



## Experimentally determined temperature thresholds for Arctic plankton community metabolism

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**Abstract.** Climate warming is especially severe in the Arctic, where the average temperature is increasing 0.4 °C per decade, two to three times higher than the global average rate. Furthermore, the Arctic has lost more than half of its summer ice extent since 1980 and predictions suggest that the Arctic will be ice free in the summer as early as 2050, which could increase the rate of warming. Predictions based on the metabolic theory of ecology assume that temperature increase will enhance metabolic rates and thus both the rate of primary production and respiration will increase. However, these predictions do not consider the specific metabolic balance of the communities. We tested, experimentally, the response of Arctic plankton communities to seawater temperature spanning from 1 °C to 10 °C. Two types of communities were tested, open-ocean Arctic communities from water collected in the Barents Sea and Atlantic influenced fjord communities from water collected in the Svalbard fjord system. Metabolic rates did indeed increase as suggested by metabolic theory, however these results suggest an experimental temperature threshold of 5 °C, beyond which the metabolism of plankton communities shifts from autotrophic to heterotrophic. This threshold is also validated by field measurements across a range of temperatures which suggested a temperature 5.4 °C beyond which Arctic plankton communities switch to heterotrophy. Barents Sea communities showed a much clearer threshold response to temperature manipulations than fjord communities.

### 1 Introduction

The European Arctic Ocean (consisting of the Barents Sea and the Fram Strait) is highly influenced by the North Atlantic Current which brings warm waters into the Arctic causing it to be a relatively ice free area and contributing significantly to summer ice melt (Loeng et al., 1997; Schauer et al., 2002). Moreover the European Arctic is characterized by the large outflow of less saline cold waters from the north, most notably from the East Greenland and East Spitsbergen Currents that have high solubility to CO<sub>2</sub>. These physical properties are responsible for the high CO<sub>2</sub> uptake in the mostly ice-free Barents Sea, which is estimated to be  $9 \times 10^{12}$  g C yr<sup>-1</sup> (Fransson et al., 2001), compared to the entire ice-covered Arctic interior ( $31 \times 10^{12}$  g C yr<sup>-1</sup>; Kaltin and Anderson, 2005). High biological production in this area also contributes to the role of the Arctic as a significant CO<sub>2</sub> sink (Loeng et al., 2005). The European Arctic corridor, is responsible for about 50 % of the primary production in the entire Arctic Ocean (Sakshaug, 2004; Ellingsen et al., 2007; Pabi et al., 2008) which has been estimated to have primary production rates between  $<30 \rightarrow 100$  g C m<sup>-2</sup> yr<sup>-1</sup> depending on the mixing properties and ice cover of the region (Wassmann et al., 2010). High primary production supports productive fisheries (Pauly and Christensen, 1995) and contributes to the high atmospheric CO<sub>2</sub> uptake in the North Atlantic (Takahashi et al., 2002).

Yet, the Arctic region is experiencing rapid climate change, warming three times faster than the global mean (ACIA, 2004; Trenberth et al., 2007). Such a steep rate of warming has resulted in severe reduction of ice cover, exceeding the range of natural variability over the past millennia and creating potentially dangerous positive feedbacks (Walsh, 2008; Duarte et al., 2012). Rapid warming is expected to continue in the future, with up to 6 °C warming throughout the 21st century (ACIA, 2004), and revised forecasts suggest that the Arctic will be ice free in the summer before 2050 (Holland et al., 2006; Boé et al., 2009; Wang and Overland, 2009; Wadhams, 2012). The ice cover over the Arctic Ocean reached a historical minimum in September 2007 with a reduction of 43 % relative to the ice cover in 1979 (Kerr, 2007). In 2012 ice cover again approached this historical minimum (National Snow and Ice Data Center, 2011 available at: <http://nsidc.org/>). Sea ice is not only changing in extent, but is also decreasing in thickness (Johannessen et al., 1999; Kwok and Rothrock, 2009; Wadhams, 2012) as well as increasing in the duration of the ice melt season (Belchansky et al., 2004). These factors are expected to affect the primary productivity in the region by changing light regimes or affecting the timing of the spring bloom (Wassmann et al., 2006, 2008, 2010; Ellingsen et al., 2008). In fact, previous studies have reported an increase in primary productivity for the Arctic as a whole for these reasons (Arigo et al., 2008; Pabi et al., 2008), however closer inspection actually reveals a decline in primary production in the Greenland and Barents Seas in 2007 due to increased ice-cover moving out of the Arctic's interior (Wassmann et al., 2010).

Besides light availability, temperature also plays a major role in regulating metabolic processes (Iriberry et al., 1985; White et al., 1991; Brown et al., 2004), as described by the metabolic theory of ecology (MTE; Brown et al., 2004), which predicts that primary production and respiration rates should increase at different rates with increasing temperature (Harris et al., 2006; Lopez-Urrutia et al., 2006). Noting that metabolic theory predicts that the activation energy for respiration should be twice as high as that for photosynthesis, Harris et al. (2006) predicted that a four degree increase in water temperatures should result in a 20 % increase in net primary production and a 43 % increase in heterotrophic metabolism, resulting in a 16 % decrease of the photosynthesis/respiration ratios (P/R). Moreover, there is evidence that respiration rates show very steep responses to increased temperature at the low ambient temperatures found in Arctic waters (Pomeroy and Wiebe, 2001; Vaquer-Suynner et al., 2010). Indeed, the mean activation energy for community respiration in the Greenland Sea, derived from 13 independent experiments, has been reported to be  $1.05 \pm 0.3$  eV (Vaquer-Suynner et al., 2010), well above the value of 0.65 eV predicted from theory (López-Urrutia et al., 2006). On the basis of these results, Vaquer-Suynner et al. (2010) postulated that warming may lead to Arctic communities shifting

from acting as an intense sink for atmospheric CO<sub>2</sub>, as they do at present, to becoming CO<sub>2</sub> sources to the atmosphere due to enhanced respiration rates, and suggest that this shift may occur within 6 °C of warming, with consequences for the global carbon budget and climate (Duarte et al., 2012).

Here we test the hypothesis (Vaquer-Suynner et al., 2010; Duarte et al., 2012) that Arctic plankton communities shift from acting as CO<sub>2</sub> sinks to acting as CO<sub>2</sub> sources at a temperature threshold within 6 °C of current temperatures. We do so through an experimental examination of the temperature-dependence of the response of Arctic community metabolism along the temperature range of 1 to 10 °C, encompassing the range of seawater temperature expected for the Arctic Ocean along the 21st Century (ACIA, 2004). To examine the possible role of temperature acclimation and adaptation of the communities, two separate experiments were conducted, one with a plankton community sampled in the Arctic water close to the marginal ice zone of the Barents Sea and another experiment with a community collected in warmer, Atlantic-influenced fjords.

## 2 Methods

### 2.1 Experimental overview

We designed the experiments to compare the responses of an open-ocean Arctic community and an Arctic community already acclimated to warm temperatures. We were conscious of the limitations of experimental manipulations to simulate *in situ* changes, such as the short temporal scales of experiments that do not allow for genetic changes and community restructuring to occur as well as the risk of creating a “shock” treatment resulting in unexpected responses. In the Barents Sea community, we allowed the communities to adjust to the experimentally imposed temperature regime, by incubating the microcosms containing the communities for 10 to 15 days, imposing warming rates (°C day<sup>-1</sup>) comparable to those observed in nature, thereby allowing the responses to be expressed. Using a time series of sea surface temperature (SST) from NOAA's Climate Prediction Center ([http://nomad2.ncep.noaa.gov/ncep\\_data/](http://nomad2.ncep.noaa.gov/ncep_data/)), we extracted weekly average SST values for the last 2 decades during the months of June and July for each sampling station using a 1° square grid cell. Over two decades, the range of temperature experienced by the Barents Sea community in June and July was from -1.03–5.68 °C while the average range of temperature experienced in any one year during June and July is 0.98–4.25 °C, thus suggesting that these communities already experience temperature variability and thereby the temperature treatments used encompassed a June-July variation range plus 5 °C. Hence, the responses evaluated here have two components: (1) a physiological component, reflecting the effect of temperature on metabolic processes; and

(2) a community component, reflecting the effect of temperature on community composition and biomass.

Seawater samples were collected in 60 L polypropylene carboys previously treated with HCl for at least 48 h and thoroughly rinsed with the seawater from the sampling site. The experimental evaluation of temperature effects on the community metabolism of an open-sea planktonic community was performed with a plankton community found in water collected on 27 June 2009 at 26 m depth in the Barents Sea (77° N, 28° E), southeast of the Svalbard archipelago, using the CTD (conductivity, temperature, depth) rosette sampling system available on R/V *Jan Mayen* (water temperature  $-1.19^{\circ}\text{C}$ , salinity 33.92). A second experiment was conducted using fjord water sampled from a boat using a pump at 2 m depth in Isfjorden (78° N, 14° E), the second largest fjord in Svalbard. In contrast to the first experiment with the Barents Sea plankton community, the community sampled at Isfjorden was expected to represent an Atlantic-influenced community growing at warmer temperatures, thereby we aimed to assess the responses of both Arctic communities and the Atlantic community expected to invade an Arctic polar ocean free of ice. Indeed, water temperature at Isfjorden ( $6.2^{\circ}\text{C}$ ) on the sampling date (8 July 2009) was much higher than that of the Barents Sea community, whereas the salinity was comparable (32.73).

## 2.2 Experimental design and set-up

The experiments were conducted in temperature regulated cold rooms (set at  $4\text{--}5^{\circ}\text{C}$ ) at the University Center in Svalbard (UNIS), Longyearbyen. All plastic and glassware used for the incubations was previously cleaned with dilute HCl and thoroughly rinsed with seawater. Seven experimental temperatures, ranging from  $1.5^{\circ}\text{C}$  to  $10.5^{\circ}\text{C}$ , in  $1.5^{\circ}\text{C}$  increments, were tested, thereby encompassing the full range of temperatures forecasted for the Arctic over the 21st Century. The water from the 60 L carboys was mixed in 280 L containers and transferred to duplicate acid-washed 20 L clear polycarbonate Nalgene™ bottles. Both of the duplicate bottles for each experimental temperature treatment were submersed in a 280 L tank connected to a temperature control unit (Poly-Science 9600 series, precision  $0.1^{\circ}\text{C}$ ) with an impelling and expelling pump. Temperature data loggers were submersed in each tank to monitor the resulting water temperature. The setup was completed with two fluorescent light tubes per tank as to provide an appropriate, continuous light environment. The light emitted from fluorescent lamps was measured to be  $90\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$  using a LI-1000 Li-Cor radiation sensor. The experimental irradiance used was similar across all tanks and remained constant 24 h a day throughout both experiments. This irradiance was selected so as to reproduce a light environment similar to where the plankton communities were collected, based on measurements from earlier cruises in this season. The Barents Sea waters sampled are characterised by a relatively high light attenuation due to

the large amount of suspended particles and sediments from river and glacier run-off, that combined with the cloudiness in May–June as well as the incident angle of the sun during that time of year (Sakshaug et al., 2009) suggests that the light irradiance used throughout the experiment was within the range of possible light regimes experienced by a community collected at 26 m depth in the open ocean during that time of year. As for the fjord water, radiation likely to be experienced at 2 m depth should not have exceeded  $350\text{--}200\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$  (Sakshaug et al., 2009), is likely higher than the irradiance from the fluorescent lights. This may be an explanation for the general increase in chlorophyll *a* concentrations for all treatments in the fjord water experiment. However, the overall trend of chlorophyll *a* concentrations in both experiments would not be confounded by photoadaptation as all temperature treatments were exposed to the same irradiance throughout the experiment.

The temperature treatments for the Barents Sea community, sampled at  $-1^{\circ}\text{C}$  in situ temperature, were achieved by gradually warming over three days to reach the target temperature while avoiding a temperature shock response of the communities. We did not raise the temperature gradually for the fjord community as the water was collected at  $6.2^{\circ}\text{C}$ . Due to the unstable temperature conditions in the cooling rooms, the temperatures fluctuated somewhat along both experiments, but the average temperature was successfully maintained in the different tanks (Tables 2 and 3). The experiment was maintained during 15 days for the Barents Sea community and 10 days for the Isfjorden community. The Arctic community was maintained longer due to a slower response time, which was determined using daily chlorophyll *a* measurements, to evaluate the time-course of the response. The duplicate samples for the Barents Sea community were pooled after day 10 to have sufficient water volume to continue the experiment on to day 15. The  $7^{\circ}\text{C}$  temperature treatment was lost in the middle of the experiment with the Isfjorden community due to technical problems leading to a sharp increase in temperature. Hence, this treatment was discontinued.

## 2.3 Variables measured

Samples of 50 mL for chlorophyll *a* determination were collected on the same days that metabolism samples were collected and filtered through Whatmann GF/F (glass fiber) filters. Chlorophyll *a* on the filters was extracted in 90 % acetone for 24 h. The concentration was measured fluorometrically following Parsons et al. (1984).

Other parameters such as nutrients, cell counts, and bacterial abundance were measured throughout the experiments at 2–3 day intervals. Nutrient samples were collected and kept frozen until later analysis. Phosphorus, nitrate + nitrite, and silicate concentrations were analyzed using standard methods (Hansen and Koroleff, 1999) in a Bran Luebe AA3 autoanalyzer. Bacterial abundance was determined in 10 mL

samples fixed with formaldehyde (2 % final concentration) and filtered onto 0.2 µm pore size, black polycarbonate filters. Filters were stained with 4',6-diamidino-2-phenylindole (DAPI) and bacteria were counted using an epifluorescence microscope following the methods described by Porter and Feig (1980). Heterotrophic bacterial production was estimated by measuring the rates of incorporation of <sup>3</sup>H-leucine into biomass in microcentrifuge tubes (Smith and Azam, 1992). Three replicates and two blanks containing 1.2 mL seawater and 40 nM leucine (final concentration) were processed for each sample. Blanks were killed by the addition of trichloroacetic acid (5 % final concentration) before the radioactive tracer was added. Samples were incubated at the corresponding temperatures for 2–4 h and processed as described in Smith and Azam (1992). Rates of leucine incorporation were transformed into biomass production by using a conversion factor of 1.5 kg C per mol of leucine incorporated, assuming no intracellular dilution of the tracer (Simon and Azam, 1989).

Community metabolism (gross primary production, community respiration and net community production) was determined from changes in oxygen over a 24 h period using the micro-Winkler method for determining dissolved oxygen concentration (Oudot et al., 1988). During the experiment with the open-ocean Arctic community, metabolic rates were determined on days 3, 4, 8, and 15 of the experimental period. To avoid the depletion of the water in the microcosms, measurements on day three were performed in only one of the two duplicate microcosms for each treatment, measurements on days 4 and 8 are based on both duplicate microcosms and those on day 15 were based on pooled samples from both duplicates. Isfjorden communities were sampled in each of the replicate microcosms on days 4 and 8 of the experimental period. Water samples from each of the 14 experimental units were carefully siphoned into narrow-mouth 25–35 ml borosilicate Winkler bottles under low light conditions in the temperature regulated cold rooms. After sampling, five replicates were immediately fixed and used to determine the initial oxygen concentration. Simultaneously, five replicates each were incubated for 24 h in “dark” and “light” and exposed to the same temperature and irradiance conditions as the corresponding microcosms from which they were sampled. Dark bottles were wrapped in black electrical tape and incubated in a submerged black plastic bag, while light bottles were incubated in submerged transparent plastic bags. Oxygen concentrations were analyzed by Winkler titration using a potentiometric electrode and automated endpoint detection (Mettler Toledo, DL28 titrator) following Oudot et al. (1988). Community respiration (CR) and net community production (NCP) were calculated by subtracting initial dissolved oxygen concentrations from dissolved oxygen concentrations measured after incubation in the dark and light conditions respectively. Gross primary production (GPP) was calculated by solving the mass balance equation  $GPP = NCP + CR$  (Carpenter, 1965; Carritt and Carpenter,

1966). The mean analytical precision of oxygen determinations across both experiments was 0.9 % (median = 0.7 %), which is well above the analytical limit of the method determined to be 0.02 % (Robinson and Williams, 2005), but comparable to the error reported in other efforts to resolve metabolic rates in the Arctic (Cotrell et al. 2006). The low precision is attributable to the small volume of the Winkler bottles (25–35 mL) used compared to standard volumes (100–250 mL) which were chosen to avoid depleting the microcosms of water. We examined using a Monte Carlo resampling approach, the contribution of the relatively low precision of our oxygen measurements to the error in the metabolic rates determined, and found that the analytical precision contributed between 45 and 66 % to the standard deviation of the rate measurements, suggesting that the low precision of our oxygen measurements plays a modest role in our capacity to resolve significant differences in metabolic rates. Standard errors for metabolic rates (NCP, CR, and GPP) were calculated using error propagation.

## 2.4 Experimental threshold detection

In the two threshold responses detected, data were adjusted by non-linear regression to the following sigmoid model function:

$$y = r_2 + \frac{r_1 - r_2}{1 + e^{s(t - T_p)}}, \quad (1)$$

where  $y$  is the actual value of the variable being fitted, in this case, NCP and CR, and  $t$  the independent variable, temperature. The other parameters are estimates by non-linear regression and describe different properties of the sigmoid function.  $r_1$  and  $r_2$  are the mean of the values of the variable at the two different regimes (high or low),  $s$  describes the slope of the changing part of the curve (how steep the change is), and  $T_p$  is the experimental “tipping point” or threshold value, defined as the temperature corresponding to the center of the shifting part of the curve. An  $R^2$  value for the curve was determined as 1 minus the squared sum of the residuals divided by  $y$  minus the mean of  $y^2$ .

## 2.5 Experimental validation with field data

In order to validate the experimental results, an extensive data base including 249 estimates of net community metabolism obtained between 2006 and 2011 from eight cruises conducted in the Greenland Sea and Svalbard Island region (see Vaquer-Sunyer et al., 2012, for details), was used to examine the relationship between Arctic plankton net community production and temperature, and derive the associated threshold of temperature separating autotrophic from heterotrophic communities.

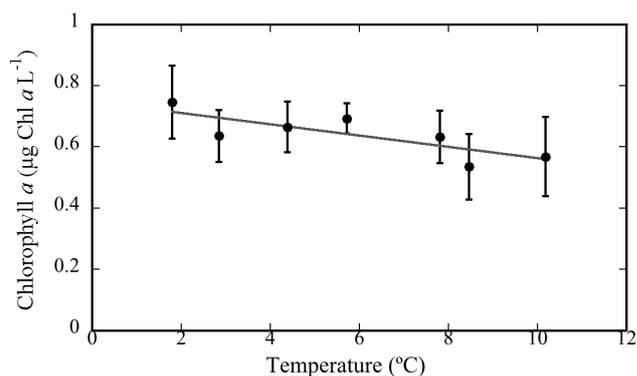
### 3 Results

#### 3.1 Response of the Barents Sea community

The Barents Sea community showed a significant decline in chlorophyll *a* concentrations along the temperature range (Fig. 1), as described by a fitted regression equation with a slope of  $-0.02 \mu\text{g Chl } a \text{ L}^{-1} \text{ } ^\circ\text{C}^{-1}$  ( $R^2 = 0.68$ ,  $p = 0.02$ ) using mean chlorophyll *a* concentrations from all days sampled.

Initial and average nutrient concentrations for all days sampled from each temperature treatment are presented in Table 1. In the experiment with open-sea communities silicate and phosphate concentrations remain similar across all temperature treatments, however average nitrate + nitrite concentrations are slightly negatively related to temperature. Bacterial abundance appears to increase with increasing temperatures while bacterial production appears to be strongly positively related to temperatures. Community metabolism rates fluctuated greatly throughout the time course of the experiment, as expected, as the communities acclimated to their new temperature treatments. Most notable differences in temperature treatments were found in the last measurement with pooled microcosms at day 15 (Fig. 2a, b, and c), as clear differences in chlorophyll *a* concentrations began to be seen (Fig. 2a). CR for the lowest temperatures (1.5, 3 and 4.5 °C) remained low throughout the experiment, while CR for medium temperatures (6 and 7.5 °C) rose throughout, reaching their highest rates at day 15 (Fig. 2b). CR for 9 °C appeared to respond positively at day 9, but further incubation resulted in a low CR at day 15. CR for the 10.5 °C treatment decreased throughout the time course (Fig. 2b). Patterns for NCP show similar patterns across treatments throughout the time course of the experiment, however, emerging differences strengthened as time increased, resulting in the highest NCP for the 3, 4.5 and 10.5 °C treatments (Fig. 2c) at day 15. These treatments also resulted in autotrophic communities (i.e., where  $\text{NCP} > 0$ ; Fig. 2c) by day 15.

When measured initially, the replicates of the Barents Sea plankton community samples were different, with one replicate acting strongly heterotrophic (i.e.,  $\text{NCP} < 0$ ;  $\text{NCP} \pm \text{SE} = -9.31 \pm 0.10$ ) and the other acting autotrophic (i.e.,  $\text{NCP} > 0$ ;  $\text{NCP} \pm \text{SE} = 4.41 \pm 0.18$ ). When the first experimental measurements were taken on day 3 there was no noticeable difference between the replicates, probably due to thermal acclimation, so further analysis was carried out averaging the replicates together. Community respiration (CR) showed a variable response to experimental temperature increase with mean CR rates ( $\pm\text{SE}$ ). Rates remained low for the lower temperatures tested while reaching their highest CR rate at an intermediate temperature of 5.8 °C and declining somewhat with additional warming (Fig. 3a; Table 2). Net community metabolism was balanced across the experiment (i.e.,  $H_0$ :  $\text{NCP} = 0$ , t-test,  $p = 0.41$ ) at low temperatures, but the community became net heterotrophic



**Fig. 1.** Mean ( $\pm\text{SE}$ ) chlorophyll *a* concentration ( $\mu\text{g Chl } a \text{ L}^{-1}$ ) of the Barents Sea plankton community tested here, averaged over the days when samples for determination of metabolic rates were taken, versus the mean temperature ( $^\circ\text{C}$ ) recorded for each experimental treatment. The solid line shows a significant ( $p = 0.02$ ) decrease in chlorophyll *a* concentration with temperature ( $R^2 = 0.68$ ).

( $\text{NCP} < 0$ ,  $\text{CR} > \text{GPP}$ ) at temperatures above 4.2 °C (Fig. 3c; Table 2). The temperature-dependence of NCP was driven by changes in CR, since GPP was variable and apparently independent of temperature changes. GPP values at  $\sim 1$  °C (Fig. 4b; Table 2) are lacking a standard error estimate due to lack of viable replicates (i.e., undetectable or negative values of GPP) and thus no trend with temperature was able to be deduced from the GPP data, although there appears to be a non-linear relationship no trend was found, which is most likely due to the limited degrees of freedom and large variance.

Since chlorophyll *a* concentrations declined across temperature treatments (Fig. 1), the responses in community metabolism may reflect changes in community biomass rather than physiological responses forced by temperature treatments. Hence, we examined the response of metabolic rates standardized to chlorophyll *a* concentrations measured in each microcosm on the same sampling day in an attempt to extract any physiological signal from the community responses. Indeed, CR rates standardized per unit chlorophyll *a* increased significantly with increasing temperature ( $R^2 = 0.64$ ,  $p = 0.03$ ). However, inspection of the relationship between CR per unit chlorophyll *a* and experimental temperature suggested that the relationship was best modeled as a logistic relationship (Fig. 4). Indeed, the changes in CR per unit chlorophyll *a* with temperature was well described by a non-linear regression characterized by low CR per unit chlorophyll *a* at low temperatures ( $3.75 \pm 0.90 \mu\text{mol O}_2 \mu\text{g Chl } a^{-1} \text{ day}^{-1}$ ) and an abrupt increase, to double the rates ( $7.71 \pm 0.74 \mu\text{mol O}_2 \mu\text{g Chl } a^{-1} \text{ day}^{-1}$ ), beyond a mean ( $\pm\text{SE}$ ) experimental threshold temperature of  $5.06 \pm 3.02$  °C ( $R^2 = 0.84$ ,  $p = 0.19$ ; Fig. 4).

Specific GPP rates, standardized per unit biomass also showed a lot of variation. Mean ( $\pm\text{SE}$ ) specific

**Table 1.** Initial ( $t_0$ ) and temperature-treatment averaged nutrient concentrations (phosphate, silicate, and nitrate + nitrite) bacterial abundance, and bacterial production averaged across all days sampled (every 2–3 days) for each experiment for both the open-sea and fjord communities. \* signifies number with out SE due to lack of viable replicates.

Temperature (°C)	Phosphate μM	NO <sub>3</sub> + NO <sub>2</sub> μM	Silicate μM	Bacterial Abundance 10 <sup>9</sup> cells L <sup>-1</sup>	Bacterial Production μmol CL <sup>-1</sup> day <sup>-1</sup>
Open-sea					
$t_0$	0.122 ± 0.015	0.152*	0.197 ± 0.009	0.375 ± 0.059	0.039 ± 0.002
1.72 ± 0.26	0.108 ± 0.005	0.326 ± 0.099	0.181 ± 0.008	0.969 ± 0.054	0.248 ± 0.009
2.60 ± 0.5	0.103 ± 0.004	0.172 ± 0.056	0.184 ± 0.021	0.870 ± 0.057	0.271 ± 0.005
4.15 ± 0.06	0.114 ± 0.004	0.147 ± 0.042	0.197 ± 0.010	0.829 ± 0.043	0.260 ± 0.005
5.76 ± 0.10	0.118 ± 0.019	0.268 ± 0.733	0.170 ± 0.013	1.220 ± 0.093	0.406 ± 0.010
7.77 ± 0.15	0.109 ± 0.008	0.109 ± 0.034	0.188 ± 0.010	1.230 ± 0.086	0.515 ± 0.007
8.53 ± 0.05	0.110 ± 0.008	0.155 ± 0.059	0.176 ± 0.018	1.070 ± 0.067	0.460 ± 0.022
10.42 ± 0.23	0.121 ± 0.009	0.098 ± 0.029	0.176 ± 0.010	1.200 ± 0.075	0.524 ± 0.015
Fjord					
$t_0$	0.122 ± 0.004	0.065 ± 0.026	0.411 ± 0.012	0.80 ± 0.252	0.364 ± 0.051
1.11 ± 0.01	0.070 ± 0.012	0.245 ± 0.095	0.403 ± 0.018	1.14 ± 0.108	0.172 ± 0.009
2.86 ± 0.06	0.066 ± 0.013	0.081 ± 0.046	0.381 ± 0.012	1.13 ± 0.086	0.184 ± 0.005
4.03 ± 0.05	0.073 ± 0.013	0.074 ± 0.051	0.392 ± 0.011	1.38 ± 0.151	0.186 ± 0.005
5.48 ± 0.03	0.060 ± 0.012	0.075 ± 0.030	0.375 ± 0.014	1.40 ± 0.147	0.244 ± 0.009
8.33 ± 0.11	0.076 ± 0.010	0.036 ± 0.014	0.373 ± 0.021	1.56 ± 0.176	0.229 ± 0.005
9.92 ± 0.05	0.070 ± 0.012	0.060 ± 0.017	0.412 ± 0.014	1.14 ± 0.090	0.247 ± 0.005

GPP rates per unit chlorophyll *a* ranged between  $4.14 \pm 0.86 \mu\text{mol O}_2 \mu\text{g Chl } a^{-1} \text{ day}^{-1}$  at  $2.6^\circ\text{C}$  and  $1.37 \pm 0.69 \mu\text{mol O}_2 \mu\text{g Chl } a^{-1} \text{ day}^{-1}$  at  $7.8^\circ\text{C}$ , without any clear relationship with the experimental temperature (Table 2). Thus, the specific NCP per unit chlorophyll *a* was also driven by changes in CR, and therefore, also showed a non-linear relationship with experimental temperature (Fig. 5) with a mean ( $\pm$ SE) experimental threshold temperature at  $4.78 \pm 1.26^\circ\text{C}$  ( $R^2 = 0.78$ ,  $p = 0.032$ ; Fig. 5) with a mean ( $\pm$ SE) specific NCP rate at colder temperature of  $-0.72 \pm 1.31 \mu\text{mol O}_2 \mu\text{g Chl } a^{-1} \text{ day}^{-1}$ , indicative of balanced metabolism, and a strongly heterotrophic community with a mean ( $\pm$ SE) specific NCP of  $-5.52 \pm 1.05 \mu\text{mol O}_2 \mu\text{g Chl } a^{-1} \text{ day}^{-1}$  developing at warmer temperatures (Table 2; Fig. 5).

For further validation of experimental results we tested, Arctic plankton net community production (averaged in  $1^\circ\text{C}$  bins) from eight cruises conducted in the region (Vaquer-Sunyer et al., 2012). NCP rates from field measurements also showed a strong negative relationship with temperature (Fig. 7), as described by the fitted model II regression equation:

$$\text{NCP } (\mu\text{mol O}_2 \text{ L}^{-1} \text{ day}^{-1}) = 11.87 (\pm 1.49) \\ - 2.19 (\pm 0.37) \text{ Temperature } (^\circ\text{C}),$$

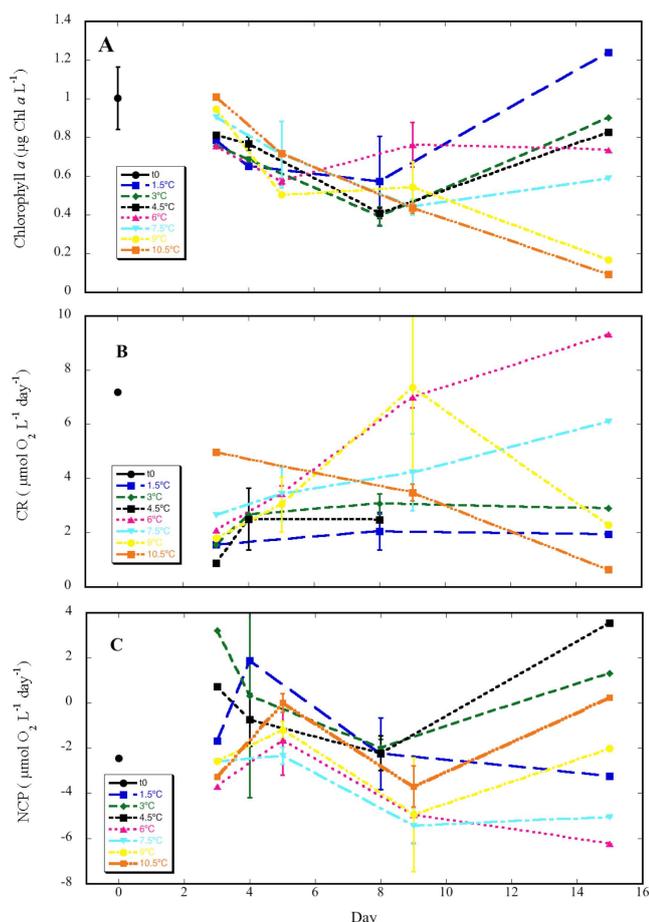
$$R^2 = 0.79, F = 26.8, p < 0.0001,$$

with a threshold NCP calculated by solving the equation to obtain the temperature for  $\text{NCP} = 0$ , of  $5.4^\circ\text{C}$ , similar to that obtained experimentally here.

### 3.2 Atlantic-influenced fjord water community

The Atlantic community showed no significant trend in chlorophyll *a* concentrations along the experimental temperature range (Fig. 6), with the highest mean biomass of about  $1.5 \mu\text{g Chl } a \text{ L}^{-1}$  developed at the temperature of  $6.2^\circ\text{C}$  at which the sampled community was growing (Table 3). Nutrient concentrations for each temperature treatment were averaged across the experiment and are presented in Table 1. In the experiment with fjord water, silicate and phosphate concentrations are similar across all temperature treatments and nitrate + nitrite concentrations are low but similar across treatments with the exception of the  $1.5^\circ\text{C}$  treatment. Bacterial abundance appears to be higher at higher temperatures, while bacterial production appears have a strongly positive relationship with temperature.

Atlantic communities were originally close to being balanced ( $\text{NCP} \pm \text{SE} = -0.73 \pm 0.35$ ) while specific community metabolic rates were heterotrophic ( $\text{NCP} \pm \text{SE} = -3.49 \pm 1.65$ ). Community respiration (CR) for the Atlantic influenced community showed high variation and no clear relationship with experimental temperature, similar to gross primary production (Table 3). As a consequence, net community production was independent of



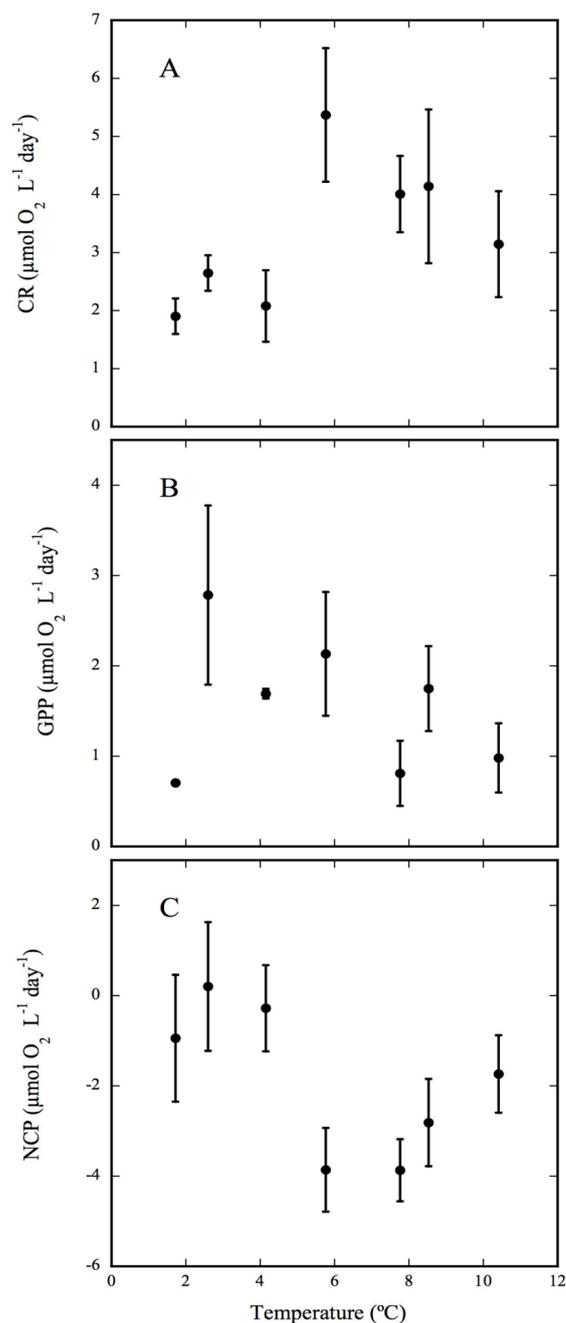
**Fig. 2.** Representation of the time course of mean biomass (a) mean CR rate (b) and mean NCP rate (c) of Barents Sea plankton community throughout 15 days of experimental treatment. Colors represent different temperature treatments as indicated.

experimental temperature, with some temperature treatments (i.e., 3 and 8.5°C) resulting in strong heterotrophic community metabolism (Table 3).

Since chlorophyll *a* concentrations were independent of the experimental temperature, the chlorophyll *a* specific rates showed the same patterns as those of the volumetric rates, with no significant relationship to the experimental temperature (Table 3).

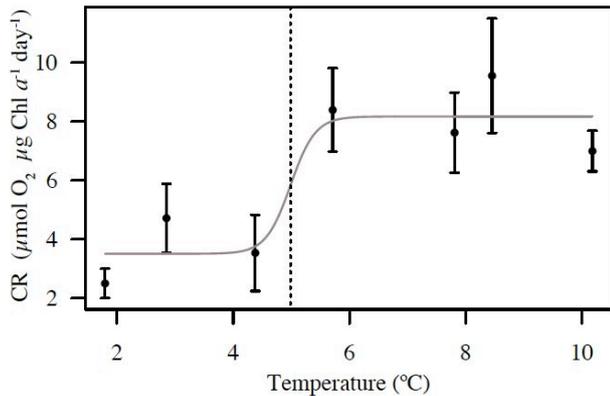
### 3.3 Analysis of other driving factors

For both experiments, nutrient dynamics were assessed as the possible alternative driving factor of metabolic rates. There was, however, no direct relationship between metabolic rates (NCP, CR, GPP) and nutrient concentrations (see the Supplement for details, Figs. S5 and S7). Furthermore, in the open-ocean community nutrient concentrations did not show any significant relationship with temperature. Nitrate + nitrite concentrations may have a slight negative relationship with temperature, however due to their variability over time and



**Fig. 3.** Barents Sea plankton community mean ( $\pm$ SE) volumetric community metabolic rates: CR (a), GPP (b), and NCP (c) averaged over the days when samples for determination of metabolic rates were taken, versus the mean temperature ( $^{\circ}\text{C}$ ) recorded for each experimental treatment.

across temperatures these relationships were not significant ( $p = 0.40$ ; Fig. S2). Nitrate + nitrite concentrations also show high variability over time, but there is no evident trend (Fig. S1). Phosphate and silicate concentrations also show no tendency over the experimental days (Fig. S1). For the fjord community, concentrations of nitrate + nitrite do have

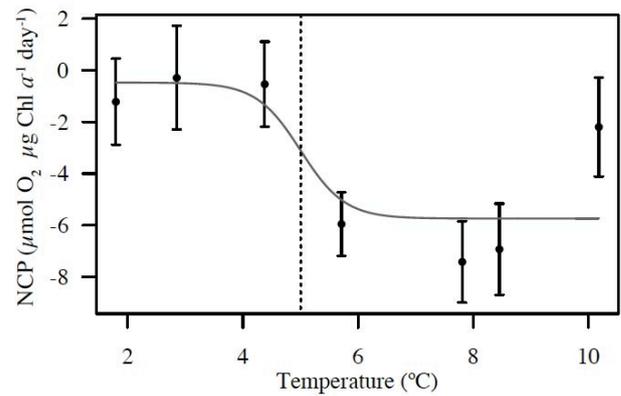


**Fig. 4.** The relationship between the mean Chl *a*-specific community respiration (CR) rate of the Barents Sea community along the experiment and the average temperature treatments. The solid line shows the fitted non-linear sigmoid model function, which defines a threshold temperature ( $\pm$ SE) of  $5.06 \pm 3.02$  °C (represented by the vertical dashed line) above which average specific CR rates ( $\pm$ SE) approximately double from a mean rate of  $3.75 \pm 0.90$   $\mu\text{mol O}_2 \mu\text{g Chl } a^{-1} \text{ day}^{-1}$  at lower temperatures to a mean rate of  $7.71 \pm 0.74$   $\mu\text{mol O}_2 \mu\text{g Chl } a^{-1} \text{ day}^{-1}$  at warmer temperatures.

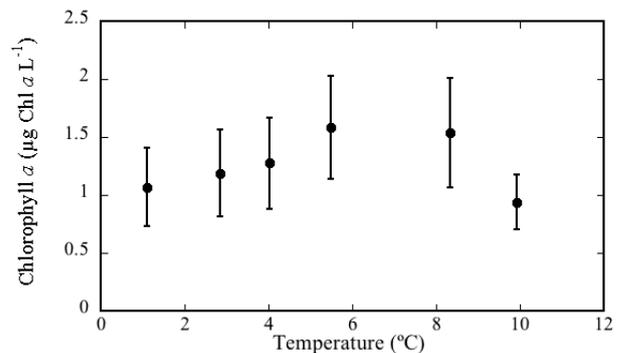
a significant ( $p = 0.006$ ,  $R^2 = 0.69$ ) relationship with temperature, however this relationship is based on only a few viable replicates over the entire experimental period ( $n = 9$ ). Silicate and phosphate concentrations decreased significantly over time (silicate:  $p < 0.005$ ;  $R^2 = 0.62$ ; phosphate:  $p < 0.005$ ;  $R^2 = 0.58$ ; Fig. S3), but did so independently of temperature ( $p = 0.85$ ,  $0.19$  respectively; Fig. S4).

We also investigated the possible relationship between bacterial abundance and production on the metabolic rates of NCP, CR, and GPP (Fig. S6). We find that in the open-ocean community, NCP is negatively related to both abundance and production ( $p = 0.03$ ,  $R^2 = 0.13$ ;  $p = 0.001$ ,  $R^2 = 0.24$  respectively) and CR is positively related to bacterial production ( $p = 0.005$ ,  $R^2 = 0.22$ ), but we find no relationship of CR to bacterial abundance ( $p = 0.09$ ). In the fjord community, both NCP and CR are independent of bacterial abundance or production (NCP:  $p = 0.16$ ,  $0.83$  respectively; CR:  $p = 0.15$ ,  $0.68$ ; Fig. S8).

Finally, as there was some relationship of metabolic rates to bacterial abundance and production in the open-ocean community, we also analyzed the NCP and CR values standardized by bacterial abundance for both experiments. We find that in the open-ocean community, standardizing metabolic rates per unit biomass of bacteria eradicate any previous metabolic relationship with temperature ( $p = 0.12$ ,  $0.47$ ; Fig. S9) seen from volumetric rates. Although the fjord community showed no relationship of metabolic rates to bacterial abundance and production, we tested bacterial abundance and standardized metabolic rates against temper-



**Fig. 5.** The relationship between the mean net community production (NCP) rate of the Barents Sea plankton community along the experiment and the average temperature treatments. The solid line shows the fitted non-linear sigmoid model function, which defines the threshold temperature ( $\pm$ SE) of  $4.78 \pm 1.26$  °C (represented by the vertical dashed line) above which average specific NCP rates ( $\pm$ SE) decrease from a mean rate of  $-0.72 \pm 1.31$   $\mu\text{mol O}_2 \mu\text{g Chl } a^{-1} \text{ day}^{-1}$  at lower temperature to a mean rate of  $-5.52 \pm 1.05$   $\mu\text{mol O}_2 \mu\text{g Chl } a^{-1} \text{ day}^{-1}$  at warmer temperatures.



**Fig. 6.** Mean ( $\pm$ SE) chlorophyll *a* concentration ( $\mu\text{g Chl } a \text{ L}^{-1}$ ) of the Atlantic fjord community tested here, averaged over the days when samples for determination of metabolic rates were taken, versus the mean temperature (°C) recorded for each experimental treatment.

ature and again found no relationship for either NCP or CR ( $p = 0.50$ ,  $0.71$  respectively; Fig. S10).

#### 4 Discussion

The experimental results presented here show that the metabolism of the open-ocean Arctic community collected in the Barents Sea was highly sensitive to warming, whereas that of the community already growing in the Atlantic-influenced, warm-water Arctic fjord, showed no clear relationship with experimental temperature across the 1 to 10 °C experimental range.

**Table 2.** Experiment with Arctic open-ocean community. Temperature ( $\pm$ SE), chlorophyll *a* ( $\pm$ SE), volumetric and specific NCP, CR and GPP rates ( $\pm$ SE), as well as GPP/CR ratio are presented for the initial measurements (*t*<sub>0</sub>) as well as values averaged across 15 days of experimental treatment. \* signifies number without SE due to lack of viable replicates.

Temperature (°C)	Chl <i>a</i> $\mu\text{g L}^{-1}$	NCP		CR		GPP		GPP/CR
		Volumetric $\mu\text{mol O}_2 \text{ L}^{-1} \text{ day}^{-1}$	Specific $\mu\text{mol O}_2 \mu\text{g Chl } a^{-1} \text{ day}^{-1}$	Volumetric $\mu\text{mol O}_2 \text{ L}^{-1} \text{ day}^{-1}$	Specific $\mu\text{mol O}_2 \mu\text{g Chl } a^{-1} \text{ day}^{-1}$	Volumetric $\mu\text{mol O}_2 \text{ L}^{-1} \text{ day}^{-1}$	Specific $\mu\text{mol O}_2 \mu\text{g Chl } a^{-1} \text{ day}^{-1}$	
<i>t</i> <sub>0</sub>	1.00 $\pm$ 0.16	-2.45 $\pm$ 6.86	-3.64 $\pm$ 7.43	7.18 $\pm$ 6.98	8.50 $\pm$ 8.33	4.73 $\pm$ 0.11	4.87 $\pm$ 0.90	11.58 $\pm$ 11.24
1.72 $\pm$ 0.26	0.72 $\pm$ 0.06	-0.94 $\pm$ 1.40	-0.98 $\pm$ 1.95	1.90 $\pm$ 0.31	2.74 $\pm$ 0.57	0.70*	2.06*	0.52*
2.60 $\pm$ 0.5	0.63 $\pm$ 0.08	0.20 $\pm$ 1.43	-0.59 $\pm$ 2.35	2.65 $\pm$ 0.31	5.02 $\pm$ 1.39	2.78 $\pm$ 0.99	4.14 $\pm$ 0.86	1.31 $\pm$ 0.64
4.15 $\pm$ 0.06	0.70 $\pm$ 0.06	-0.28 $\pm$ 0.95	-1.34 $\pm$ 1.70	2.08 $\pm$ 0.62	3.54 $\pm$ 1.29	1.69 $\pm$ 0.05	2.17 $\pm$ 0.11	1.20 $\pm$ 0.39
5.76 $\pm$ 0.10	0.76 $\pm$ 0.08	-3.86 $\pm$ 0.93	-5.53 $\pm$ 1.37	5.37 $\pm$ 1.15	7.68 $\pm$ 1.45	2.13 $\pm$ 0.69	3.01 $\pm$ 0.92	0.40 $\pm$ 0.16
7.77 $\pm$ 0.15	0.55 $\pm$ 0.04	-3.87 $\pm$ 0.69	-7.22 $\pm$ 1.85	4.01 $\pm$ 0.66	7.16 $\pm$ 1.52	0.81 $\pm$ 0.36	1.37 $\pm$ 0.69	0.19 $\pm$ 0.08
8.53 $\pm$ 0.05	0.48 $\pm$ 0.05	-2.81 $\pm$ 0.97	-6.07 $\pm$ 1.83	4.14 $\pm$ 1.32	8.88 $\pm$ 2.16	1.75 $\pm$ 0.47	3.52 $\pm$ 0.65	0.41 $\pm$ 0.10
10.42 $\pm$ 0.23	0.46 $\pm$ 0.06	-1.74 $\pm$ 0.86	-2.99 $\pm$ 2.06	3.14 $\pm$ 0.91	7.00 $\pm$ 0.89	0.98 $\pm$ 0.38	4.01 $\pm$ 2.78	0.61 $\pm$ 0.39

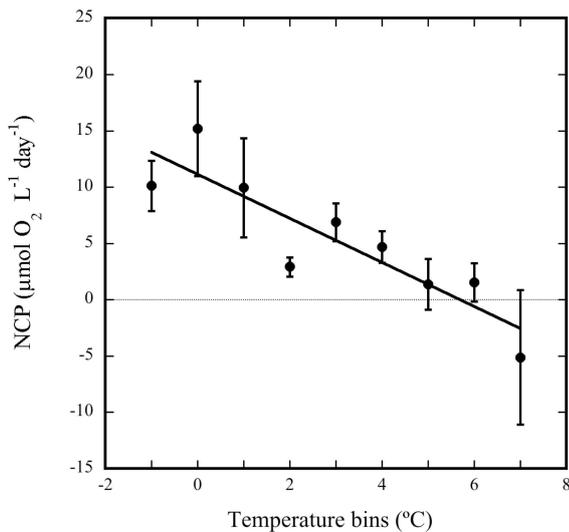
**Table 3.** Experiment with Atlantic-influenced fjord communities. Temperature ( $\pm$ SE), chlorophyll *a* ( $\pm$ SE), volumetric and specific NCP, CR and GPP rates ( $\pm$ SE), as well as GPP/CR ratio are presented for the initial measurements (*t*<sub>0</sub>) as well as values averaged across the 10 days of experimental treatment. \* signifies number with out SE due to lack of viable replicates.

Temperature (°C)	Chl <i>a</i> $\mu\text{g L}^{-1}$	NCP		CR		GPP		GPP/CR
		Volumetric $\mu\text{mol O}_2 \text{ L}^{-1} \text{ day}^{-1}$	Specific $\mu\text{mol O}_2 \mu\text{g Chl } a^{-1} \text{ day}^{-1}$	Volumetric $\mu\text{mol O}_2 \text{ L}^{-1} \text{ day}^{-1}$	Specific $\mu\text{mol O}_2 \mu\text{g Chl } a^{-1} \text{ day}^{-1}$	Volumetric $\mu\text{mol O}_2 \text{ L}^{-1} \text{ day}^{-1}$	Specific $\mu\text{mol O}_2 \mu\text{g Chl } a^{-1} \text{ day}^{-1}$	
<i>t</i> <sub>0</sub>	0.21 $\pm$ 0.002	-0.73 $\pm$ 0.35	-3.49 $\pm$ 1.65	1.79*	8.61*	1.41*	6.78*	0.79*
1.11 $\pm$ 0.01	1.07 $\pm$ 0.34	2.78 $\pm$ 4.19	6.27 $\pm$ 9.26	1.59 $\pm$ 0.61	1.45 $\pm$ 0.25	3.93*	2.14*	1.78*
2.86 $\pm$ 0.06	1.19 $\pm$ 0.38	-1.56 $\pm$ 1.89	-3.59 $\pm$ 3.80	6.14 $\pm$ 0.85	7.16 $\pm$ 2.69	4.58 $\pm$ 1.75	3.57 $\pm$ 1.30	0.79 $\pm$ 0.26
4.03 $\pm$ 0.05	1.28 $\pm$ 0.39	2.07 $\pm$ 4.11	5.94 $\pm$ 6.95	5.27 $\pm$ 0.34	4.57 $\pm$ 1.98	3.29 $\pm$ 1.30	3.58 $\pm$ 2.51	0.60 $\pm$ 0.22
5.48 $\pm$ 0.03	1.58 $\pm$ 0.45	0.37 $\pm$ 1.73	1.22 $\pm$ 1.39	5.21 $\pm$ 1.21	3.06 $\pm$ 0.45	4.22 $\pm$ 1.05	3.04 $\pm$ 1.19	0.10 $\pm$ 0.86
8.33 $\pm$ 0.11	1.54 $\pm$ 0.47	-5.24 $\pm$ 5.51	-1.78 $\pm$ 2.59	9.02 $\pm$ 6.69	4.31 $\pm$ 2.66	1.44 $\pm$ 0.41	1.14 $\pm$ 0.58	0.62 $\pm$ 0.34
9.92 $\pm$ 0.05	0.94 $\pm$ 0.23	1.14 $\pm$ 2.10	3.44 $\pm$ 3.11	2.34 $\pm$ 1.36	1.86 $\pm$ 0.87	3.48 $\pm$ 1.15	5.29 $\pm$ 2.37	10.74 $\pm$ 6.76

We report metabolic rates, standardized for chlorophyll *a*, to be able to test our results against the metabolic theory, as the MTE is based on physiological processes and refers to metabolic rates per unit biomass of the same individual (Brown et al., 2004). It may be argued that chlorophyll *a* is not the most relevant parameter to standardize for biomass as community respiration has both components of autotrophic respiration and heterotrophic respiration. However, we found no significant relationships between temperature and metabolic rates standardized for bacterial abundance (Figs. S9 and S10). Chlorophyll *a* normalized rates of NCP and CR do however show a strong relationship with temperature (Figs. 4 and 5). These observations are comparable to those in a recent global assessment, where rates of both GPP and CR standardized by chlorophyll *a* yield patterns with temperature (Regaudie-de-Gioux and Duarte, 2012). Regaudie-de-Gioux and Duarte (2012) show that chlorophyll *a* is an appropriate normalization parameter for both GPP and CR, whereas other properties that could be in principle related to CR, such as bacterial abundance, when used

as a normalization parameter, do not yield any patterns with temperature similar to our findings. This may be due to the fact that a majority of bacterial cells in marine plankton communities are metabolically inactive (Gasol et al., 1995) or may also be due to the inherent covariation of both bacterial abundance and production with chlorophyll *a* concentrations (Li et al., 2004; Lopez-Urrieta and Morán, 2007) that confounds the relationship of community respiration with temperature (Lopez-Urrieta and Morán, 2007). We argue that chlorophyll *a* is an appropriate parameter due to the fact that community respiration is constrained by the flow of organic matter from autotrophs, which is strongly correlated with chlorophyll *a*, and the previous relationships found between CR and chlorophyll *a* (Robinson and Williams 2005).

Consistent with predictions from the metabolic theory (Harris et al., 2006; Lopez-Urrutia et al., 2006) and short-term experiments (Vaquer-Sunyer et al., 2010), experimentally increased water temperature in the Barents Sea plankton community resulted in a shift from a balanced metabolism (NCP=0, GPP=CR) at lower temperatures to a strongly



**Fig. 7.** The relationship of the mean NCP ( $\mu\text{mol L}^{-1} \text{ day}^{-1}$ )  $\pm$ SE average across different temperature bins, taken from a data set from Vaquer-Suyner et al. (2012). Black line represents the model II regression between mean NCP and temperature ( $R^2 = 0.79$ ,  $p < 0.0001$ ) and gray dashed line drawn at  $y = 0$  intersects the regression at approximately  $5.4^\circ\text{C}$ , defining the point at which NCP rates switch from positive to negative values.

heterotrophic community (NCP < 0, GPP < CR), acting as a CO<sub>2</sub> source. This response was, however, steeper than expected. Whereas for the expectations derived from the consideration of the temperature-dependence of metabolic processes (Harris et al., 2006; Lopez-Urrutia et al., 2006; Vaquer-Suyner et al., 2010), the actual responses involved also changes at the community level, particularly a decline in chlorophyll *a* concentration. Moreover, the decline in chlorophyll *a* concentration with increasing temperature explains that, unlike the predictions by metabolic theory, gross primary production did not show significant increase with warming for the Barents Sea community, despite a tendency for increased chlorophyll *a*-specific GPP at higher temperatures (Table 2). Hence, the increase in CR and decline in NCP for the Barents Sea community with increasing warming, compounded physiologic-level with community-level responses to yield a much steeper decline in net community metabolism of the community, which becomes strongly heterotrophic. Previous examinations of the temperature-dependence of community metabolism, available only for respiration rates, used short-term, 24 h to 48 h experiments (Vaquer-Suyner et al., 2010), and did not allow, therefore, for responses in community structure to be realized.

Using the van't Hoff–Arrhenius relation, we can then estimate the activation energy ( $E_i$ ) required for the reaction of respiration across experimental temperature treatments using the equation:  $B \sim e^{-E_i/kT}$ , and the Boltzman constant,  $k$  ( $8.617343 \times 10^{-5} \text{ eV K}^{-1}$ ), where  $B$  is the metabolic rate and  $T$  the temperature in Kelvin (Gillooly et al., 2001; Brown

et al., 2004). The experiment conducted with the Barents Sea community yields an  $E_i$  of approximately 0.85 eV, higher than the value of 0.65 eV predicted from theory (López-Urrutia et al., 2006), but not different from  $E_i$  derived from short-term experiments of  $1.05 \pm 0.3 \text{ eV}$  (Vaquer-Suyner et al., 2010). The  $E_i$  of 0.85 eV derived here, confirms that respiration rates of Arctic plankton communities have  $E_i$  values above the rate of 0.41–0.74 eV suggested for organisms living at intermediate temperature regimes (Gillooly et al., 2001; Brown et al., 2004). This finding confirms the conclusion that the respiration of planktonic communities of organisms growing at the lower range of ocean temperatures show a steep response to increased temperature (Pomeroy and Wiebe, 2001; Vaquer-Suyner et al., 2010). In contrast, this could also be the reason that no significant relationships were found in the experiment with the Atlantic-influenced fjord water communities, which are exposed to much more variable temperatures throughout the spring melt season.

Most importantly, the results obtained here allowed the postulated temperature threshold beyond which Arctic communities become heterotrophic to be experimentally resolved at about  $5^\circ\text{C}$  ( $4.78 \pm 1.26^\circ\text{C}$ ). The relationship between net community metabolism and temperature was best described with a non-linear relationship where communities shift from metabolic balance to net heterotrophic beyond a temperature threshold of  $5^\circ\text{C}$ , above which the specific community respiration doubles and NCP is reduced 5-fold. These results provide, therefore, support for the proposition that Arctic plankton community metabolism shows tipping point behavior (Duarte et al., 2012), and quantifies the experimental tipping point for the community to flip from acting as a CO<sub>2</sub> sink to a CO<sub>2</sub> source at a temperature threshold of  $5^\circ\text{C}$ .

We did not detect an effect of temperature on the metabolism of Isfjorden communities along the duration or range of temperatures tested. One consideration is that Isfjorden communities were not gradually adjusted to their experimental temperatures as the open-sea communities were. We felt that temperature acclimation for the fjord communities was unnecessary due to the high fluctuation of temperatures felt in Svalbard fjords during the months of June and July. Using a time series of sea surface temperature (SST) from NOAAs Climate Prediction Center (<http://nomad2.ncep.noaa.gov/ncep.data/>), we extracted weekly average SST values for the last two decades for the sampling station in Isfjorden using a  $1^\circ$  square grid cell. Over two decades the range of temperature experienced by the fjord community in June and July ranged from  $1.9$ – $7.2^\circ\text{C}$ , while the average range of temperature experienced in any one year during June and July is  $3.0$ – $5.6^\circ\text{C}$ , thus suggesting that these communities are already well adapted to steep temperature fluctuations. Moreover, whereas the Barents Sea community was growing in situ at one extreme of the experimental temperature range used here, the fjord community was growing near the midpoint of this range, hence the maximum departure, in  $^\circ\text{C}$  from the in situ temperature was about 1/2 in the

fjord community compared to that in the Barents Sea. Isfjorden communities were growing in Arctic ecosystems invaded by warm Atlantic waters, however decreasing water temperature did not cause the metabolic rates of the Isfjorden community tested here to become autotrophic within the limitations of the duration of the experiment conducted. This may suggest the presence of hysteresis creating a resistance for communities already growing in warm waters to revert from a net heterotrophic community to an autotrophic one as waters become colder (Duarte et al., 2012).

Nutrient dynamics are often closely coupled to many metabolic and community processes (McAndrew et al., 2007; Karl, 2007), especially in the European Arctic Ocean (Sakshaug et al., 2009) which has a very acute spring bloom. The experiments reported here were conducted, however, with post-bloom communities, as the bloom occurs over a month before the time of the experiment (Vaquer-Sunyer et al., 2012). This is also supported by the relatively low nitrate and phosphate concentrations in the waters sampled (Sakshaug et al., 2009). In the open-ocean community we did not find any significant relationship between nutrient concentration and experimental temperature or over time. In the fjord experiment, nitrate + nitrite concentrations declined significantly with temperature, and silicate and phosphate concentrations declined over time. However, metabolic rates in the fjord experiment were independent of temperature, and metabolic rates showed no relationship with nutrients. Hence, nutrient dynamics did not appear to affect the metabolic responses to temperature manipulation reported here for either experiment.

Whereas variable nutrient concentrations in the open-ocean community did not have any effect on metabolic rates, a possible factor confounding the relationship of metabolic rates with temperature is the relationship of bacterial abundance and production to NCP and CR rates in the open-ocean community. NCP decreased with higher bacterial abundance and bacterial production, and CR increased with higher bacterial production. This is to be expected provided the temperature dependence of bacterial metabolism in the Arctic Ocean (Kritzberg et al., 2010).

The results here, derive from microcosm experiments and suffer, therefore, from the limitations inherent to these experimental setups (cf. Duarte et al., 1997). However, the results do not stand alone in concluding that polar plankton communities show a steep response to warming, as these results are supported by theoretical expectations (Harris et al., 2006; López-Urrutia et al., 2006; Duarte et al., 2012) and short-term warming experiments in polar communities (Pomeroy and Wiebe, 2001; Vaquer-Sunyer et al., 2010). This as well as previous short-term experiments (Vaquer-Sunyer et al., 2010) indicate that warming leads to a steep increase in respiration rates of polar plankton communities, thus increasing the threshold GPP or the primary production needed to balance out respiration at higher temperatures (i.e.,  $GPP/R > 1$ ). It has already been hypothesized that po-

lar communities may be more vulnerable to warming than temperate communities (Pomeroy and Wiebe, 2001). However the metabolic balance of the communities may be more vulnerable to warming than that of Southern Ocean communities, as Arctic communities are characterized by a large threshold for GPP ( $3.84 \mu\text{mol O}_2 \text{ L}^{-1} \text{ day}^{-1}$ ; Vaquer-Sunyer et al., 2012), much higher than that of Southern Ocean communities ( $2.05 \mu\text{mol O}_2 \text{ L}^{-1} \text{ day}^{-1}$ ; Agustí and Duarte, 2005), which is suggested to be due in part to access to large pools of dissolved organic carbon that lead to high bacterial respiration rates (Duarte and Regaudie-de-Gioux, 2009; Regaudie-de-Gioux and Duarte, 2010). Arctic glaciers are melting at an increasing pace and are expected to be a large source of ancient labile organic matter to the Arctic Ocean (Hood et al., 2009) thus increasing the pool of organic carbon available for bacterial metabolism in the future. Recent experimental work also suggests that the increased substrate availability amplifies the effect of temperature on polar bacterial metabolism (Kritzberg et al., 2010).

There is also a large amount of research dedicated to forecast the effects of changing light environments and increased stratification on carbon fluxes in a future warmer Arctic. Arrigo et al. in 2008 measured, using satellite chlorophyll *a* concentrations, an increase in primary production 30 % attributable to loss of sea ice extent. However, research carried out by Hessen et al. (2008) suggests that increasing the light environment is likely to enhance primary production but may lead to nutrient limitation, which will put a cap on the enhancement of primary production with warming, as also acknowledged by Arrigo et al. (2008). Nutrient cycling is likely to be affected by future increased vertical stratification of the Arctic, with freshening especially in the seasonal ice zone where spring blooms are strongest (Wassmann et al., 2008). This is expected to suppress new production by reducing mixing-derived nutrient supply (Wassmann et al., 2008). Furthermore, it is suggested that nutrient limitation may play a much larger role in governing primary productivity in these regions than light availability (Tremblay and Gagnon, 2009).

Whereas the role of the indirect effects of increased light availability and reduced nutrient limitation with reduced ice cover have been considered extensively, the direct effects of warming on plankton metabolism had not yet been assessed. While the experimental tipping point of  $5^\circ\text{C}$  for communities to shift from autotrophic to heterotrophic derived here will be affected by synergies with these indirect effects, including increased irradiance, reduced nutrient supply and increased DOC loads from runoff, addressing these complex synergies is beyond the capacity of experimental approaches and will require modeling exercises. These will require the input of functional responses between plankton communities and the various drivers involved. The experimental relationship between temperature and Arctic plankton community metabolic rates supplied here will be fundamental in allowing this important driving factor to be adequately

parameterized in models addressing the response of Arctic plankton communities to climate change.

The present results suggest that Arctic plankton communities may be considered, as proposed by Duarte et al. (2012), as tipping elements (*sensu* Lenton et al., 2008) triggering changes when perturbed beyond climatic tipping points. These experimental results only take into account one variable, temperature, to determine a possible tipping point for metabolic balance in the Arctic Ocean and suggest that an increase beyond 5 °C switches Arctic plankton communities to strong heterotrophy. Planktonic metabolism in the Arctic will also be affected by other direct changes related to global change (i.e., increasing  $p\text{CO}_2$ , pollution, increased UV-B radiation) as well indirect changes associated with warming of the region, such as increased irradiance with ice loss, increased water column stratification, increasing DOC loads with increased ice and permafrost melting (Duarte et al., 2012). These other variables may add complexity to the response of plankton metabolic rates to temperature and add uncertainty to the temperature beyond which Arctic plankton communities may act as  $\text{CO}_2$  sources. Further effort is needed to quantify these direct and indirect effects and their consequences on the ability of the Arctic Ocean to function as a large sink of  $\text{CO}_2$  (Takahashi et al., 2002). Although multiple factors will change as the Arctic Ocean warms, the experimentally derived threshold of temperature for communities to shift from autotrophic to heterotrophic of  $4.78 \pm 1.26$  °C is strengthened by results derived empirically from a comparative analysis of 249 estimates of net community metabolism obtained on 8 different cruises conducted in the region between 2006 and 2011 (Vaquer-Sunyer et al., 2012) which estimated a threshold value of 5.4 °C. This adds strength to our experimental results and suggests that a threshold of  $\sim 5$  °C can be used as a threshold to model the expected shift of the Arctic plankton community from acting as a sink to a source of atmospheric  $\text{CO}_2$  (Duarte et al., 2012). The implications of the results from this experiment suggest that at least temperature may have a negative effect on the sink capacity with future warming beyond 5 °C. Furthermore, these results concur with global analyses (Regaudie-de-Gioux and Duarte, 2012) to indicate that the GPP/CR ratio of plankton communities decline with warming.

**Supplementary material related to this article is available online at:** <http://www.biogeosciences.net/10/357/2013/bg-10-357-2013-supplement.pdf>.

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