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Continuous daylight in the high-Arctic summer supports high plankton respiration rates compared to those supported in the dark

Elena Mesa¹, Antonio Delgado-Huertas¹, Paloma Carrillo-de-Albornoz², Lara S. García-Corral³, Marina Sanz-Martín^{3,4}, Paul Wassmann⁵, Marit Reigstad⁵, Mikael Sejr⁶, Tage Dalsgaard⁶ & Carlos M. Duarte^{2,6}

Plankton respiration rate is a major component of global CO₂ production and is forecasted to increase rapidly in the Arctic with warming. Yet, existing assessments in the Arctic evaluated plankton respiration in the dark. Evidence that plankton respiration may be stimulated in the light is particularly relevant for the high Arctic where plankton communities experience continuous daylight in spring and summer. Here we demonstrate that plankton community respiration evaluated under the continuous daylight conditions present *in situ*, tends to be higher than that evaluated in the dark. The ratio between community respiration measured in the light (R_{light}) and in the dark (R_{dark}) increased as the 2/3 power of R_{light} so that the $R_{\text{light}}:R_{\text{dark}}$ ratio increased from an average value of 1.37 at the median R_{light} measured here ($3.62 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$) to an average value of 17.56 at the highest R_{light} measured here ($15.8 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$). The role of respiratory processes as a source of CO₂ in the Arctic has, therefore, been underestimated and is far more important than previously believed, particularly in the late spring, with 24 h photoperiods, when community respiration rates are highest.

Community respiration is the process responsible for the degradation of organic matter by organisms to extract energy to support biological processes in the ecosystem and provides, therefore, an integrated assessment of the energy requirements of the ecosystem¹. Oceanic respiration, estimated to release 66 Gt C year⁻¹ globally, is one of the main elements of the carbon flux in the biosphere², but remains the least constrained term in most models of metabolism, gas exchange and carbon mass balance in the ocean^{3,4}.

Our understanding of the respiration of plankton communities is also limited by the fact that most respiration rates have been evaluated using bulk oxygen consumption rates evaluated in the dark, thereby assuming respiration in the dark to be equivalent to that in the light⁴. However, published reports suggest that respiration in the light might be higher than that in the dark⁵⁻⁷, so current estimates of community respiration of plankton communities may be underestimated.

The severity of the bias involved in the assumption that community respiration in the dark equals that in the light involved in most estimates of plankton community respiration, depends on the photoperiod the community experiences. This shows the broadest range in the high Arctic, where there is an extended period of darkness in fall and winter, where darkness prevails, and an extended period of continuous daylight in spring and summer, when any differences between respiration in the dark and that in the light will have the highest impact on the

¹Instituto Andaluz de Ciencias de la Tierra, CSIC-UGR, Avda. de las Palmeras 4, 18100, Armilla, Spain. ²King Abdullah University of Science and Technology (KAUST), Red Sea Research Center (RSRC), Thuwal, 23955-6900, Saudi Arabia.

³Instituto Mediterráneo de Estudios Avanzados (IMEDEA), CSIC-UiB, Miquel Marqués 21, 07190, Esporles, Spain.

⁴Facultat de Geologia, Universitat de Barcelona, Barcelona, Spain. ⁵Institute of Arctic and Marine Biology, UiT The Arctic University of Norway, N-9037, Tromsø, Norway. ⁶Arctic Research Centre, Department of Bioscience, Aarhus University, Aarhus, Denmark. Correspondence and requests for materials should be addressed to E.M. (email: emesacano@gmail.com)

| Svalbard | | | | | | | | | | | | | | | |
|-------------|-------------------|--------|------|-------|----|--------------------|--------|------|-------|----|------------------------------------|--------|------|-------|----|
| | R_{dark} | | | | | R_{light} | | | | | $R_{\text{light}}:R_{\text{dark}}$ | | | | |
| | mean \pm SE | median | min | max | N | mean \pm SE | median | min | max | N | mean \pm SE | median | min | max | N |
| Surface | 2.50 \pm 0.42 | 2.06 | 0.21 | 8.25 | 28 | 3.18 \pm 0.64 | 2.68 | 0.09 | 9.56 | 20 | 2.87 \pm 1.08 | 1.40 | 0.03 | 17.88 | 18 |
| 20% PAR | 2.68 \pm 0.72 | 1.66 | 0.12 | 17.69 | 25 | 6.67 \pm 0.93 | 6.59 | 0.76 | 13.35 | 19 | 7.66 \pm 3.31 | 2.06 | 0.57 | 52.51 | 18 |
| DCM | 2.40 \pm 0.49 | 1.64 | 0.10 | 11.52 | 28 | 5.65 \pm 0.92 | 4.01 | 0.44 | 15.80 | 27 | 5.56 \pm 1.84 | 2.48 | 0.24 | 39.80 | 24 |
| Overall | 2.52 \pm 0.31 | 1.76 | 0.10 | 17.69 | 81 | 5.20 \pm 0.52 | 3.97 | 0.09 | 15.80 | 66 | 5.38 \pm 1.26 | 1.85 | 0.03 | 52.51 | 60 |
| Young Sound | | | | | | | | | | | | | | | |
| Surface | 0.30 \pm 0.09 | 0.25 | 0.03 | 0.89 | 9 | 0.51 \pm 0.24 | 0.68 | 0.12 | 0.73 | 3 | 2.27 \pm 2.09 | 0.82 | 0.32 | 5.67 | 3 |
| 20% PAR | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| DCM | 0.34 \pm 0.10 | 0.29 | 0.06 | 0.77 | 7 | 0.02 \pm 0.01 | 0.02 | 0.01 | 0.04 | 3 | 0.12 \pm 0.04 | 0.14 | 0.05 | 0.17 | 3 |
| Overall | 0.32 \pm 0.06 | 0.27 | 0.03 | 0.89 | 16 | 0.27 \pm 0.15 | 0.08 | 0.01 | 0.73 | 6 | 1.20 \pm 0.99 | 0.25 | 0.05 | 5.67 | 6 |
| Overall | | | | | | | | | | | | | | | |
| Surface | 1.96 \pm 0.35 | 1.22 | 0.03 | 8.25 | 37 | 2.83 \pm 0.59 | 2.55 | 0.09 | 9.56 | 23 | 2.78 \pm 0.94 | 1.37 | 0.03 | 17.88 | 21 |
| 20% PAR | 2.68 \pm 0.72 | 1.66 | 0.12 | 17.69 | 25 | 6.67 \pm 0.93 | 6.59 | 0.76 | 13.35 | 19 | 7.66 \pm 3.31 | 2.06 | 0.57 | 52.51 | 18 |
| DCM | 1.99 \pm 0.41 | 1.26 | 0.06 | 11.52 | 35 | 5.09 \pm 0.88 | 3.97 | 0.01 | 15.80 | 30 | 4.96 \pm 1.66 | 1.18 | 0.05 | 39.80 | 27 |
| Overall | 2.16 \pm 0.27 | 1.47 | 0.03 | 17.69 | 97 | 4.78 \pm 0.50 | 3.62 | 0.01 | 15.80 | 72 | 5.00 \pm 1.15 | 1.57 | 0.03 | 52.51 | 66 |

Table 1. Mean \pm standard error (SE), median, range and number of estimates (N) for volumetric ($\mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$) rates (respiration in the dark and in the light), and for the ratio between both.

estimates. The robust assessment of community respiration in the Arctic is particularly important, as community respiration has been predicted to rise with future Arctic warming^{8–10}. Yet, the bias introduced by the assumption that community respiration in the dark equals that in the light in the Arctic summer has not yet been assessed. Here we evaluate plankton community respiration rates in the photic zone of the Arctic ocean along several cruises conducted in the spring/summer period in the European Sector of the Arctic Ocean, during 2012, 2013 and 2014. In particular, we test the null hypothesis that community respiration rate in the dark equals that in the light. We did so by calculating respiration rate using oxygen consumption in the dark, evaluated by high-precision Winkler titration, and estimating community respiration in the light as the difference between gross primary production (GPP¹⁸O), evaluated with the ¹⁸O method and net community production (NCP), evaluated from bulk oxygen mass balance, of communities incubated under ambient incoming irradiance.

Results

Plankton community respiration varied three orders of magnitude among communities, and was significantly higher in the communities sampled in the Svalbard region compared to those sampled in Young Sound (Kruskal-Wallis test, $P < 0.01$), both when measured in the light and in the dark (Table 1). Mean monthly community respiration rates in the Svalbard region were highest in April and lowest in August (Fig. 1), although these differences were only significant for respiration rates measured in the light (Kruskal-Wallis test, $P = 0.013$), when rates measured in August were significantly lower than those measured in April and May (Dunn's test, $P < 0.05$), but not June (Dunn's test, $P = 0.27$). The statistical significance of seasonal differences could not be tested for the communities examined in Young Sound (Fig. 1), due to the limited number of estimates available and the fact that respiration rates in Young Sound were not evaluated in the spring, when the area is still fully covered by sea ice.

Community respiration rates in the light differed with depth (Kruskal-Wallis test, $P = 0.0015$, Fig. 2A,B), with the respiration rate in the light in communities sampled at the depth receiving 20% of PAR (photosynthetically active radiation) being significantly higher (Dunn's test, $P = 0.0014$) than those sampled at the depth of chlorophyll maximum, DCM, and surface samples having the minimum mean respiration rate in the light among the three depths (Table 1). In contrast, community respiration in the dark did not differ with depth (Kruskal-Wallis test, $P = 0.53$), with comparable mean values across depths (Table 1 and Fig. 2C,D).

Community respiration rates evaluated in the light and in the dark differed consistently (Wilcoxon signed rank test, $p < 0.0001$), with respiration rates in the light tending to be greater than those measured in the dark (Fig. 3A). The difference between R_{light} and R_{dark} did not differ significantly with depth (Kruskal-Wallis test, $P = 0.19$), but was greatest in May, when R_{light} tended to be much higher than R_{dark} , compared to a smaller absolute difference in June and August (Fig. 1, Kruskal-Wallis test, $P = 0.0085$; Dunn's test, $P < 0.05$). Closer examination showed that community respiration rates evaluated in the light and in the dark differed significantly for the communities evaluated in the Svalbard region (Wilcoxon signed rank test, $p < 0.001$), but not so for those in Young Sound (Wilcoxon signed rank test, $p = 0.22$), where community respiration rates were consistently low. The ratio $R_{\text{light}}:R_{\text{dark}}$ varied three orders-of-magnitude across communities (Table 1), and increased significantly ($R^2 = 0.50$, $P < 0.001$) in communities showing high respiration in the light (Fig. 3B). The fitted regression equation showed that the ratio $R_{\text{light}}:R_{\text{dark}}$ was scaled to the 2/3 power of R_{light} (Fig. 3B), so that the R_{light} and R_{dark} were similar for R_{light} of the order of $1 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$, but R_{light} was four-fold greater than R_{dark} for high R_{light} rates of the order of $10 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$ (Fig. 3B).

The difference between community respiration rates evaluated in the light and in the dark increased significantly with increasing GPP¹⁸O rates (Fig. 4), with no significant difference in community respiration rates at GPP¹⁸O rates $< 10 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$ (Fig. 4).

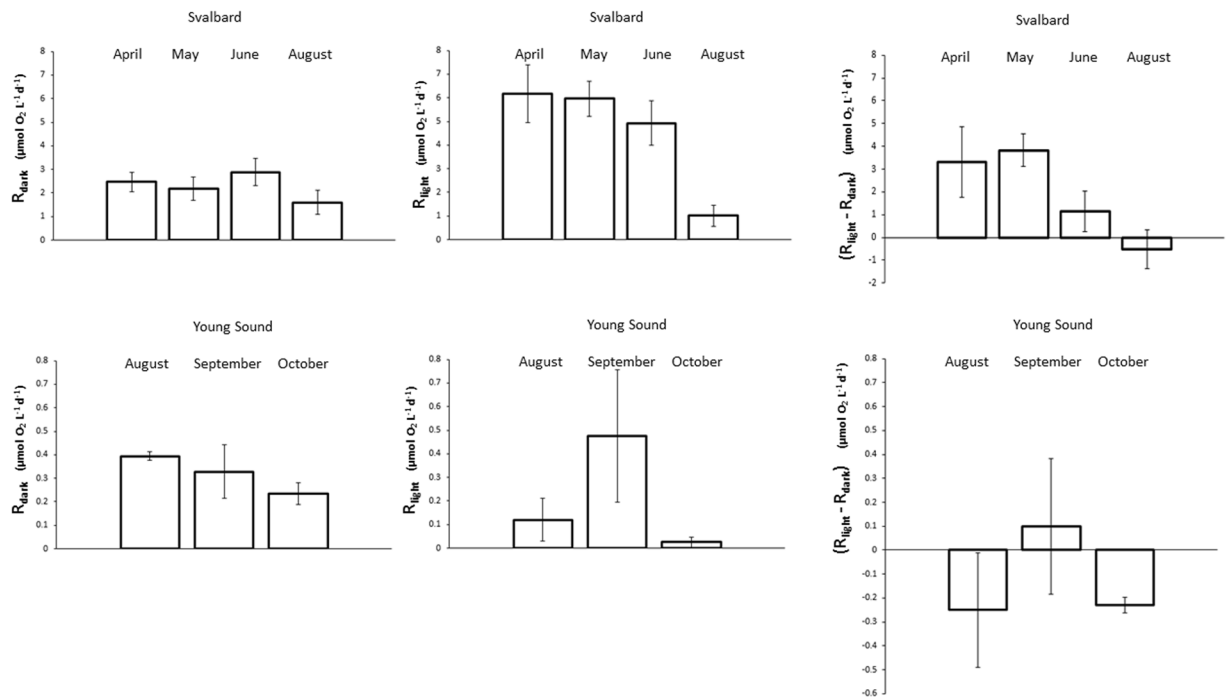


Figure 1. Mean (\pm SE) monthly respiration in the dark (R_{dark}), respiration in the light (R_{light}), and the difference between both ($R_{\text{light}} - R_{\text{dark}}$) in the Svalbard region and Young Sound.

Discussion

The respiration of plankton communities is a major component of the carbon budget of the oceans². Yet, estimates of community respiration rates are much less frequent than those of primary production, particularly in the Arctic Ocean where community respiration rates had thus far been evaluated only in the dark^{11–15}. We found that R_{light} tended to be significantly higher than R_{dark} across the Arctic plankton communities tested. This is consistent with the majority of reports concluding that respiration in the light tends to be greater than that in the dark^{6, 7, 16–18}, involving all, except two^{19, 20}, published reports comparing such rates. However, the underestimation of respiration rates derived from measuring respiration rates in the dark may be particularly acute for Arctic plankton communities, which experience a 24-hour photoperiod during much of the year.

The estimates of R_{light} provided here represent the first assessment of respiration under ambient solar radiation for Arctic plankton communities. Previous comparison of R_{light} and R_{dark} for polar plankton communities derived from the Southern Ocean, where two studies had been conducted^{6, 18}. These studies also concluded that respiration in the light tends to be greater than that in the dark. The mean vertically-integrated $R_{\text{light}}:R_{\text{dark}}$ ratio was reported to be 1.95 in a summer cruise around 76 °S in the Ross Sea⁶; and to range between 1.2 and 2 for spring and summer, respectively, in a transect from 52 to 70 °S across the Antarctic Polar Front region¹⁸. The median $R_{\text{light}}:R_{\text{dark}}$ ratio in our study was 1.57, within the range of values reported for Southern Ocean plankton communities^{6, 18}. We found, however, that the $R_{\text{light}}:R_{\text{dark}}$ ratio increased as the 2/3 power of R_{light} so that the $R_{\text{light}}:R_{\text{dark}}$ increased from an average value of 1.37 at the median R_{light} measured here ($3.62 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$) to an average value of 17.56 at the highest R_{light} measured here ($15.8 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$).

Estimates of gross primary production obtained directly using the ^{18}O method tend to be greater than those calculated as the difference between NCP and R_{dark} , which comprise all of the estimated gross primary production thus far available for the Arctic Ocean^{11–15}. This was interpreted to indicate that R_{light} tends to be higher than R_{dark} ²¹, as confirmed by our results. Indeed, when the estimates of R_{dark} obtained here are corrected for the underestimation derived from estimating this rate in the dark by multiplying them by the $R_{\text{light}}:R_{\text{dark}}$ ratio predicted from the regression equation in Fig. 3, the NCP predicted as the difference between GPP^{18}O and this corrected R estimate is strongly consistent with the observed NCP (Fig. 5). Hence, whereas reported NCP for plankton communities in the Arctic Ocean^{11–15} are robust, previous estimates of gross primary production and respiration rates are underestimates. The reason for this is that the assumption, rejected by our experimental results, that R_{light} equals R_{dark} is particularly inadequate for the high Arctic, where plankton communities do not experience darkness within the photic zone during the 24 h photoperiods in spring and summer.

It has been suggested that R_{light} rates are higher than those in the dark due to the contribution of autotrophic metabolic processes, such as photoenhanced mitochondrial respiration, chlororespiration, photorespiration and/or the Mehler reaction²². Autotrophic respiration has also been proposed to dominate community R during the pre-bloom and bloom phases of the seasonal cycle in the Southern Ocean²³. These observations are consistent with the observation that the difference between R_{light} and R_{dark} estimates increased with increasing gross primary production (Fig. 4). Figure 4 also shows that for $\text{GPP}^{18}\text{O} < 10 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$, most R_{dark} tend to be higher than

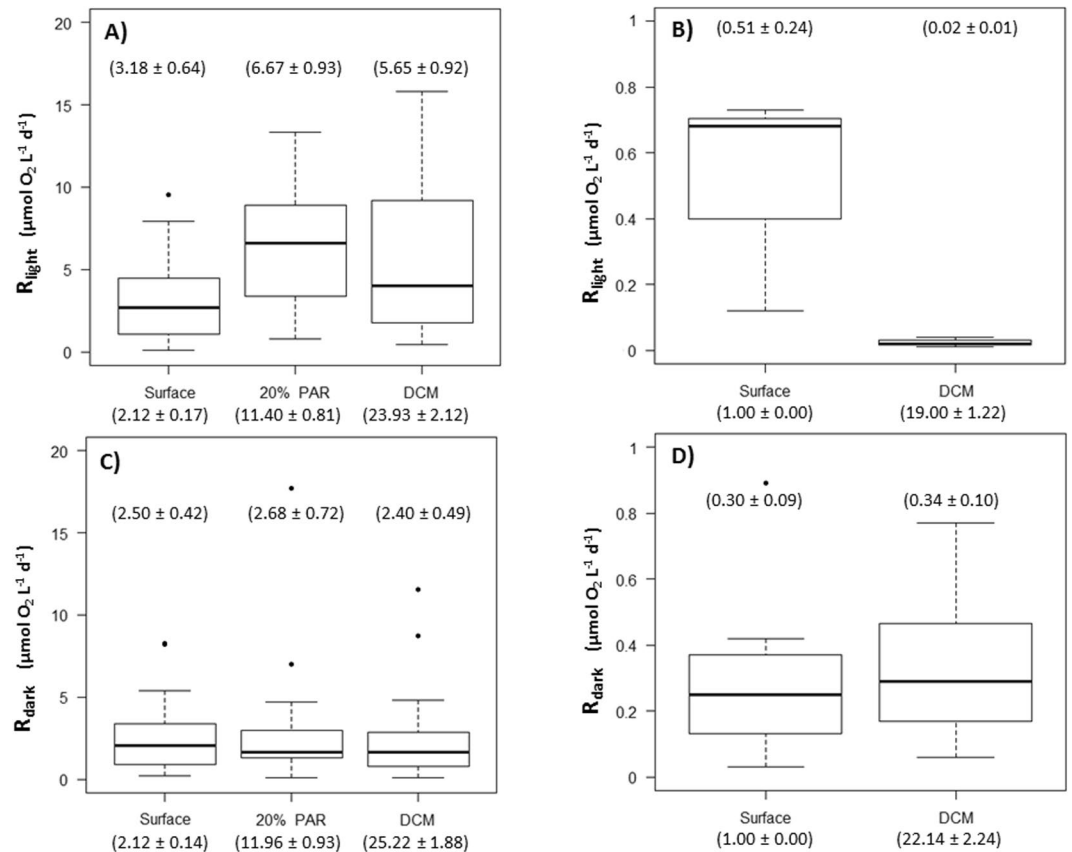


Figure 2. Box-and-Whisker plots showing the distribution of community respiration (**A**) in the light in Svalbard, (**B**) in the light in Young Sound, (**C**) in the dark in Svalbard and (**D**) in the dark in Young Sound, for the depths sampled (mean depth \pm SE in parentheses). The boxes show the median rate plus the lower (25%) and upper (75%) quartiles, the whiskers indicate 1.5 times the interquartile range, and the points show outliers. Numbers above the boxes are the mean rates.

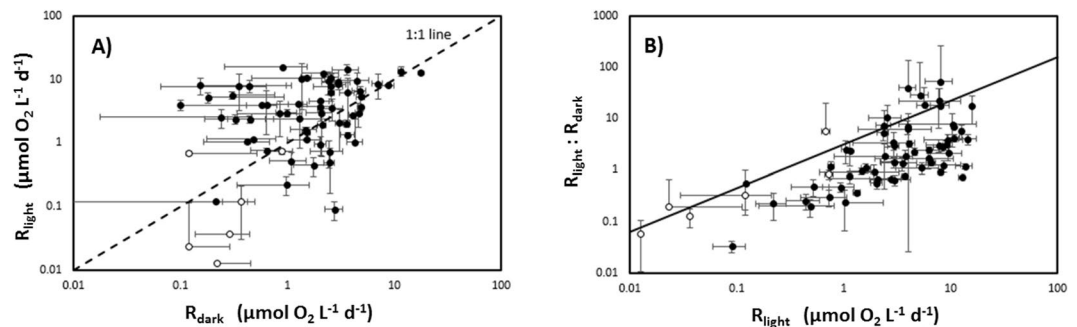


Figure 3. The relationship between (**A**) respiration in the dark (R_{dark}) and that in the light (R_{light}), and (**B**) respiration in the light (R_{light}) and the ratio between respiration in the light and that in the dark ($R_{\text{light}}:R_{\text{dark}}$) in the Svalbard region (black symbols) and in Young Sound (white symbols). The solid line in (**B**) shows the fitted regression line $\text{Ln}(R_{\text{light}}:R_{\text{dark}}) = -0.02 + 0.68 * \text{Ln}(R_{\text{light}})$ ($\mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$), $R^2 = 0.50$, $P < 0.001$. Error bars are the SE.

R_{light} , reflecting that metabolic processes supporting R_{light} may be specially enhanced over a GPP^{18}O threshold of $10 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$, below which dark processes prevail.

In conclusion, the results presented show that respiration in the light tends to be much higher than that in the dark in productive communities, whereas both values are low in communities with low productivity. Periods of high production, particularly the spring bloom, contribute disproportionately to the annual metabolic budget of the Arctic Ocean¹¹. Estimates of net community production in the Arctic^{11,15}, which are derived from incubations in the light, are not affected by the bias introduced by dark incubations to estimate respiration rates. However,

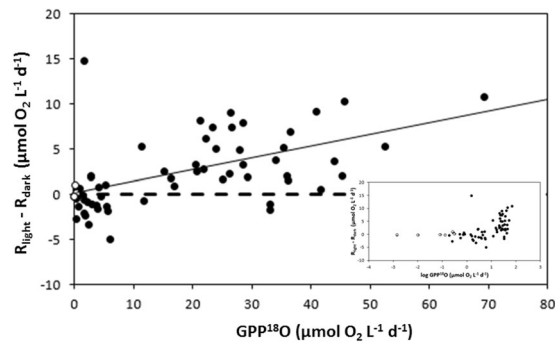


Figure 4. The relationship between gross primary production (GPP^{18O}) and the difference between community respiration in the light and that in the dark ($R_{light} - R_{dark}$) in the Svalbard region (black symbols) and in Young Sound (white symbols). The insert shows the same figure with log-transformed gross primary production ($\log GPP^{18O}$), to allow examination of the values at low GPP^{18O} values.

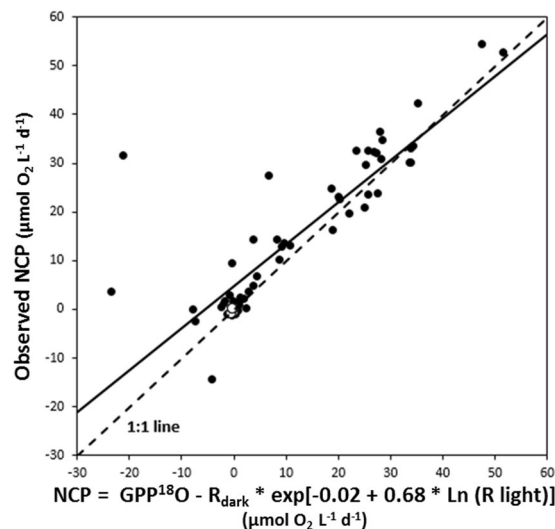


Figure 5. The relationship between NCP calculated as $GPP^{18O} - R_{dark} * \exp[-0.02 + 0.68 * \ln(R_{light})]$ and observed net community production (NCP) in the Svalbard region (black symbols) and in Young Sound (white symbols). The solid line shows the fitted regression equation: $y = 0.86 (\pm 0.06) \times + 4.81 (\pm 1.19)$ ($R^2 = 0.74$, $p < 0.001$, $N = 66$).

these procedures would have led to underestimate the gross primary production of Arctic communities in the summer, where this was derived as the difference between NCP and respiration rates.

Methods

Plankton community respiration (R) in the dark and under ambient irradiance conditions was evaluated in both sides of the Greenland Sea, the western margin of Svalbard region and Young Sound fjord, in NE Greenland (Fig. 6). R was evaluated in five cruises in Svalbard, in 2012 (from 9 to 16 June), 2013 (27 April to 4 May; and 6 to 14 June) and 2014 (16 to 27 May; 8 to 14 August). Four stations were sampled in Young Sound in each of August, September and October 2014.

In the cruises conducted in the Svalbard region, water samples were collected using a Rosette sampler system fitted with 5 L Niskin bottles and a calibrated CTD, at three different depths: surface (2.12 ± 0.13 m), DCM (24.56 ± 1.63 m), which receives, on average, 1% of the incident irradiance, and at an intermediate depth (13.56 ± 0.93 m) between surface and DCM, receiving 20% of the incident radiation on the surface. Only two depths (surface and DCM) were sampled in Young Sound, where temperature and salinity were collected from a CTD cast, and water samples were collected with 5 L Niskin bottles.

Plankton community respiration rates were estimated using two methods: (1) respiration in the dark (R_{dark}) was assessed by evaluating oxygen consumption after incubation of samples in the dark, by high-precision Winkler titration^{24, 25} in Svalbard cruises and by visual end-point detection²⁶ in Young Sound, and (2) respiration in the light (R_{light}) was assessed as the difference between gross primary production (GPP^{18O}), evaluated using H_2^{18O} additions²⁷, and net community production (NCP), evaluated from oxygen changes resolved using high-precision Winkler titration^{24, 25} in Svalbard and using visual end-point detection²⁶ in Young Sound, of

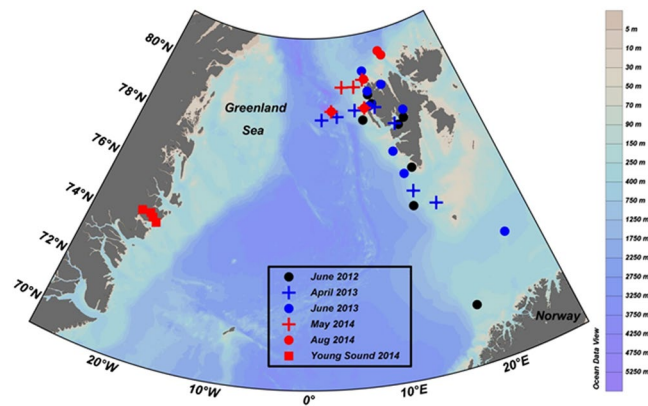


Figure 6. Location of the stations sampled. Map created with Ocean Data View software (version 4.6.3, <http://odv.awi.de/>).

samples incubated under the incident solar radiation. Daily R_{light} rates were corrected for those communities that were exposed to less than 24 hours of light (only five communities in September and October in Young Sound). The rates determined based on dissolved oxygen changes in Young Sound, 12 out of 147 respiration rates reported here, carry considerable error, as the titration end point was determined visually, as a titration was not available. The precision obtained (expressed as SD of average in %) for O_2 concentration measurements with this procedure was 0.15%.

Per each depth, a set of seven replicated 100-mL narrow-mouth Winkler bottles was fixed immediately to evaluate the initial oxygen content, and two sets were incubated under light and dark conditions for 24 hours. Incubations were done in water baths on deck (maintained at the *in situ* temperature of the surface water, $\pm 1^\circ\text{C}$, through continuous water flow from the surface) in Svalbard; and *in situ* in Young Sound. Neutral screens were used to reduce incident irradiance as to mimic the light environment *in situ*. Dissolved oxygen concentrations were determined by automated high-precision Winkler titration with a potentiometric end-point Metrohm 808 Titrando²⁸ in the Svalbard communities and using starch as indicator for end-point detection in the Young Sound communities. R_{dark} and NCP were calculated from changes in dissolved oxygen concentrations, before and after incubation of samples under “dark” and “light” conditions, respectively, for 24 h in Svalbard and 48 h in Young Sound. As a consequence of the low rates and low precision of dissolved oxygen determination in Young Sound, the communities were incubated for 48 h, thereby experiencing changes that could be resolved with the techniques used there. On the other hand, long incubations may increase the risk of artifacts derived from bottle effects. Rates are reported in $\mu\text{mol } O_2 \text{ L}^{-1} \text{ d}^{-1}$ and standard errors were calculated using error propagation. In order to compare the $R_{\text{light}}:R_{\text{dark}}$ ratios obtained here with those observed in the past, we surveyed the literature for results reported in the past^{6,7,16–18,27}. An extreme value reported by one of the studies⁷ (ratio $R_{\text{light}}:R_{\text{dark}} = 19$), 8-fold higher than the rest, was excluded from the comparison.

For evaluation of $GPP^{18}\text{O}$, eight 12-ml glass vials were filled per depth. Four replicate samples were immediately fixed (biological activity stopped) with $80 \mu\text{l}$ of saturated HgCl_2 solution for later analysis of initial $\delta^{18}\text{O}(O_2)$ values, and stored upside down in darkness. The other four vials, containing beads inside to ensure mixing, were spiked with $80 \mu\text{l}$ and $200 \mu\text{l}$ of 98% H_2^{18}O in Svalbard and Young Sound communities, respectively. After being closed, these spiked vials were immediately agitated, to ensure that H_2^{18}O was homogeneously distributed inside the vial. The spiked samples were incubated together with the Winkler bottles under “light” conditions. After the 24-hour incubation, vials were fixed with $80 \mu\text{l}$ of saturated HgCl_2 solution and stored upside down in darkness.

At the stable isotope laboratory, a 4-ml headspace was generated in each vial, by flushing with a helium flow. The vials were left for equilibration at room temperature for 24 hours. The $\delta^{18}\text{O}$ of dissolved oxygen in the headspace was measured in a Finnigan GasBench II attached to a Finnigan DeltaPlusXP isotope ratio mass spectrometer, with precision better than 0.1%. $\delta^{18}\text{O}-\text{H}_2\text{O}$ of spiked samples was measured in a liquid water isotope analyzer (Los Gatos Research), with precision of 0.1%, and $GPP^{18}\text{O}$ was calculated²².

Statistical analysis were based on non-parametric tests (Wilcoxon signed rank test, Kruskal-Wallis test and Dunn’s test), as the data were skewed and not normally distributed, or log-transformed to homogenize the variance prior to fitting least squares linear regression equations.

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Author Contributions

C.M.D., P.W., M.R., M.S., and T.D. designed the experiments. E.M., P.C.A., L.S., M.S.M., M.S. and T.D. performed the field work. E.M. and A.D. measured the ¹⁸O values. E.M. and C.M.D. analyzed the data and wrote the manuscript. All authors contributed to the writing and editing of the manuscript.

Additional Information

Competing Interests: The authors declare that they have no competing interests.

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