (21832.2)	12451.0 (1685.0)
	24	No	12.9	2314.3	2247.2	7.8	807.1		1243892.1	11405.5
	96	Yes	10.1	2318.3	2234.5	7.8	716.7	179.3 (18.1)		
	96	No	1.0	2325.3	2209.9	7.9	577.5			
E03 274	24	Yes	15.4	2295.2	2206.9	7.9	528.0	5.0 (1.1)	824785.6 (18588.5)	2981.2 (163.9)
	24	No	15.2	2296.9	2211.3	7.92	540		481463.0	2800.4
	96	Yes	16.0	2297.8	2210.6	8.0	493.0	18.9 (2.4)		
	96	No	16.1	2292.2	2201.4	7.93	516			
E04 274	24	Yes	15.6	2294.1	2208.6	7.9	538.0	70.5 (2.9)	1060930.4 (125832.4)	3279.4 (228.2)
	24	No	15.3	2298.4	2203	7.98	463		1043688.3	3384.3
	96	Yes	16.1	2297.2	2199.0	8.0	453.0	120.0 (0.8)		
	96	No	16.3	2303.3	2184.4	8.04	390			

Figure(s)

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8