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Discussions

Interactive comment on "Temperature thresholds for Arctic plankton community metabolism: an experimental assessment" *by* J. M. Holding et al.

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Interactive Author Comment on "Temperature thresholds for Arctic plankton community metabolism: and experimental assessment" by J.M. Holding et al.

First we would like to thank Reviewers 1 and 2 for their comments, suggestions and criticisms, which have aided greatly in improving the manuscript. Here we address all comments from each reviewer point by point with a reply followed by relevant actions taken to improve the quality of the manuscript following the suggestions of the reviewers.

Response to Reviewer 1

Reviewer 1 General Comments 1: 'I am very concerned that the authors didn't measure

a sufficient number of parameters associated with the plankton community to unambiguously evaluate the community response to temperature. For example, the authors don't provide any information about nutrient concentrations. There is no mention of nutrient additions so I assume that the populations had to exist on whatever nutrients were available at the time of collection. Given a 15-day incubation period, it is very likely that nutrients in some of the treatments may have been exhausted. Is it possible that this might explain the reduction in chlorophyll in the 9 and 10 degree treatments? We don't know because nutrient data were not presented. In addition, phytoplankton biomass was only characterized using measurements of chlorophyll and heterotrophic biomass wasn't characterized at all.'

Reply: We agree with reviewer 1's concerns and have now added a table (Table 1) where we show average nutrient concentrations, bacterial abundance and production for both experiments. The responses of the phytoplankton and microheterotroph community, as well as the dynamics of nutrient concentrations are reported in detail in a manuscript submitted by Coello-Camba, et al.

Action: To address Reviewer 1's questions about characterization of the communities we have now included Table 1 with the following table heading to the manuscript as well as including text to the methods and results section of the paper:

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

Methods: "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-nitrate, 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 0.2μm-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 3H-leucine into biomass in microcentrifuge tubes (Smith and Azam, 1992). Three replicates and two blanks containing 1.2 mL seawater and 40nM leucine (final concentration) were processed for each sample. Blanks were killed by the addition of tricholoroacetic 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 producion 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)."

Results: "Average nutrient concentrations for 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 nitrate + nitrate 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." ...

"Nutrient concentrations for each temperature treatment were averaged across the experiment and 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°C treatment. Bacterial abundance appears to be higher at higher temperatures while bacterial production appears have a strongly positive relationship with temperature."

Reviewer 1 General Comments 2: 'Because chlorophyll/cell can change under different light regimes (independent of temperature), it is important to measure phytoplankton

cell number and the light levels that the community experienced at the start of the experiment and the light levels produced by the fluorescent lights used in the incubations. This is the only way to know whether changes in chlorophyll are due to photoacclimation by the phytoplankton community during a shift to a new light regime or to changes in phytoplankton abundance.'

Reply: We agree with reviewer 1's concerns about photoacclimation, however irradiance experienced by each treatment was constant ($\sim$90 μmol m-2 s-1) throughout the experiment. The irradiance used during the experiment is within the approximate radiation that the communities would have experienced in situ. Moreover, all tanks were exposed to the same light environment. Surface irradiance in midsummer in the Barents Sea can reach 1500 μmol m-2 s-1 at latitudes above 70° on a clear day (Sakshaug et al., 2009). The water used in the mesocosms was collected at a depth of 26 m just above the deep chlorophyll maximum. Even the clearest Barents Sea water has a vertical diffuse attenuation coefficient (KPAR) of $\sim$0.7 m-1 in the upper 50 m (Sakshaug et al., 2009), which would imply that the highest irradiance at this depth would be $\sim$240 μmol m-2 s-1. The Barents Sea is characterized by high reflection and attenuation due to large amounts of suspended particles and sediments from glacier run-off especially in the coastal areas (Sakshaug et al., 2009). Furthermore, given the average cloudiness in May- June and the low incident angle of the sun at this time of year, radiation likely to be experienced at 2m depth should not have exceeded 350-200 μmol m-2 s-1 (Sakshaug et al., 2009). This suggests that light irradiance used throughout the experiment ($\sim$90 μmol m-2 s-1) falls within the possible range of irradiance that the phytoplankton community may experience at 26 m depth. Indeed, it was designed to do so. Even if the phyotoplankton communities were responding to a change in light regimes when taken out of the field, this does not take away from the overall trend of decreased chlorophyll a concentrations along the temperature gradient, as all temperature treatments were exposed to the same irradiance ($\sim$90 μmol m-2 s-1).

Action: We have now added the following text to the methods to address this issue:

"The light emitted from florescent lamps was measured to be 90 μmol photons m-2 s-1 using the LI-1000 LI-COR radiation sensor. The experimental irradiance used was similar across all tanks and remained constant 24 hours a day through out 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 relative 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 experiences by a community collected at 26m depth in the open ocean during that time of year. As for the fjord water, radiation likely to be experienced at 2m depth should not have exceeded 350-200 μmol m-2 s-1 (Sakshaug et al. 2009) likely higher than the irradiance from the florescent 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."

Reviewer 1, Specific Comments 1: 'In the Introduction, the authors ignore the impact of cooling of northward-advecting waters and how this would impact air-sea CO2 exchange. The CO2 sink is driven in part by biological drawdown of CO2, but also by the fact that cooling waters have a lowered pCO2 and facilitate greater air-sea exchange. This should be mentioned in the paper.'

Reply: We agree with Reviewer 1, that this information is indeed left out of the introduction.

Action: We have now changed the first paragraph of the introduction to include this information. It now reads: "The Barents sea region of the Arctic Ocean 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 Barents Sea is characterized by the large outflow of less saline cold waters from the north most notable from the East Greenland and East Spitsbergen Currents that a have high solubility to CO2. These physical properties are responsible for the high CO2 uptake in the mostly ice-free Barents Sea, which is estimated to be 9 * 1012 g C yr-1 (Fransson et al., 2001), compared to the entire ice-covered Arctic interior (31* 1012 g C yr-1; Katlin and Anderson 2005). High biological production in this area also adds to the role of the Arctic as a significant CO2 sink (Loeng et al., 2005)."

Reviewer 1, Specific Comments 2: 'None of the oxygen data used to calculate community metabolism are presented. How often during a 24-hour period was O2 measured? And the authors never describe how GPP, CR, and NPP are actually calculated. A diagram showing the experimental set-up would also be helpful. It was hard to envision from the text alone.'

Reply: We believe that due to our poor explanation, Reviewer 1 has misunderstood the methodology used for measuring dissolved oxygen concentrations.

Action: We have now re-written the text to make the methodology more clear. It now reads: "Community metabolism (gross primary production, community respiration and net community production) was determined from changes in oxygen over a 24h 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 day 3, 4, 8, and 15 of the experimental period. To avoid the depletion of the water in the mesocosms, measurements on day three were performed in only one of the two duplicate mesocosms for each treatment, measurements on days 4 and 8 are based on both duplicate mesocosms and those on day 15 were based on pooled samples from both duplicates. Isfjorden Communities were sampled in each of the replicate mesocosm 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 hours in “dark” and “light” and exposed to the same temperature and irradiance conditions as the corresponding mesocosms 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 analysed 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 concentration 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).”

Reviewer 1, Specific Comments 3: ’Because the paper is based on the balance between heterotrophic and autotrophic processes, it would be highly desirable to characterize the heterotrophic and autotrophic communities. Only chlorophyll was measured, but chlorophyll is an insufficient quantity to use as a normalization parameter for characterizing community responses. Most of the heterotrophic processes will be from non-phytoplankton and it is not clear how relevant a community respiration term normalized by chlorophyll is. Particulate organic carbon would have been a much better choice for normalization parameter.’

Reply 1: We agree with Reviewer 1 that a brief characterization of the autotrophic and heterotrophic communities is desirable. Action: In response to this comment as well as in response to Reviewer 1’s General Comment 1, we have now added Table 1, which gives an overview of the bacterial abundance at the initiation of the experiment as well as throughout. Also, initial chlorophyll a concentrations are given for both experiments in Tables 2 and 3. Further characterization of the phytoplankton and microheterotroph

communities are reported in detail in a manuscript submitted by Coello-Camba, et al.

Reply 2: We understand reviewer 1’s concerns about normalizing community respiration rates by chlorophyll a, however we feel that this parameter is the only one suitable, and that it is a suitable parameter for measuring the community response here. First of all, as reviewer 2 points out in his/her Specific Comment 5, patterns of per volume rates are rather similar to pattern per chlorophyll so performing our analysis on volumetric rate would yield similar results for the threshold response. However we believe that is important to standardize rates for biomass and not just leave the normalized rates out, as suggested by Reviewer 2. The Metabolic Theory of Ecology (MTE) is based on physiological processes and refers to metabolic rates per unit biomass of the same individual (Brown et al., 2004), thus to really test our results against the MTE, we must present normalized rates. We have available data of bacterial abundance (BA) and bacterial production (BP) that we have attempted to use for biomass standardization however when standardizing rates for either of these parameters the result is no apparent relationship with temperature, which we would not expect from the MTE, suggesting that bacterial abundance and production are poor descriptor factors of respiration. Chlorophyll a normalized rates do however show a strong relationship with temperature. Indeed, these observations are comparable to those in recent global assessments, which have shown that chlorophyll a is an appropriate normalization parameter for both GPP and R, whereas other properties that could be considered to be related to CR, such as bacterial abundance, do not yield any patterns with temperature (Regaudie-de-Gioux and Duarte 2012), similar to our own observations for this experiment. This is most likely due to the inherent co-variation of both BA and BP with chlorophyll concentrations (Li et al., 2004; Lopez- Urrutia and Morán, 2007) that confounds the relationship of respiration with temperature (Lopez- Urrutia and Morán, 2007) and the fact that community respiration is constrained by the flow of organic matter from autotrophs, that is strongly correlated with chlorophyll a.

Action: We have now addresses this concern in the discussion section of the

manuscript. The text reads as follows:

"The MTE is based on physiological processes and refers to metabolic rates per unit biomass of the same individual (Brown et al., 2004). We report metabolic rates standardized for chlorophyll a to be able to test our results against metabolic theory. 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 CR nor NCP rates standardized for bacterial abundance (BA) nor bacterial production (BP). Chlorophyll-a normalized rates of NCP and CR do however show a strong relationship with temperature. Indeed, these observations are comparable to those in a recent global assessment, which has shown that chlorophyll a is an appropriate normalization parameter for both GPP and R, whereas other properties that could be in principle related to R, such as bacterial abundance, do not yield any patterns with temperature (Regaudie-de-Gioux and Duarte 2012). In this global study, rates of both GPP and R standardised by chlorophyll a do yield patterns with temperature (Regaudie-de-Gioux and Duarte 2012), similar to our own observations in this experiment. This is most likely due to the inherent co-variation of both BA and BP with chlorophyll concentrations (Li et al., 2004; Lopez- Urrieta and Morán, 2007) that confounds the relationship of community respiration with temperature (Lopez- Urrieta and Morán, 2007) and the fact that community respiration is constrained by the flow of organic matter from autotrophs, that is strongly correlated with chlorophyll a (Regaudie-de-Gioux and Duarte 2012)."

Reviewer 1, Specific Comments 4: 'The authors stated that, "When measured initially, the replicates of the Barents Sea plankton community samples were different, with one replicate acting strongly heterotrophic and the other acting autotrophic". However, somehow after that initial period, both replicates behaved the same over time. How was that possible? Something must have shifted during the initial phase of the experiment and it is important to know what it was.'

Reply: We understand Reviewer 1's concerns about the initial difference between duplicate replicates at the initiation of the experiment. Samples at the initial stage of the experiment were taken just after mixing of the water and separation into separate mesocosms took place. These initial measurements were taken before any acclimation to temperature or light had taken place. Although the measurements came from the same mixed water for each of the duplicate replicates used in each temperature treatments, the replicates from which the initial samples were taken were not continued into the experimental set up. Samples taken at day 3 after the temperature acclimation period are more representative of a time zero for each mesocosm. At this point there were no longer significant differences between the two duplicate replicates for each treatment, thus replicates were averaged together for each temperature treatment. Indeed, something shifted during the initial three days, which must be due to the thermal acclimation to the respective treatments by the community.

Reviewer 1, Specific Comments 5: 'On page 11293, the authors state that GPP is independent of temperature. However, Figure 4b appears to show that GPP is low at the lowest temperature but then increases dramatically to its peak at about 3 degrees and then declines steadily with temperature thereafter. There certainly appears to be a possible relationship to me. How was the p value of 0.50 for the relationship between GPP and T determined? Using a linear model? Clearly a non-linear model would fit these data much better.'

Reply: We appreciate the point raised by Reviewer 1. The first value in figure 4b at $\sim$1°C has no standard error estimate due to lack of viable replicates, thus we were hesitant to suggest that any relationship exists. Although the value at 1°C has no SE, we have attempted to fit a polynomial curve to the average data as presented in figure 4b and find that no significant relationship exists for either (p=0.60) most likely due to the limited degrees of freedom. We have attempted to fit both a linear and polynomial curve to the data using all data points, which also resulted in no significant relationships (linear: p= 0.14; polynomial: p= 0.35), which is most likely due to the high variance of

the data. GPP values are the result of as calculation using NCP and CR values which are directly measured and are already confounded with error, high variance in GPP estimates is likely due to the propagation of error when making calculations with these variables.

Action: We have now excluded the p-value and the linear regression reporting for the GPP trend depicted in figure 4b. We have noted in the results section that a trend cannot be determined due to lack of viable replicates and limited degrees of freedom. We have now inserted text into the results section to explain, it reads:

"GPP values at $\sim$1°C (Fig. 4b; Table 1) 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 a non-linear relationship no trend was found which is most likely due to the limited degrees of freedom and large variance"

Reviewer 1, Specific Comments 6: 'For Figure 7, the authors state that there is no significant relationship between chlorophyll and temperature, yet there is a curved line drawn through the data giving the impression that chlorophyll peaks at some intermediate temperature. This line should be removed if no significant trend exists.'

Reply: We appreciate Reviewer 1 calling attention to this erroneous trend line. We agree with Reviewer 1 that the line should be removed.

Action: We have now removed the line from Figure 7.

Reviewer 1, Specific Comments 7: 'I don't think that these experiments provide sufficient evidence to conclude that the Arctic will become net heterotrophic when water temperatures reach 5 degrees. It will depend on whether the Arctic Ocean becomes more productive in the future.'

Reply: We agree with Reviewer 1 that a complex system should not necessarily be simplified down to a single temperature based solely on this experiment.

Action: We have now toned done the text, copied below, in the discussion to reflect this concern. Also, in response to Reviewer 2's General Comment 2, we have included a section in the discussion to discuss the potential affects of loss of sea ice affecting nutrient mixing and light environment.

"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. This experiment only takes into account one variable, temperature, to determine the tipping point for metabolic balance in the Arctic Ocean, and suggests that an increase beyond 5 °C in the Arctic Ocean could lead plankton communities to become strongly heterotrophic in the Arctic summer, leading to a shift of the Arctic Ocean region from a large sink of atmospheric CO2 (Takahashi et al., 2002) to a CO2 source with further future climate warming. 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. However, planktonic metabolism will be also affected by other indirect changes associated with warming of the region, such as increased irradiance with ice loss and 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 CO2 sources."

Reviewer 1, Technical Comments 1: 'Page 11286 Line 25. This sentence makes it sound like CO2 is capable of sinking. I think the meant to say the high capability of the Arctic Ocean to act as a CO2 sink.'

Reply: We thank the Reviewer 1 for his/her close attention to technical detail.

Action: We thank Reviewer 1 for pointing out this error. The line now reads: "High biological production in this area also adds to the role of the Arctic as a significant CO2 sink (Loeng et al., 2005)."

Reviewer 1, Technical Comments 2: 'Page 11292 Line 13. Change forth to fourth; Page 11293 Line 5. Temperature was left off the slope unit – it is a change in chlorophyll per degree change in temperature. Line 8. Change adapted to acclimated. Line 16. Change patters to patterns'

Reply: We thank the Reviewer 1 for his/her close attention to technical detail.

Action: We have now corrected all errors as suggested.

Reviewer 1, Technical Comments 3: 'Page 11301. The first four values for GPP/CR in Table 1 are wrong (4.87/8.50 does not equal 11.58).' Reply: We believe Reviewer 1 misunderstood the way these values were calculated. The GPP/CR values are not wrong in the tables. They represent an average of individual GPP/CR values from each treatment and their standard errors. They are not a calculation of average GPP values divided by average CR values.

Action: We have revised the table heading to avoid confusion. It reads as follows: "Table 2. Experiment with Arctic open-water community. Temperature (±SE), Chlorophyll a (±SE), volumetric and specific NCP, CR and GPP rates (±SE), as well as GPP/CR (±SE) ratio are presented for the initial measurements (t0) as well as values averaged across 15 days of experimental treatment. * signifies number with out SE due to lack of viable replicates."

Reviewer 1, Technical Comments 4: 'Page 11302. Either the volumetric or the specific NCP for the t0 has a wrong sign. One can't be positive while the other is negative.'

Reply: We thank Reviewer 1 for his/her close attention, both values should indeed be negative.

Action: We have now corrected the error in Table 2.

Response to Reviewer 2

Reviewer 2, General Comments 1: 'A serious limitation, which cannot be fixed, is that

the authors apparently did not do a control mesocosm held at the in situ temperatures. Some of the changes they observed were undoubtedly due to simply putting water in the mesocosms.'

Reply: The lowest temperature treatment was sufficiently close (1.5 °C in the experiment compared to -1.19 °C at the time of sampling) to the in situ temperature to represent the behaviour expected. Moreover, the in situ temperature is derived from a one-time measurement and is likely to experience variability comparable to that difference over the two weeks elapsed between sampling and completion of the experiment, so it is unlikely that the experimental temperature would differ significantly from the in situ temperature if an estimate of variability of the in situ temperature was available. Furthermore, this was the closest temperature we could maintain to the in situ temperature avoiding the risk of freezing of the water in the temperature-controlled tanks, which would have lead to failure of the experiment. Indeed, we used a time series of sea surface temperature (SST) from NOAA's Climate Prediction Center (http://nomad2.ncep.noaa.gov/ncep_data/), to extract 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 ranged from -1.03- 5.68°C while the average range of temperature experienced in one year during June and July is 0.98-4.25°C, thus suggesting that these communities already experience significant temperature variability and that the temperature treatments we used encompassed a June-July variation range plus 5°C.

Actions: We now include the following text in the Methods section of the manuscript: "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 changes, such as their short temporal scales 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 mesocosms containing the communities for 10 to 15 days, imposing warming rates (°C day-1) 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/), 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 ranged from -1.03- 5.68°C while the average range of temperature experienced in 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 we 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."

Reviewer 2, General Comments 2: 'The authors make this very interesting observation that the "tipping point" when the system turns heterotrophic is about 5 °C. The specific comments below have several technical complaints about this temperature. A more general criticism is that it reduces a very complex system to a single number. I'm all for reducing complexity, but not when doing so is misleading. Perhaps the biggest problem with the paper is that it implies that the direct effect of temperature on metabolic rates will have the biggest impact on these microbial communities. This paper cannot review the entire literature on the problem, but it certainly has space for a couple paragraphs about how warming and the loss of sea ice will affect mixing and thus nutrient supplies and the light environment. Other studies have argued that these indirect effects are likely to be greater than the direct effects.'

Reply: We agree with Reviewer 2's concerns about simplifying a complex system to

a single number if doing so could be misleading. In fact, we believe that the argument possibly should be viewed the other way around, whereas much discussion has focussed on these indirect effects, such as increased irradiance and possible nutrient limitation with reduced ice cover, the direct effects of warming on plankton metabolism had not been assessed as yet. We point out that discussion of the experimental "tipping point" includes calculated standard errors. Furthermore, we have now added an explanation and formula of the model used to derive the experimental "tipping point" in the methods section in response to Reviewer 2's concerns outlined in his/her Specific Comment 7. Also, we agree that a section should be added to the discussion to discuss the potential affects of loss of sea ice affecting nutrient mixing and light environment. Whereas we agree that the experimental tipping point derived here may not apply when considering the synergies with multiple changes, the functional relationship between plankton community metabolism and temperature derived here allows models addressing the responses of Arctic communities to climate change to incorporate the temperature dependence of these processes, and important component of these models that was as yet lacking.

Action: We have included the following paragraphs in the discussion to address this concern as well as Reviewer 1's Specific Comment 7.

"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 buy Hessen et al.(2008) suggests that increasing 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 e al. (2008). Indeed, 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 °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 modelling exercises. These will require the input of functional responses between plankton communities and the various drivers involved. The experimental relationship between community metabolism and Arctic 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."

Reviewer 2, Specific Comments 1: p11290, line 16: 'What was the light intensity for the mesocosms, especially relative to the depth where the water for the mesocosms was taken? Any change in this light intensity could affect chlorophyll concentrations (photoadaptation), independent of changes in biomass, and phytoplankton production.'

Reply: We agree with reviewer 1's concerns about photoacclimation, however irradiance experienced by each treatment was the same ($\sim$90 μmol m-2 s-1). Furthermore, we feel that given the intrinsic qualities of the water and natural low incident irradiance the experimental irradiance during the experiment is with in the approximate radiation that the communities would have experienced in situ. Moreover, all tanks were exposed to the same light conditions such that resulting tendencies would not change. This reply is also elaborated in the response to Review 1's General Comment 2.

Action: As indicated in the response to Review 1's General Comment 2, we have now

added the following text to the methods to address these concerns: "The light emitted from florescent lamps was measured to be 90 μmol photons m-2 s-1 using the LI-1000 LI-COR radiation sensor. The experimental irradiance used was similar across all tanks and remained constant 24 hours a day through out 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 relative 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 experiences by a community collected at 26m depth in the open ocean during that time of year. As for the fjord water, radiation likely to be experienced at 2m depth should not have exceeded 350-200 μmol m-2 s-1 (Sakshaug et al. 2009) likely higher than the irradiance from the florescent 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."

Reviewer 2, Specific Comments 2: p11290, line 18: 'Was the gradual warming over three days done before the first measurements were taken? This warming period is not in time course data, such as those presented in Figure 3? The authors argued that it was not necessary to gradually raise the temperature of the fjord water because it was already at 6 C. But fjord communities did experience large changes in temperature in going from the in situ temperature of 6 C to as low as 1 and as high as 10 C. The authors should have had an acclimation period with these waters as they did for the Barents Sea experiment.'

Reply: Yes, the gradual warming over three days was achieved before the first mea-

surements were taken. No measurements were taken during this period, which is why they do not appear in figure 3. Also, we agree with Reviewer 2 about the gradual warming in the experiment with fjord water. Ideally both communities would have experiences a gradual warming period, however we feel that this would not have had strong effects on the on the outcome of the experiment with fjord communities. We feel that temperature acclimation in fjord community was unnecessary due to the high fluctuation of temperature felt in Svalbard fjords during the months of June and July. Using a time series of sea surface temperature (SST) from NOAA's Climate Prediction Center (http://nomad2.ncep.noaa.gov/ncep_data/), we extracted weekly average SST values for the last 2 decades for each of the sampling stations using a 1° square grid cell. We found that in the last two decades during the months of June and July the range of temperature experienced by the fjord community can be from 1.9- 7.2°C while the average range of temperature experienced in one year during June and July is 3.0-5.6°C. The difference between the experiment with the Barents Sea community and the fjord community is that 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 °C from the in situ temperature was about $\frac{1}{2}$ in the fjord community compared to that in the Barents Sea.

Action: We have now added the following text to the discussion to address this issue: "We did not detect an affect 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. 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 NOAA's 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° 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°C while the average range of temperature experienced in one year during June and July is 3.0-5.6°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 °C from the in situ temperature was about $\frac{1}{2}$ 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 here. 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)."

Reviewer 2, Specific Comments 3: 'P11291, line 17: What was the light intensity for the NCP experiments (oxygen changes in light bottles)? Was only one intensity examined?'

Reply: NCP was calculated as the change in oxygen concentration measured in light bottles. These bottles were incubated in the same tanks as the mesocosms where they were sampled and thus exposed to the same light and temperature conditions.

Action: The methods section was improved in the text so as to clarify this. It now reads: "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 hours in "dark" and "light" and exposed to the same temperature and irradiance conditions as the corresponding mesocosms from which they were sampled. Dark bottles were wrapped in black electrical tape and incu-

bated in a submerged black plastic bag while light bottles were incubated in submerged transparent plastic bags."

Reviewer 2, Specific Comments 4: 'p11292, line 5: The slope of chlorophyll versus time must also have units of time, i.e. per day.'

Reply: The slope is referring to chlorophyll verses temperature, we thank reviewer 2 for identifying this error.

Action: The units have been changed accordingly.

Reviewer 2, Specific Comments 5: 'p11293: The authors calculated NCP and CR (community respiration) per chlorophyll because they wanted to look at changes in rates independent of changes in biomass. The problem is, many non-chlorophyll-containing organisms contribute to respiration. Chlorophyll most likely does not track these other organisms (e.g. heterotrophic bacteria) very well. So, there is not much value in these per chlorophyll rates. In fact, the patterns for the per volume rates in Figure 4 look the same as the per chl rates in Figures 5 and 6. The authors could mention that they did the calculations, say the pattern is similar, without showing the results. They could do the same curve fitting thing in Figure 4 as they did in Figures 5 and 6.'

Reply: We understand reviewer 2's concerns about normalizing community respiration rates by chlorophyll a, however we feel that this parameter is the only one suitable, and that it is a suitable parameter for measuring the community response here. First of all reviewer 2 points out in this comment that patterns of per volume rates are rather similar to pattern per chlorophyll so performing our analysis on volumetric rate would yield similar results for the threshold response. However we believe that is important to standardize rates for biomass and not just leave them normalized rates out, as suggested. The Metabolic Theory of Ecology (MTE) is based on physiological processes and refers to metabolic rates per unit biomass of the same individual (Brown et al., 2004), thus to really test our results against the MTE, we must present normalized rates. In response

to Reviewere 1's Specific Comment 3, we have attempted to use bacterial abundance (BA) and bacterial production (BP) for biomass standardization however when standardizing rates for either of these parameters the result is no apparent relationship with temperature, which we would not expect from the MTE, suggesting that bacterial abundance and production are poor descriptor factors of respiration. Chlorophyll a normalized rates do however show a strong relationship with temperature. Indeed, these observations are comparable to those in recent global assessments, which have shown that chlorophyll a is an appropriate normalization parameter for both GPP and R, whereas other properties that could be considered to be related to CR, such as bacterial abundance, do not yield any patterns with temperature (Regaudie-de-Gioux and Duarte 2012), similar to our own observations for this experiment. This is most likely due to the inherent co-variation of both BA and BP with chlorophyll concentrations (Li et al., 2004; Lopez- Urrutia and Morán, 2007) that confounds the relationship of respiration with temperature (Lopez- Urrutia and Morán, 2007) and the fact that community respiration is constrained by the flow of organic matter from autotrophs, that is strongly correlated with chlorophyll a.

Action: We have now addresses this concern in the discussion section of the manuscript. The text reads as follows:

"The MTE is based on physiological processes and refers to metabolic rates per unit biomass of the same individual (Brown et al., 2004). We report metabolic rates standardized for chlorophyll a to be able to test our results against metabolic theory. 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 CR nor NCP rates standardized for bacterial abundance (BA) nor bacterial production (BP). Chlorophyll-a normalized rates of NCP and CR do however show a strong relationship with temperature. Indeed, these observations are comparable to those in a recent global assessment, which has shown that chlorophyll a is

an appropriate normalization parameter for both GPP and R, whereas other properties that could be in principle related to R, such as bacterial abundance, do not yield any patterns with temperature (Regaudie-de-Gioux and Duarte 2012). In this global study, rates of both GPP and R standardised by chlorophyll a do yield patterns with temperature (Regaudie-de-Gioux and Duarte 2012), similar to our own observations in this experiment. This is most likely due to the inherent co-variation of both BA and BP with chlorophyll concentrations (Li et al., 2004; Lopez- Urrieta and Morán, 2007) that confounds the relationship of community respiration with temperature (Lopez- Urrieta and Morán, 2007) and the fact that community respiration is constrained by the flow of organic matter from autotrophs, that is strongly correlated with chlorophyll a (Regaudie-de-Gioux and Duarte 2012)."

Reviewer 2, Specific Comments 6: P11293, line 21: 'The authors here used the GP and NCP rates per chlorophyll to talk about the autotrophic/heterotrophic balance. This is incorrect. This balance is defined by per volume rates or better, rates integrated over a water column, not the biomass- normalized rates. They should use the data in Figure 4C for the discussion here.'

Reply: We agree with reviewer 2 the rate reported in this paragraph should be the volumetric rates.

Action: We have no changed all rates in this paragraph to volumetric rates.

Reviewer 2, Specific Comments 7: P11293, line 27: Here the authors give the "mean ($\pm$SE) threshold temperature at 4.78 $\pm$ 1.26 C. They need to explain how this threshold was calculated. My guess is that they set NCP per chlorophyll to zero and solved for temperature with the logistic equation. But the logistic equation may be misleading and not the right way to analyze the data. The data seem to follow a step function. The values are roughly the same (within the errors) below 4C and then decrease abruptly to another level of values at the next tested temperature (a bit below than 6 C). There are no values in between the two temperatures following the curve of the logistic equa-

tion. So, the authors should consider other analyses which would find objectively the inflection point in their data.

Reply: We agree with Reviewer 2, the calculation for the threshold was not explained in the text. However, we did not use a simple logistic function to calculate the threshold temperatures. We actually adjusted the data by nonlinear regression to a sigmoid model function.

Action: We have now added the following text to the methods section of the manuscript: "In the two threshold responses detected data were adjusted by non-linear regression to the following sigmoid model function:

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 nonlinear regression and describe different properties of the sigmoid function. r1 and r2 are the means 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 Tp is the experimental 'tipping point' or threshold value, defined as the temperature corresponding to the center of the shifting part of the curve. An R2 value for the curve was determined as 1 minus the squared sum or the residuals divided by y minus the mean of y2."

Reviewer 2, Specific Comments 8: p11295, line 18: The authors can't use the Arrhenius equation to calculate activation energies because their day, their data of rates versus temperature clearly don't follow that equation very well at all. If the logistic equation describes changes in rates versus temperature (which I don't think it really does) how could the Arrhenius equation? If that equation is not applicable, they can't calculate activation energies with these data.

Reply: We understand Review 2's comments, but believe that this does not rule out the capacity to use the Arrhenius equation to calculate activation energy. It is true that a non linear equation better fit our data, however a linear model (R2= 0.64, p= 0.0306)

can also fit the CR data almost equally well. When fitting the Arrehenius equation, all data points were used as opposed to data averaged across the entire experiment and they were natural log transformed and significantly (p= 0.004) negatively correlated with 1/kT (R2= 0.22). In addition, the parameterisation using the Arrhenius equation is important, because it allows for the inclusion of these experimentally determined temperature dependences in models evaluating the response of Arctic plankton communities to climate change.

Reviewer 2, Specific Comments 9: 'p11297, line 2: I don't buy this argument that Arctic "communities" (the relevant organisms are bacteria, which may be helpful to say) are more sensitive to change because DOC pools are large in the Arctic. DOC pools are large because of the input of refractory organic carbon from terrestrial sources. The authors have to also argue that this DOC somehow becomes more labile with increasing temperatures.'

Reply: We thank reviewer 2 for his/ her comment and have now further explained and elaborated the argument

Action: The text now reads: "This as well as previous short term experiments (Vaquer-Suyner 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 polar 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 Arctic community, as Arctic communities are characterized by a large threshold for GPP (3.84 μmol O2 L-1 day-1; Vaquer-Suyner, R., unpublished data) much higher than that of Southern Ocean communities (2.05 μmol O2 L-1 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 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. Recent experimental work also suggests that the increased substrate availability amplifies the effect of temperature on bacterial metabolism (Kritzberg et al., 2010)."

Reviewer 2, Specific Comments 10: 'Table 1 and 2: It seems that only one light intensity was examined, so it's not clear how the authors can calculate an integrated rate and present those values here in these tables. Regardless, nothing new is learned with those rates. They just make the table more complicated than necessary. My reservations about the per chl values were mentioned already.'

Reply: We believe that Reviewer 2 has misunderstood the values represented in Tables 1 and 2. Rates represented are per volume (i.e. volumetric) rates as well as chlorophyll a normalized rates. We did not attempt to calculate any integrated rates. As for Reviewer 2's reservations about chlorophyll a normalized rates, we have addressed this concern in the response to Reviewer 2's Specific Comment 5 as well as Reviewer 1's Specific Comment 3. We argue that it is necessary to include biomass normalized rates to test our results against the Metabolic Theory of Ecology which describes physiological processes and refers to rates per unit biomass.

Reviewer 2, Specific Comments 11: Figure 1: This figure should be deleted. It's not needed for this mesocosm experiment. The authors can give the lat-lon in the Methods for readers who need to find out where the water was taken.

Reply: We agree with Reviewer 2, this figure is unnecessary and can be replaced with the latitudes and longitudes in the Methods section.

Action: We have now excluded Figure 1 from the manuscript and added the latitudes and longitudes of the sampling sites in the Methods section.

Reviewer 2, Specific Comments 12: Figure 3: The data in this figure are difficult to see,

unless I blow it up on my screen by 200%. The data points, not just lines, should be given.

Reply: We appreciate Reviewer 2's criticism of this figure and we agree that the data points are difficult to see

Action: We have now re-drawn figure 3 increasing the contrast between the lines and the data points so that the individual data points are clearly visible.

Reviewer 2, Specific Comments 13: Figure 5 and 6: These should be deleted and replaced with the per volume equivalents for reasons discussed above. The vertical dashed lines need to be explained.

Reply: We understand Reviewer 2's views about the chlorophyll a normalized rates. We have previously addressed these concerns in the responses to both Reviewer1's Specific Comment 3 and Reviewer 2's Specific Comment 5. We argue that it is necessary to include biomass normalized rates to test our results against the Metabolic Theory of Ecology (MTE) which describes physiological processes and refers to rates per unit biomass. While we can apply the same sigmoid model function to the volumetric data we still feel that chlorophyll a normalized data better explain the trends we have found and allow for better relation to the MTE, as such, we have not re-drawn the figures with the volumetric data as suggested by Reviewer 2. However, we agree with Reviewer 2 that the vertical dashed lines in figure 5 and 6 need be explained.

Action: We have now amended the figure legends of figures 5 and 6 to explain the vertical dashed lines. They now read: "Figure 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 μmol O2 μg Chl a-1 day-1 at lower temperatures to a mean rate of 7.71 $\pm$ 0.74 μmol O2 μg Chl a-1

day-1 at warmer temperatures. ... Figure 5. The relationship between the mean net community production (NCP) rate of the Barents Sea plankton community along the experiment and 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 μmol O2 μg Chl a-1 day-1 at lower temperature to a mean rate of -5.52 $\pm$ 1.05 μmol O2 μg Chl a-1 day-1 at warmer temperatures."

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