Universidad de Granada

PhD thesis:

ASSESSMENT OF PLANKTONIC METABOLISM IN THE ARCTIC AND SUBTROPICAL OCEANS BY THE ^{18}O METHOD

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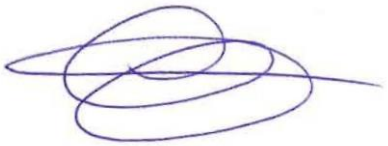

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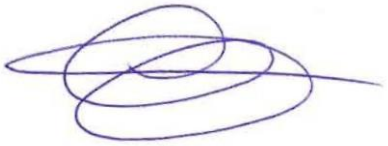

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Contents

General abstract

The metabolism of oceanic phytoplankton, consisting of gross primary production (GPP) and respiration (R), regulates biogeochemical cycles and climate. Plankton photosynthesis is responsible for half of the world primary production and fuels the marine food web and the biological CO_2 pump, which makes oceanic phytoplankton primary production a fundamental process at the global scale. However, there is a current debate over the estimations coming from different methods to assess GPP. The ^{18}O method is the most accurate one and the only one that measures GPP directly, but has been used in very few studies (less than twenty, to the best of our knowledge) which are not representative of the global ocean. The other metabolic process, plankton respiration, is a major component of global CO_2 production. At the ecosystem level, respiration integrates so many aspects of the functioning, that long-term shifts in respiration may provide the best warning system for global change. However, global respiration data are much more scarce than primary production data, there are around 20,000 estimates of oceanic production for each estimate of respiration rate. In addition, respiration is usually measured in dark conditions, assuming that it is equivalent to respiration in the light, which has not been proved and might thus be biasing global models of gas fluxes. The main aim of this PhD thesis is to go a step further in the global assessment of planktonic metabolism, by evaluating $GPP^{18}O$ (i.e. GPP measured with the ^{18}O method) in the Arctic and in the tropical and subtropical ocean, and by testing the hypothesis of equal respiration in the dark and in the light.

We assessed $GPP^{18}O$ in the context of three different studies: in the tropical and subtropical ocean (Malaspina Expedition); in the European Arctic Sector (to the west of Svalbard islands); and in a fjord in the east coast of Greenland (Young Sound). We evaluated $GPP^{18}O$ in 84 stations across the Atlantic, Pacific and Indian Oceans, in the framework of the 2010 Malaspina Expedition, occupying four of the five subtropical gyres. The mean (± SE) $GPP^{18}O$ was 0.60 ± 0.06 µmol O_2 L^{-1} d^{-1} (range 0.01 - 11.49). This mean rate is lower than previously published estimates, suggesting that mean values previously inferred from existing data did not characterize the global ocean adequately. The Pacific Ocean was more heterogeneous, in relation to $GPP^{18}O$ rates, than the Atlantic and Indian Oceans, which results from very high rates in the Northern compared to very low rates in the Southern Pacific Ocean.

The mean $GPP^{18}O$ for the European Arctic Sector was 14.00 ± 1.49 µmol O_2 L^{-1} d^{-1} (range: 0.19 - 69.15), with a strong seasonal variability. The data were consistent with estimates

by the dark-light method ($GPPO_2$). Mean $GPP^{18}O$ for Young Sound fjord was very low, 0.123 ± 0.026 µmol O_2 L^{-1} d^{-1} (range 0.001 – 0.330), which are by far the lowest estimates reported with this method to date. These low $GPP^{18}O$ rates agree well with earlier investigations suggesting that the system is net heterotrophic, requiring additional carbon input to balance mineralization.

Plankton community respiration rates were evaluated in the dark (R_{dark}) and in the light (R_{light}), in the European Sector of the Arctic Ocean (in a 24-h photoperiod during summer). The mean R_{light} (5.20 ± 0.52 µmol O_2 L^{-1} d^{-1}, range 0.09 - 15.80) was significantly higher than mean R_{dark} (2.52 ± 0.31 µmol O_2 L^{-1} d^{-1}, range 0.10 - 17.69), showing that the common assumption of equal R_{light} and R_{dark} is incorrect. The mean ratio R_{light}:R_{dark} (5.07 ± 1.15) was higher than the one reported to date (2.72 ± 0.75) in studies outside the Arctic.

Finally, we measured $\delta^{18}O$ and concentration of dissolved oxygen in Arctic waters. The mean oxygen concentration (360.98 ± 2.59 µmol L^{-1}; range 303.80 - 431.39 µmol L^{-1}) correlated well with $\delta^{18}O$ (O_2) values (mean 23.91 ± 0.12 ‰, range 21.17 - 25.83 ‰). This correlation might be explained by gross primary production, respiration and ice melting.

Resumen general

El metabolismo del fitoplancton oceánico, el balance entre producción primaria bruta (GPP) y respiración (R), regula los ciclos biogeoquímicos y el clima. La mitad de la producción primaria mundial corresponde a la fotosíntesis del plancton e impulsa la cadena alimentaria marina y la bomba biológica de CO_2, lo que hace de la producción primaria del fitoplancton oceánico un proceso clave a escala global. Sin embargo, actualmente hay un debate sobre las estimas procedentes de los distintos métodos para evaluar la GPP. El método del ^{18}O es el más preciso y el único que mide la GPP directamente, pero se ha usado en muy pocos estudios (menos de veinte estudios, según nos consta) que no son representativos del océano global. El otro proceso metabólico, la respiración planctónica, es un componente principal de la producción global de CO_2. A nivel de ecosistema, la respiración integra tantos aspectos del funcionamiento, que los cambios a largo plazo en la respiración pueden ser el mejor sistema de alerta respecto al cambio global. Sin embargo, los datos globales de respiración son mucho más escasos que los datos de producción primaria, habiendo unas 20,000 estimas de producción oceánica por cada estima de respiración. Además, la respiración se suele medir en oscuridad, asumiendo que ésta es equivalente a la respiración en la luz, lo cual no se ha probado y podría, por tanto, estar sesgando los modelos globales de flujos de gases. El principal objetivo de esta tesis es dar un paso más en la evaluación global del metabolismo planctónico, evaluando la $GPP^{18}O$ (GPP medida con el método ^{18}O) en el Ártico y en el océano tropical y subtropical, y comprobando la hipótesis de igual respiración en luz que en oscuridad.

Evaluamos la $GPP^{18}O$ en el contexto de tres estudios: en el océano tropical y subtropical (Expedición Malaspina); en el sector europeo del Ártico (al oeste de las islas Svalbard); y en un fiordo en la costa este de Groenlandia (Young Sound). Medimos la $GPP^{18}O$ en 84 estaciones abarcando el océano Atlántico, Pacífico e Índico, en el marco de la Expedición Malaspina 2010, ocupando cuatro de los cinco giros subtropicales. La $GPP^{18}O$ media (± SE) fue 0.60 ± 0.06 µmol O_2 L^{-1} d^{-1} (rango 0.01 - 11.49). Esta tasa media es más baja que las estimas publicadas hasta ahora, sugiriendo que los valores medios previamente inferidos de los datos existentes, no caracterizaban el océano global adecuadamente. El océano Pacífico fue más heterogéneo, en relación a las tasas de $GPP^{18}O$, que el Atlántico y el Índico, debido a las altas tasas en el Pacífico Norte en comparación a bajas tasas en el Sur.

La $GPP^{18}O$ media para el sector europeo del Ártico fue 14.00 ± 1.49 µmol O_2 L^{-1} d^{-1} (rango: 0.19 - 69.15), con una gran variabilidad estacional. Los datos fueron consistentes con

las estimas del método de incubaciones en luz-oscuridad ($GPPO_2$). La $GPP^{18}O$ media para el fiordo Young Sound fue muy baja, 0.123 ± 0.026 µmol O_2 L^{-1} d^{-1} (rango 0.001 – 0.330), siendo éstas, muy de lejos, las estimas más bajas dadas por este método hasta la fecha. Estas bajas tasas de $GPP^{18}O$ son coherentes con investigaciones anteriores que sugerían que el sistema es netamente heterotrófico, requiriendo una entrada de carbono adicional para equilibrar la mineralización.

La respiración de la comunidad planctónica fue evaluada en oscuridad (R_{oscura}) y en la luz (R_{luz}), en el sector europeo del océano Ártico (durante el verano con un fotoperiodo de 24 horas). La R_{luz} media (5.20 ± 0.52 µmol O_2 L^{-1} d^{-1}, rango 0.09 - 15.80) fue significativamente más alta que la R_{oscura} media (2.52 ± 0.31 µmol O_2 L^{-1} d^{-1}, rango 0.10 - 17.69), demostrando que la frecuente asunción de igual respiración en la luz y en la oscuridad, es incorrecta. El ratio R_{luz}:R_{oscura} medio (5.07 ± 1.15) fue mayor que el publicado hasta la fecha (2.72 ± 0.75) en estudios fuera del Ártico.

Finalmente, medimos la $\delta^{18}O$ y concentración de oxígeno disuelto en aguas del Ártico. La concentración media de oxígeno (360.98 ± 2.59 µmol L^{-1}; rango 303.80 - 431.39 µmol L^{-1}) se correlacionó bien con valores $\delta^{18}O$ (O_2) (media 23.91 ± 0.12 ‰, rango 21.17 - 25.83 ‰). Esta correlación podría explicarse por la producción primaria bruta, la respiración y la fusión de hielo.

GENERAL INTRODUCTION

General introduction

The ocean is a dynamic part of the biosphere, as marine biota have a key role in the recycling of organic matter, consuming and producing an important proportion of it. Phytoplankton, the microscopic flora on which all aquatic food webs are based, converts inorganic carbon to organic carbon in a process called primary production [*Marra,* 2009]- performed mainly through photosynthesis. In fact, half of the world primary production is done by plankton photosynthesis [*Field et al.,* 1998], a fundamental process that influences climate and biogeochemical cycles, because it changes the atmospheric concentration of oxygen and carbon dioxide [*Chavez et al.,* 2011].

The biological pump is the process in which the materials generated in primary production sink down out of the euphotic zone, with their later decay or deposition on the bottom of the ocean [*Chavez et al.,* 2011]. Thanks to the biological pump, the ocean has been a strong sink for CO_2 even during the industrial era, removing around 2 Gt C yr^{-1} from the atmosphere [*Siegenthaler and Sarmiento,* 1993; *Sarmiento and Gruber,* 2002].

However, if future warmer temperatures lead to stratification and a higher increase in the respiration rate than in photosynthesis, the biological pump might change [*Sarmiento et al.,* 1998; *López-Urrutia et al.,* 2006]. The prediction of 4°C warmer for the 21st century might reduce the production/respiration ratio by 16% [*Harris et al.,* 2006], shifting the metabolism of many planktonic communities to now act as a source of CO_2 to the atmosphere [*Duarte et al.,* 2011]. This imbalance might not be just punctual, as there is enough organic carbon in the ocean so as to allow an excess respiration for a few decades [*Duarte et al.,* 2011]. Moreover, because of the large fluxes that oceanic gross primary production and respiration consist of, this stimulation of CO_2 production has the potential to alter the carbon cycle even more than the anthropogenic cause, thereby contributing to warmer temperatures [*Duarte et al.,* 2011].

Lenton et al. [2008] define a tipping element as "a subsystem of the Earth system that can be switched into a qualitatively different state by small perturbations". The point at which the qualitative alteration takes place is the "tipping point" [*Lenton et al.,* 2008]. Therefore, planktonic metabolism may function as a tipping element of our planet because of the reasons mentioned above- metabolism would shift towards an increased heterotrophy, releasing to the atmosphere extra CO_2 that would intensify global warming on a continuous feedback [*Duarte et al.,* 2011].

Indeed, some evidences that the CO_2 sink role of the ocean is weakening have already been reported: recent decreases in the CO_2 absorption in the Southern Ocean, North Atlantic, and Equatorial Pacific [*Doney et al.,* 2009]; fewer communities acting as a strong carbon sink [*Waycott et al.,* 2008; *Duarte,* 2008]; or unproductive gyres expanding [*Polovina et al.,* 2008].

Therefore, accurate evaluation of ocean metabolism is essential because it regulates the global CO_2 and O_2 fluxes, and because of its potential to affect the Earth climate. Gross primary production (GPP) and respiration (R), the two processes defining planktonic metabolism, will be the focus of the different chapters in this thesis.

Metabolism rates can be assessed by a number of methods, each of them giving different estimates. This has fostered an ongoing debate within the scientific community over what method gives the most valid estimate. What follows is an analysis of the various methods available to measure primary production. After that, respiration will be commented.

Available methods to evaluate **primary production** are either *in vitro* (incubation) or *in situ* (incubation-free). *In vitro* methods measure inorganic carbon incorporation or oxygen production at discrete depths for a 24 h incubation; while *in situ* methods integrate rates for a fixed column depth (usually the surface mixed layer) and a period of time (usually weeks or months) [*Robinson and Williams,* 2005; *Regaudie-de-Gioux et al.,* 2014].

In vitro oxygen-based techniques evaluate the oxygen released in photosynthesis by measuring the change in either 1) total O_2 concentration in the incubation, and correcting it for respiration (i.e., dark-light method); or 2) $^{18}O_2$ when the sample is spiked with ^{18}O-labeled H_2O ($H_2{}^{18}O$) (i.e., ^{18}O method) [*Regaudie-de-Gioux et al.,* 2014]. *In vitro* carbon-based methods to measure primary production add ^{14}C or ^{13}C labeled bicarbonate as a tracer (i.e., ^{14}C and ^{13}C methods) and then evaluate how much tracer has been assimilated onto organic carbon (particulate or total) [*Regaudie-de-Gioux et al.,* 2014].

In situ methods assess either 1) the oxygen field based on the data provided by moored sensors [*Dickey,* 1991] or sensors placed in gliders [*Nicholson et al.,* 2008], correcting for atmospheric exchange; or 2) triple oxygen isotopes ($^{16}O_2$, $^{17}O_2$, $^{18}O_2$) to evaluate GPP by comparing the measured isotope values with the ones predicted only from atmospheric equilibrium [*Regaudie-de-Gioux et al.,* 2014].

The estimates from these *in situ* techniques are not precise because they depend on assumptions about the mixed layer and about exchange with with the atmosphere and adjacent

waters. First, these methods assume that the water column is mixed, which is questioned, as *Calleja et al.* [2013] reported variable levels of pCO_2 in the upper open ocean. Second, the estimation of the O_2 exchanged with the atmosphere has an uncertainty of 30% or higher because it depends on certain parameters [*Calleja et al.,* 2009]. Third, bubbles modify transport and exchanges, so they can significantly influence the calculations in most O_2-based *in situ* methods [*Emerson et al.,* 2008]. Fourth, with these techniques, metabolic rates are commonly integrated for the mixed layer. However, if the euphotic zone extends beyond the mixed layer (such as in the oligotrophic subtropical gyres, for instance), the metabolic rate would then be underestimated [*Duarte et al.,* 2013]. Consequently, metabolic rates evaluated by *in situ* methods carry important uncertainty [*Duarte et al.,* 2013].

Incubation techniques might experience artifacts because the community is enclosed [*Duarte et al.,* 2013]. In fact, *Calvo-Díaz et al.* [2011] suggested that metabolic rates estimated with these techniques might be biased, as the ratio of autotrophic/heterotrophic biomass decreased over the incubation. However, both autotrophs and bacteria have been reported to experience in situ diel variability [*Gasol et al.,* 1998; *Llabrés et al.,* 2011, *Loisel et al.,* 2011]. Therefore, changes reported during incubations agree with those observed in situ, so they do not necessarily reflect bias due to the confinement of the community [*Duarte et al.,* 2013].

Incubation methods do exclude the contribution of zooplankton, which implies that R is underestimated by around 3% [*Robinson and Williams,* 2005]. The light and temperature environment can also raise concern. The use of borosilicate bottles alters the light field because this material blocks UVB radiation, which is known to impact metabolic rates [*Godoy et al.,* 2012; *Agustí et al.,* 2014; *Regaudie-de-Gioux et al.,* 2014]. Regarding temperature, incubations are rarely set at the temperature found in their environment, so, providing that this is a key factor conditioning metabolism, rates might be over- or underestimated if performed at a different temperature from the one *in situ* [*Regaudie-de-Gioux et al.,* 2014].

One of the most widely used methods to assess primary production is performed through incubations: the ^{14}C method [*Steeman Nielsen,* 1952]. It consists of adding $NaH^{14}CO_3$ to the sample, and then measuring how much ^{14}C was incorporated (into particulate and total organic carbon) through photosynthesis [*Regaudie-de-Gioux et al.,* 2014]. The method has always been questioned, because of the influence of heterotrophs and the effect of respiration, among others [*Marra,* 2009]. Furthermore, other issues like international law or the risk of

contaminating the ship might prevent future use of this method [*Regaudie-de-Gioux et al.,* 2014].

The dark-light method (or bulk oxygen evolution) has been applied to evaluate primary production for around one century [*Gaarder and Gran,* 1927]. This technique calculates the difference in oxygen concentration before and after a 24-hour incubation where plankton communities are placed in dark and clear bottles, and the measurement is done by a high-precision Winkler titration with an end-point detection [*Regaudie-de-Gioux et al.,* 2014]. The difference between initial and final oxygen concentration allows to calculate R (in dark bottles) and net community production, NCP (in clear bottles). However, GPP is assessed indirectly by summing NCP and R dark, assuming equal respiration in the dark than in the light [*Regaudie-de-Gioux et al.,* 2014]. This is why the dark-light method can underestimate GPP, as respiration has been reported to be higher in the light [*Harris and Lott,* 1973].

The ^{18}O method evaluates GPP by measuring the ^{18}O produced in photosynthesis after previous enrichment of the sample with $H_2{}^{18}O$ [*Bender et al.,* 1987]. The ratio $^{18}O/^{16}O$ of the oxygen generated in photosynthesis is the same as in the water from which it came from [*Bender et al.,* 1987; *Guy et al.,* 1993], which allows GPP to be calculated by mass balance [*Bender et al.,* 1987]. The ^{18}O method has several advantages:

1) Unlike the dark-light method, it calculates GPP directly without relying on the assumption of equal respiration in the dark than in the light [*Regaudie-de-Gioux et al.,* 2014].

2) If the dark-light method is also performed for the same water samples, the ^{18}O method allows respiration in the light to be calculated as the difference between $GPP^{18}O$ (i.e. GPP assessed with the ^{18}O method) and NCP [*Bender et al.,* 1987].

3) Along the 24-hour incubation, the recycling of labeled O_2 will be weak (2%) compared with the large pool of $PO^{14}C$ that can be recycled in the ^{14}C method [*Bender et al.,* 1999].

4) In addition, unlike the ^{14}C method, the use of the ^{18}O method poses no health hazards because ^{18}O is a stable isotope.

For all the former reasons and because it is based on the fewest assumptions to evaluate the rate, the ^{18}O method has been reported to be the most apropriate one to evaluate GPP [*Marra,* 2002; *Regaudie-de-Gioux et al.,* 2014].

However, only a small proportion of all *in vitro* measurements done so far, used the ^{18}O method. To the best of our knowledge, only 19 published studies reported estimates by this method. One of them consisted of laboratory incubations of different phytoplanktonic species [*Grande et al.,* 1989]; 3 of them were in lakes [*Luz et al.,* 2002; *Ostrom et al.,* 2005; *Yacobi et al.,* 2007], one in estuaries [*Gazeau et al.,* 2007], 4 in coastal areas [*Bender et al.*, 1987; *Sarma et al.,* 2006; *González et al.,* 2008; *Goldman et al.,* 2015], and the other 10 studies were in the open ocean [*Grande et al.,* 1989a; *Kiddon et al.,* 1995; *Bender et al.,* 1999; *Laws et al.,* 2000; *Bender et al.,* 2000; *Dickson and Orchardo,* 2001; *Dickson et al.,* 2001; *Juranek and Quay,* 2005; *Robinson et al.,* 2009; *Quay et al.,* 2010]. None of these studies was in the Arctic Ocean.

With respect to **respiration**, much less has been done in comparison with primary production: the ratio in the number of respiration measurements per primary production measurements in the ocean is as unbalanced as 1:20,000 [*Williams and Del Giorgio,* 2005]. The reason for such different numbers might be that oceanographers have never had a valid method to evaluate phytoplankton respiration [*Marra,* 2009].

Moreover, respiration tends to be evaluated in darkness, assuming that the rate in light conditions would be the same, which could be adding a bias when modelling gas fluxes [*Marra,* 2009]. Respiration is a key process to understand because many aspects are involved in it, so long-term changes would be very informative in the current context of global change [*Del Giorgio and Williams,* 2005]. Therefore, it is critical to improve our knowledge of respiration rates by improving our current scarce database [*Del Giorgio and Williams,* 2005].

Regaudie-de-Gioux and Duarte [2013] put together a large data set of plankton metabolism rates evaluated with different methods. The dark-light method was the most frequent one for GPP and R rates (83% and 100%, respectively), with very few estimates coming from the other techniques. This database improves the global ocean coverage of planktonic metabolism data, but it is still very unbalanced geographically (Fig. I.1.) [*Regaudie-de-Gioux and Duarte,* 2013]. The vast majority of estimates are located in the Atlantic Ocean, while the Pacific and Indian oceans have much fewer data. Unquestionably, getting a balanced picture of planktonic metabolism in the global ocean still remains as a goal to be fulfilled [*Regaudie-de-Gioux and Duarte,* 2013].

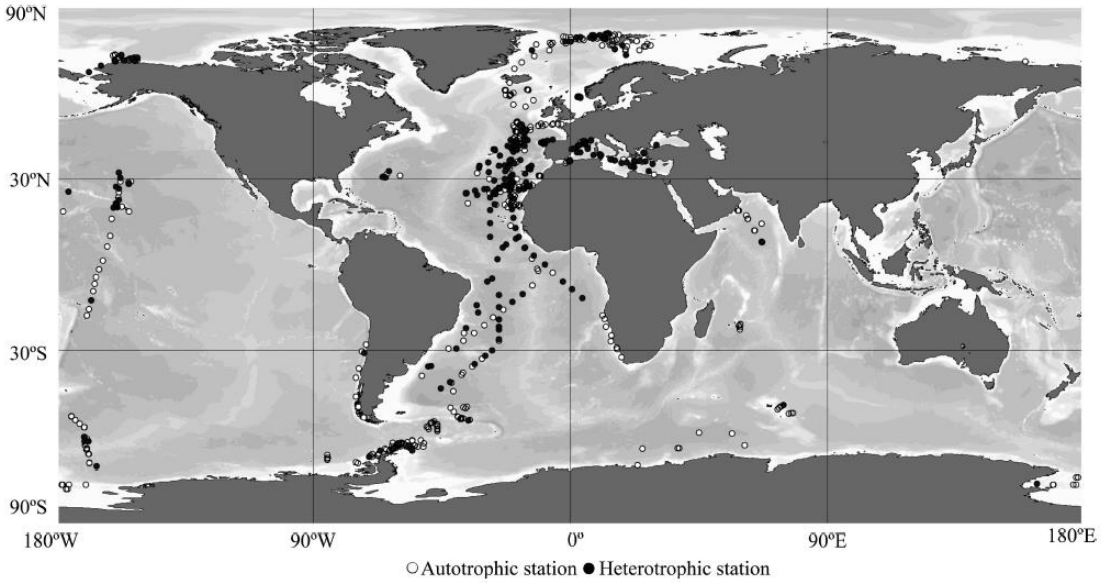

Fig. I.1. Stations where planktonic metabolism rates have been reported with the dark-light method. White and black dots refer to autotrophic and heterotrophic stations, respectively [*Regaudie-de-Gioux and Duarte,* 2013].

The main objective of this thesis is to go a step further in the global assessment of planktonic metabolism, by evaluating GPP^{18}O in the Arctic Ocean and in the tropical and subtropical ocean, and by testing the hypothesis of equal respiration in the dark and in the light.

The specific goals for each chapter are the following:

- Chapter 1: Evaluate GPP^{18}O in the subtropical ocean, in the framework of the Malaspina Expedition.

- Chapter 2: Evaluate GPP^{18}O in the European sector of the Arctic ocean (to the west of Svalbard islands).

- Chapter 3: Assess GPP^{18}O in Young Sound, a high-arctic fjord in North East Greenland.

- Chapter 4: Assess respiration in the dark and in the light in the Arctic Ocean, testing the hypothesis of equal respiration in both conditions.

- Chapter 5: Examine the concentration and δ^{18}O values of dissolved oxygen in Arctic waters, providing insights on its relationship with metabolic processes.

GENERAL METHODOLOGICAL APPROACH

General methodological approach

The evaluation of community metabolism in this thesis comes from different oceanographic cruises. Some data were produced in the Malaspina expedition and the rest come from work in the Arctic Ocean. The Malaspina expedition covered the Atlantic, Pacific and Indian Oceans between December 2010 and July 2011. Work conducted in the Arctic took place in the Svalbard region in cruises in 2012, 2013 and 2014; and in a Northeast Greenland fjord in 2014. Specific details of the methods used vary among different cruises (see the different chapters for these), so only the general method will be described under the present section.

Seawater was sampled at three different depths within the photic layer: surface; the deep chlorophyll maximum (DCM), which receives, on average, 1% of the incident irradiance; and an intermediate depth between surface and DCM, receiving 20% of the incident radiation on the surface (this intermediate depth was not sampled in the Greenland fjord). Both DCM and intermediate depths were sampled using a Rosette sampler system fitted with a calibrated Sea-Bird CTD. Surface waters were sampled manually with a Niskin bottle. All seawater samples were directly siphoned from Niskin bottles into calibrated 100 ml borosilicate glass narrow-mouth Winkler bottles and 12-ml borosilicate Exetainer vials using a silicon tube.

For each depth, different sets of replicates were taken for each method. For the Winkler bottles, a set of replicates (seven or eight) was fixed (biological activity immediately stopped) to provide the initial oxygen concentration. The two other sets of replicates, dark and transparent, were filled and incubated for 24 h. For the 12-ml vials, a set of replicates (three or four) was fixed with a saturated solution of $HgCl_2$ to provide the initial $\delta^{18}O$ (O_2) values. The other set was spiked with $H_2{}^{18}O$ and incubated along with the transparent Winkler bottles. Incubations were done either on deck or in situ, depending on the campaign. For those samples incubated on deck, neutral density screens were used to mimic the light environment in situ or they were incubated in the dark, in the case of the ‘‘dark’’ bottles. At the end of the incubation period, all samples were fixed to determine final O_2 concentrations (Winkler bottles) or final $\delta^{18}O$ (O_2) values (^{18}O vials) in the case of $GPP^{18}O$ (i.e. gross primary production assessed with the ^{18}O method).

For the winkler bottles, planktonic metabolism was evaluated from changes in dissolved oxygen concentrations, which were determined by automated high-precision Winkler titration with a potentiometric end-point Metrohm 808 Titrando [*Oudot et al.,* 1988]. Respiration (R)

and net community production (NCP) were calculated from changes in dissolved oxygen concentrations, before and after incubation of samples under "dark" and "light" conditions, respectively, and GPP was calculated as NCP + R. We used the reagents recommended by *Carrit and Carpenter* [1966]. Standard errors were calculated using error propagation.

The ^{18}O vials were measured back in the laboratory. A 4-ml headspace was generated in each vial, by flushing with a helium flow. Special attention was paid not to contaminate samples with atmospheric oxygen at any time. The vials were left for equilibration for 24 hours at room temperature, so that the dissolved gases in the water equilibrated with the headspace, originally helium 100%. After 24 hours, the $\delta^{18}O$ 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 ‰. The flow was passed through a liquid nitrogen trap before entering GasBench II to retain water vapour. Oxygen and nitrogen were separated in a Molecular Sieve 5Å chromatographic column. Data, which were corrected with atmospheric air, are reported as $\delta^{18}O$ value (‰) relative to V-SMOW (Vienna Standard Mean Ocean Water) standard.

The $\delta^{18}O$ (H_2O) of spiked samples was measured in a liquid water isotope analyzer (Los Gatos Research), with precision of 0.1 ‰. In order to avoid contamination of the analyzer with highly ^{18}O-enriched H_2O, the spiked samples were diluted with a laboratory standard of known isotopic composition. These $\delta^{18}O$ (H_2O) data, corrected with internal working standards, are also expressed as $\delta^{18}O$ value (‰) relative to V-SMOW standard.

Gross primary production measured by the ^{18}O method, $GPP^{18}O$, was calculated using the equation of *Bender et al.* [1999]:

$$GPP^{18}O = [O_2]_{initial} \times [(\delta^{18}O_{final} - \delta^{18}O_{initial})/(\delta^{18}O_{water} - \delta^{18}O_{initial})]$$

where $\delta^{18}O(O_2)_{initial}$ and $\delta^{18}O(O_2)_{final}$ are the initial and final $\delta^{18}O$ of dissolved O_2 (‰), respectively; $\delta^{18}O_{water}$ is the $\delta^{18}O$ of the spiked sea water (‰); and $[O_2]_{initial}$ is the initial O_2 concentration (mmol O_2 m^{-3}). Respiration in the light was calculated as the difference between $GPP^{18}O$ and NCP. Standard errors of $GPP^{18}O$ and total respiration were calculated using error propagation. Volumetric metabolic rates in this PhD thesis are expressed as mmol O_2 $m^{-3}d^{-1}$ (or µmol O_2 $L^{-1}d^{-1}$, which is equivalent). Integrated rates are reported in mmol O_2 m^{-2} d^{-1}.

CHAPTER 1

Evaluation of planktonic gross primary production with the ^{18}O method in the global ocean

Evaluation of planktonic gross primary production with the ^{18}O method in the global ocean

Abstract

Planktonic gross primary production (GPP) was assessed with the ^{18}O method in 84 stations in the Atlantic, Pacific and Indian Oceans, in the framework of the 2010 Malaspina Expedition. Our study, which occupied four of the five subtropical gyres, is the first report of GPP^{18}O (GPP assessed with the ^{18}O method) rates in the Indian Ocean gyre and the North and South Atlantic Ocean gyres. As such, our mean (± SE) GPP^{18}O rate, 0.60 ± 0.06 µmol O_2 L^{-1} d^{-1} (range 0.01-11.49), is lower than previously published estimates. The average GPP^{18}O rates did not differ significantly among depths. The communities sampled in the Pacific Ocean had a significantly higher GPP^{18}O (1.24 ± 0.20 µmol O_2 L^{-1} d^{-1}) than those sampled in the Atlantic (0.36 ± 0.04 µmol O_2 L^{-1} d^{-1}) and Indian (0.35 ± 0.02 µmol O_2 L^{-1} d^{-1}) Oceans.

Introduction

Half of the world primary production corresponds to plankton photosynthesis [*Field et al.,* 1998], which is the main process fueling the marine food web and the biological CO_2 pump [*Duarte et al.,* 2011; *Sarmiento et al.,* 2011]. Hence, plankton photosynthesis is a key factor regulating the ocean ecosystem and its role in biogeochemical cycles and climate regulation [*Chavez et al.,* 2011], rendering oceanic phytoplankton primary production a fundamental process at the global scale.

Primary production rates have been resolved through a number of methods, including ^{14}C and ^{13}C additions, bulk oxygen production and ^{18}O additions, providing different estimates [see *Regaudie-de-Gioux et al.,* 2014]. The differences in estimates resulting from the application of different methods has fostered an ongoing debate over the suitability of different estimates and what each method actually measures [*Quay et al.,* 2010; *Marra,* 2012; *Regaudie-de-Gioux et al.,* 2014]. One of the least applied techniques is the ^{18}O method, first introduced in 1987 [*Bender et al.,* 1987]. Labeling experiments had previously been conducted, where labeling the dissolved oxygen in water served to determine photosynthesis and respiration rates through the increase in $^{16}O_2$ concentration and the decrease in ^{18}O:^{16}O ratios [e.g. *Mehler and*

Brown, 1952; *Brown*, 1953]. The label was then switched from O_2 to H_2O [*Bender et al.*, 1987]. The ^{18}O method measures the gross primary production (GPP) after spiking the sample with $H_2{}^{18}O$, which is then split during photosynthesis, with the subsequent sample enrichment in ^{18}O [*Bender et al.*, 1987]. Some of the O_2 produced is consumed by the Mehler reaction, photorespiration and Ptox (plastid terminal oxidase) [see *Laws et al.*, 2000], so that ^{18}O estimates still provide underestimates of GPP. The GPP evaluated with this method ($GPP^{18}O$) does not involve the assumption of equal respiration in the light and in the dark or possible losses of GPP due to respiratory processes, giving the most precise estimate of GPP with the fewest assumptions needed [*Duarte et al.*, 2013; *Regaudie-de-Gioux et al.*, 2014]. Other methods providing an estimate of GPP are the Winkler bulk oxygen mass balance and those that use *in situ* geochemical fields, including the triple oxygen method [e.g. *Luz and Barkan*, 2000; *Juranek and Quay*, 2013], which provides values integrated across the mixed layer. However, these methods also involve assumptions. For instance, the triple oxygen method assumes an aquatic mixed layer in steady state in relation to O_2 and neglects vertical mixing with deeper water [*Luz and Barkan*, 2000]. It also assumes that the mixed layer is actively mixing [*Duarte et al.*, 2013], an assumption that was violated in one of three of the stations where microturbulence profiles were conducted in our circumnavigation cruise [*Fernández-Castro et al.*, 2015]. The Winkler bulk oxygen mass balance, unlike the ^{18}O method, does not provide a direct estimate of GPP, but GPP is calculated as net community production plus community respiration, so that error in each of these estimates as well as assumptions, such as that respiration in the light equals that on the dark, compounds on the resulting GPP estimates.

Despite the superiority of the ^{18}O method to estimate GPP [*Duarte et al.*, 2013; *Regaudie-de-Gioux et al.*, 2014], less than twenty studies, to the best of our knowledge, have used this method to date. Only punctual estimates are available, most of them being in productive oceanic locations or in the Northern hemisphere, with very limited data in the subtropical gyres of the ocean, where estimates have been only reported for the North Pacific gyre [*Grande et al.*, 1989; *Juranek and Quay*, 2005; *Quay et al.*, 2010].

Here we provide a global assessment of gross primary production in the subtropical and tropical ocean, evaluated with the ^{18}O method. We do so on the basis of nine cruises along the Atlantic, Pacific and Indian oceans, conducted under the framework of the Malaspina 2010 Circumnavigation Expedition.

Methods

The study was part of the Malaspina 2010 Expedition, a circumnavigation project sampling the tropical and subtropical Atlantic, Pacific and Indian Oceans [*Duarte,* 2015]. The cruises were conducted on board Spanish R/V *Sarmiento de Gamboa* and R/V *Hespérides*, from 27 December 2010 to 10 July 2011. Out of the total 9 legs involved in the cruises, five were conducted in the Atlantic Ocean (43 stations), two in the Pacific Ocean (25 stations) and two in the Indian Ocean (16 stations), including communities sampled within 16 different Longhurst biogeographical provinces [*Longhurst,* 1998; *Longhurst,* 2007].

Water samples for assessment of $GPP^{18}O$ were collected in 87 stations at three different depths within the photic layer: surface (3.53 ± 0.12 m), depth of chlorophyll maximum (DCM, 99.39 ± 4.12 m), receiving on average 1% of the incident photosynthetically active radiation (PAR), and at an intermediate depth (38.85 ± 1.33 m) between surface and DCM, receiving 20% of the PAR reaching the surface. A Rosette sampler consisting of 12 L Niskin bottles and a calibrated CTD, was used to sample DCM and intermediate depths. Surface waters were sampled with a 30 L Niskin bottle on board R/V *Hespérides* and with a Rosette sampler on board R/V *Sarmiento de Gamboa*.

Six 12-ml glass vials were filled per each depth for the assessment of gross primary production ($GPP^{18}O$). Three replicate samples were immediately fixed with 50 µl of saturated $HgCl_2$ solution to stop all biological activity and stored upside down in darkness for later analysis of initial $\delta^{18}O(O_2)$ values. The other three vials, containing washed glass beads inside to ensure mixing during the incubation, were spiked with 12 or 25 µl of 98% $H_2{}^{18}O$, depending on the cruise leg. The $H_2{}^{18}O$ spike was not distilled to remove trace metals and nutrients. The certificate of analysis of the $H_2{}^{18}O$ water stated the concentration (mg L^{-1}) of the following elements: F^-: 0.002, Cl^-: 0.002, Br^-: 0.002, I^-: 0.003, Ca: 0.01, Mg: 0.02, Na: 0.2, K: 0.02, Cu: 0.01, Fe: 0.02, PO_4^{3-}: 0.01, NO_3^-: 0.01. Accordingly, the input of trace metals and nutrients associated to the 12 or 25 µl of spike added is negligible. The spiked vials were closed and immediately shaken to ensure that $H_2{}^{18}O$ was homogeneously distributed inside the vial. The spiked samples were incubated on board in the light for 24 h. $GPP^{18}O$ measurements from ship-board incubations may have 2x biases when compared with estimates derived from parallel *in situ* incubations, possibly because of differences in light quality [*Grande et al.,* 1989]. Whereas we could not make such comparisons, as we could not stay at station for > 24 h required to derive the *in situ* estimates, we carefully adjusted the light received to the on deck incubations

to minimize such biases. The surface communities were incubated on deck in a large 2,000 L tank with running surface seawater to keep the natural temperature and solar radiation of the surface water. For intermediate and DCM depths, the vials were incubated inside transparent polycarbonate incubation cylinders which had neutral density and blue filters to mimic the irradiance levels received by the community at the depth where they were originally sampled. The tubes were filled with surface seawater and attached to a closed-circuit incubator provided with a chiller controlling water temperature within ± 0.5 ºC of that *in situ* and circulated through the tubes with a pump. After 24-hour incubation, vials were fixed with 50 µl of saturated $HgCl_2$ solution and stored upside down in darkness for subsequent analysis.

In the laboratory, a 4-ml headspace was generated in each vial, by flushing with a helium flow. Special attention was paid not to contaminate samples with atmospheric oxygen at any time. The vials were left to equilibrate for 24 hours at room temperature, so that the dissolved gases in the water equilibrated with the headspace (initially 100% helium). After 24 hours, the $\delta^{18}O$ of dissolved oxygen in the headspace was measured in a Finnigan GasBench II conected to a Finnigan DeltaPlusXP isotope ratio mass spectrometer, with a precision better than 0.1 ‰. The flow was passed through a liquid nitrogen trap before entering GasBench II to retain water vapour. Oxygen and nitrogen were separated in a Molecular Sieve 5Å chromatographic column. Data, which were corrected with atmospheric air, are reported in the $\delta^{18}O$ notation (‰) relative to V-SMOW (Vienna Standard Mean Ocean Water) standard.

The $\delta^{18}O(H_2O)$ of spiked samples was measured in a liquid water isotope analyzer (Los Gatos Research), with a precision of 0.1 ‰. In order to avoid contamination of the analyzer with highly ^{18}O-enriched H_2O (≈ 420 or 900 ‰, depending on the spike added), the samples were diluted with a laboratory standard of known isotopic composition.

$GPP^{18}O$ in 24 h (µmol O_2 L^{-1} d^{-1}) was calculated as follows [*Bender et al.*, 1999]:

$$GPP^{18}O\ (24\ h) = [O_2]_{initial} \times [(\delta^{18}O_{final\ (24\ h)} - \delta^{18}O_{initial})/(\delta^{18}O_{water\ (24\ h)} - \delta^{18}O_{initial})],$$

thereby yielding $GPP^{18}O$ estimates with units mmol O_2 m^{-3} day^{-1}, where $\delta^{18}O_{initial}$ and $\delta^{18}O_{final(24\ h)}$ are the initial and final $\delta^{18}O$ of dissolved O_2 (‰), respectively; $\delta^{18}O_{water(24\ h)}$ is the $\delta^{18}O$ of the spiked sea water (‰); and $[O_2]_{initial}$ is the initial O_2 concentration (µmol O_2 L^{-1}) measured by high-precision Winkler titration. Rates are expressed as the mean and standard error of replicate samples (µmol O_2 L^{-1} d^{-1}). $GPP^{18}O$ was integrated down to the depth receiving 1% of surface irradiance (DCM) using the trapezoidal method (integrated rates expressed in mmol O_2

m^{-2} d^{-1}). The resulting uncertainty in the integrated rates (expressed as SD) was calculated by error propagation, combining the variance of the different terms involved in the calculation.

Results

The communities sampled in the subtropical and tropical ocean were characterized by relatively warm waters (mean temperature (± SE) 22.72 ± 0.33, 20.45 ± 0.49, and 24.74 ± 0.37 ^{o}C in the Atlantic, Indian and Pacific Ocean, respectively), with temperature declining by, on average 3.36 ^{o}C, from surface to the DCM depth, which was located at about 100 m (mean DCM depth = 99.39 ± 4.12 m, range 19 m to 165 m). The cruise track occupied four of the five subtropical gyres and, accordingly, the waters sampled were characterized by low chlorophyll *a* concentrations (0.26 ± 0.02 µg Chl *a* L^{-1}, range 0.02 – 1.63 µg Chl *a* L^{-1}, Fig. 1.1), characteristic of the oligotrophic ocean.

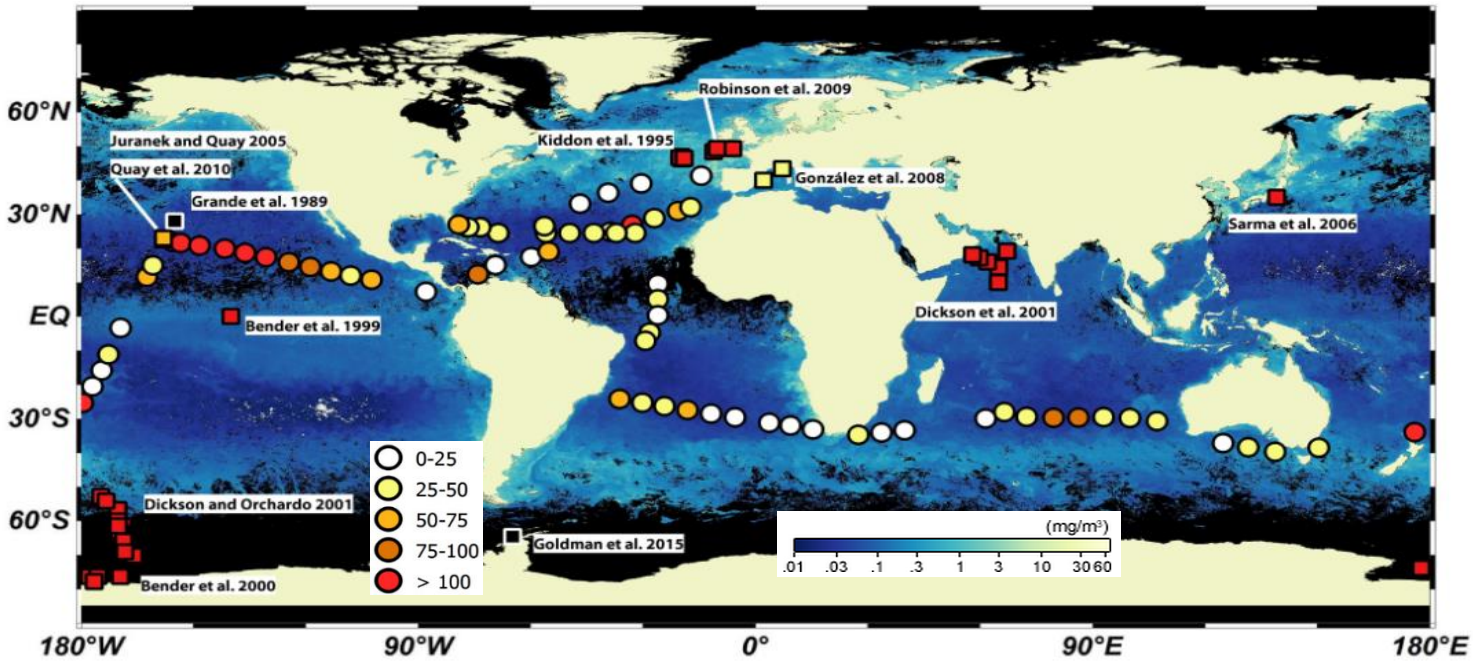

Fig. 1.1. Integrated GPP^{18}O (mmol O_2 m^{-2} d^{-1}) for the stations occupied along the Malaspina 2010 Expedition (circles) and those reported in the literature (squares, labels identify the studies reported in Table 1.2). The color scale for the symbols represents bines of GPP^{18}O whereas that in the background represents the mean chlorophyll concentration reported for April 2011, when the study was being conducted. Chlorophyll data obtained from Moderate Resolution Imaging Spectroradiometer (MODIS) instrument aboard NASA's Terra and Aqua satellites http://neo.sci.gsfc.nasa.gov/view.php?datasetId=MY1DMM_CHLORA&year=2011

Volumetric $GPP^{18}O$ rates were low, ranging three orders of magnitude from 0.01 ± 0.02 (mean ± SE) to 11.49 ± 2.45 µmol O_2 L^{-1} d^{-1}, with a mean (± SE) GPP of 0.60 ± 0.06 µmol O_2 L^{-1} d^{-1} and 86 % of the communities examined (i.e. 205 out of the 237 communities where GPP was measured) supporting $GPP^{18}O$ < 1 µmol O_2 L^{-1} d^{-1}. GPP rates did not differ significantly among depths (Fig. 1.2, mean 0.56 ± 0.07, 0.59 ± 0.09 and 0.66 ± 0.16 µmol O_2 L^{-1} d^{-1} for surface, 20% PAR and DCM depths, respectively) and were independent of chlorophyll *a* concentration, salinity and water temperature (F-test, $p > 0.05$). Yet, the $GPP^{18}O$ rates standardized for chlorophyll a concentration declined significantly (paired t-test, $P < 0.0001$) with depth (mean 4.82 ± 0.55, 4.92 ± 0.77 and 1.72 ± 0.40 µmol O_2 µg Chl a^{-1} d^{-1} for surface, 20% PAR and DCM depths, respectively).

The communities sampled in the Pacific Ocean supported significantly higher volumetric $GPP^{18}O$ rates (1.24 ± 0.20 µmol O_2 L^{-1} d^{-1}) than those sampled in the Atlantic (0.36 ± 0.04 µmol O_2 L^{-1} d^{-1}) and the Indian Oceans (0.35 ± 0.02 µmol O_2 L^{-1} d^{-1}) (Kruskal-Wallis test, $p < 0.001$, Figs. 1.1 and 1.3). However, the minimum mean volumetric and integrated $GPP^{18}O$ rates were found in the Pacific Equatorial Divergence Province (PEQD, 0.20 ± 0.05 µmol O_2 L^{-1} d^{-1} and 10.08 mmol O_2 m^{-2} d^{-1}, respectively), whereas the maximum mean volumetric and integrated $GPP^{18}O$ rates were found in the North Pacific Tropical Gyre Province (NPTG, 1.60 ± 0.21 µmol O_2 L^{-1} d^{-1} and 182.71 ± 46.65 mmol O_2 m^{-2} d^{-1}, respectively) (Table 1.1, Fig. 1.1).

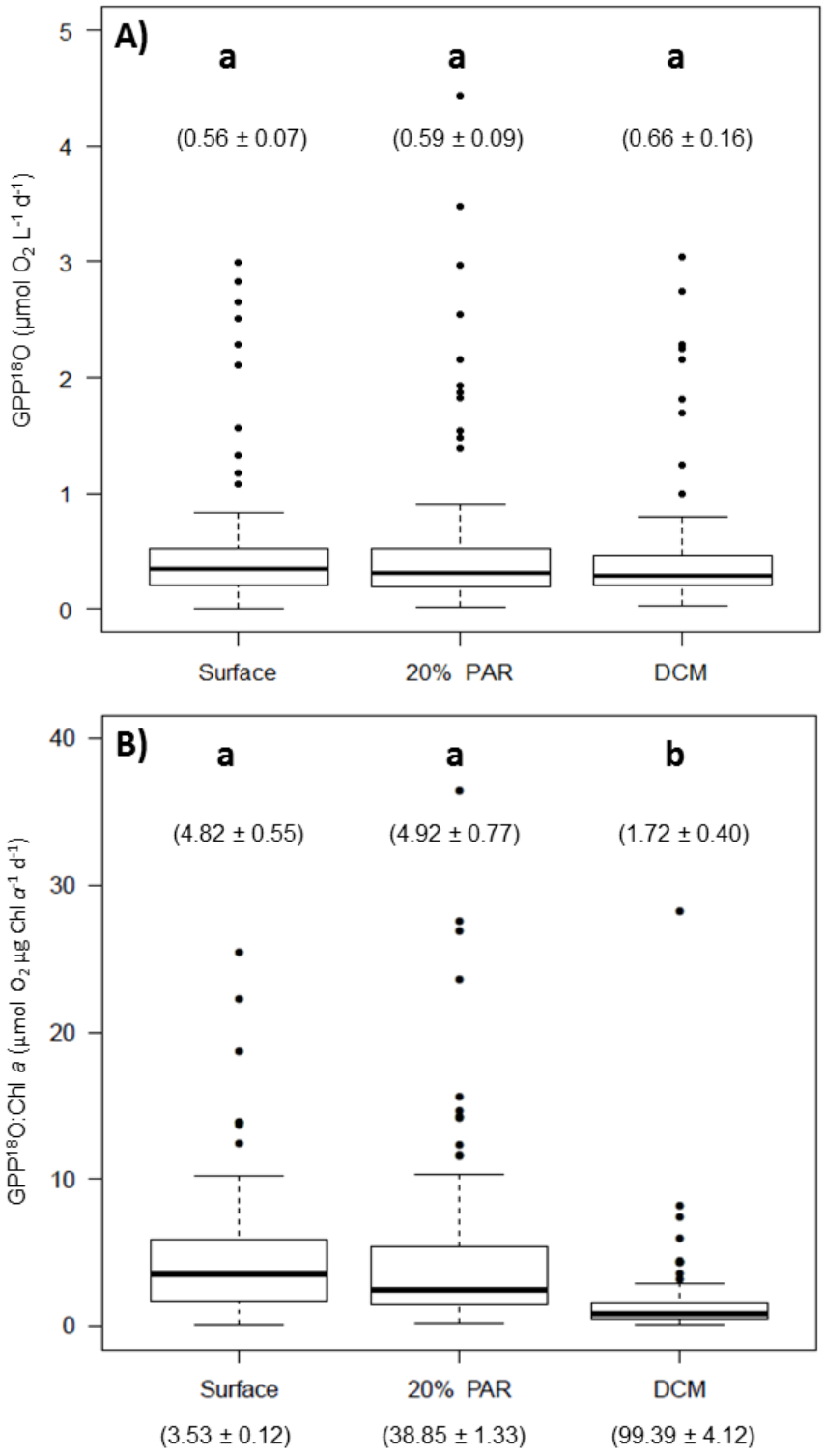

Fig. 1.2. Boxplot showing the distribution of A) $GPP^{18}O$ (µmol $O_2 L^{-1} d^{-1}$), and B) $GPP^{18}O$:Chl *a* (µmol O_2 µg Chl a^{-1} d^{-1}) at each of the three layers sampled. The central lines represent the median, and the boxes encompass the lower (25%) and upper (75%) quartiles, the whiskers extend to 1.5 times the interquartile range, and extreme rates are shown as closed circles. Values in parentheses represent the mean ± SE $GPP^{18}O$ and $GPP^{18}O$:Chl *a*. The mean depth ± SE corresponds to each sampled layer. Groups with different letters were significantly different (Tukey post hoc HSD test, $P < 0.05$).

		$GPP^{18}O$ (vol) ($mmol\ O_2\ m^{-3}\ d^{-1}$)		$GPP^{18}O$ (int) ($mmol\ O_2\ m^{-2}\ d^{-1}$)	
Longhurst province	**N**	**Mean**	**SE**	**Mean**	**SE**
BENG	1	0.24	0.02	13.28	----
CARB	4	0.51	0.15	41.89	23.74
NASE	6	0.46	0.12	49.33	24.35
NASW	5	0.22	0.05	17.20	3.47
NATR	13	0.29	0.02	40.95	2.98
NWCS	1	0.50	0.10	52.48	----
SATL	9	0.31	0.03	34.88	6.08
WTRA	4	0.31	0.04	26.93	9.46
Atlantic	43	0.34	0.03	36.38	4.38
NPTG	10	1.60	0.21	182.71	46.65
PEQD	2	0.20	0.05	10.08	----
PNEC	7	0.79	0.20	35.82	11.71
SPSG	5	1.41	0.79	145.56	99.53
Pacific	24	1.24	0.20	135.41	33.88
AUSE	2	0.42	0.05	28.20	----
EAFR	1	0.27	0.04	25.06	----
ISSG	10	0.34	0.03	40.62	8.23
SSTC	3	0.41	0.05	28.91	3.32
Indian	16	0.35	0.02	36.41	5.57
Total	83	0.60	0.06	63.90	10.79

Table 1.1. Mean and standard error (SE) of volumetric and integrated $GPP^{18}O$. SE is not given in cases with only one value. N = number of stations.

AUSE = East Australian Coastal Province. AUSW = Australia-Indonesia Coastal Province. BENG= Benguela Current Coastal Province. CARB= Caribbean Province. EAFR= E. Africa Coastal Province. ISSG= Indian S. Subtropical Gyre Province. NASE= N. Atlantic Subtropical Gyral Province (East). NASW= N. Atlantic Subtropical Gyral Province (West). NATR= N. Atlantic Tropical Gyral Province. NPTG= N. Pacific Tropical Gyre Province. NWCS= NW Atlantic Shelves Province. PEQD= Pacific Equatorial Divergence Province. PNEC= N. Pacific Equatorial Countercurrent Province. SATL= South Atlantic Gyral Province . SPSG= S. Pacific Subtropical Gyre Province. SSTC= S. Subtropical Convergence Province. WTRA= Western Tropical Atlantic Province.

Discussion

To the best of our knowledge, only twelve published studies have assessed GPP with the ^{18}O technique in the ocean thus far (Fig. 1.1, Table 1.2). However, none of these studies included the subtropical oligotrophic gyres of the Indian Ocean and the North and South Atlantic Ocean (Fig. 1.1), so the results presented here represent a significant contribution to estimating GPP^{18}O rates in the tropical and subtropical ocean. Indeed, the mean Chl *a* concentration of the communities sampled in our study (0.26 ± 0.02 µg Chl *a* L^{-1}) is below the mean Chl *a* concentration reported in previous reports of GPP^{18}O [*Kiddon et al.,* 1995; *González et al.,* 2008; *Robinson et al.,* 2009; *Goldman et al.,* 2015]. Accordingly, the mean volumetric GPP^{18}O reported here for the tropical and subtropical ocean of 0.60 ± 0.06 µmol O_2 L^{-1} d^{-1} is the lowest among assessments published thus far, which were biased towards productive ocean waters in the north-temperate zone. The mean integrated GPP^{18}O rate of 63.90 ± 10.79 mmol O_2 m^{-2} d^{-1} we report here for the tropical and subtropical ocean is also the lowest yet reported, with the exception of an integrated GPP^{18}O rate from an oligotrophic site in the NW Mediterranean [*González et al.,* 2008]. In addition, published reports were biased towards the northern hemisphere, as all except three studies in the Southern Ocean, were conducted in the northern hemisphere. Hence, the GPP^{18}O estimates derived here contribute to balance available data on this process, by representing both the northern and southern hemisphere, and focusing on tropical and subtropical ocean, including the subtropical gyres, where GPP^{18}O rates had only been reported for the North Pacific gyre.

The average GPP^{18}O rates reported for the tropical and subtropical ocean did not differ significantly among the three depths sampled (Fig. 1.2), they did decline significantly with depth when standardized for chlorophyll *a* concentration (Fig. 1.2). The higher phytoplankton biomass at depth, conforming the deep chlorophyll maximum, compensates for the reduced productivity at depth, resulting in relatively uniform GPP rates across the three depths examined here when compared across the circumnavigation cruise.

	$GPP^{18}O$ (vol) ($mmol\ O_2\ m^{-3}\ d^{-1}$)		$GPP^{18}O$ (int) ($mmol\ O_2\ m^{-2}\ d^{-1}$)	
Source	**Mean**	**SE**	**Mean**	**SE**
Grande et al., 1989[5]	1.57	0.11	----	----
Kiddon et al., 1995[1]	4.91	0.48	213.08	16.54
Bender et al., 1999[1]	3.96	0.36	286.14	23.52
Bender et al., 2000[1]	7.73	1.06	108.62	26.27
Dickson and Orchardo, 2001[1]	2.45	0.33	129.70	28.06
Dickson et al., 2001[2]	6.50	0.51	245.93	25.01
Juranek and Quay, 2005[3]	0.78	0.10	111.25	29.29
Sarma et al., 2006[4]	----	----	109.00	25.75
González et al., 2008[1]	2.27	0.34	49.51	4.75
Robinson et al., 2009[1]	35.95	7.59	1278.48	552.59
Quay et al., 2010[3]	----	----	71.00	16.00
Goldman et al., 2015[5]	32.03	10.70	----	----

Table 1.2. Mean and SE of volumetric and integrated $GPP^{18}O$ in published studies. (1) Integrated over the euphotic zone (defined as the depth receiving 1% of surface irradiance); (2) integrated to the depth receiving 4% of surface irradiance; (3) integrated for the first 100 m of the euphotic zone; (4) integrated to the mixed layer; (5) cannot be integrated as they report rate for only one depth.

Previous studies evaluated $GPP^{18}O$ rates in locations close to some of those we sampled in the Pacific Ocean (Fig. 1.1). Comparison between our results and those reported earlier shows good agreement, including two studies conducted in the same site [*Juranek and Quay*, 2005; *Quay et al.*, 2010]. Another study reported a mean volumetric rate similar to the rate of of 2.56 ± 0.34 µmol $O_2\ L^{-1}\ d^{-1}$ we obtained at the stations closest to that they sampled [*Grande et al.*, 1989] (cf. Table 1.2). Despite this agreement, the results presented here depict the Pacific Ocean as more heterogeneous, in relation to $GPP^{18}O$ rates, than the Atlantic and Indian Oceans,

having a much wider range in GPP^{18}O values than the other two oceans (Fig. 1.3), resulting from very high rates in the Northern compared to very low rates in the Southern Pacific Ocean (Fig. 1.1).

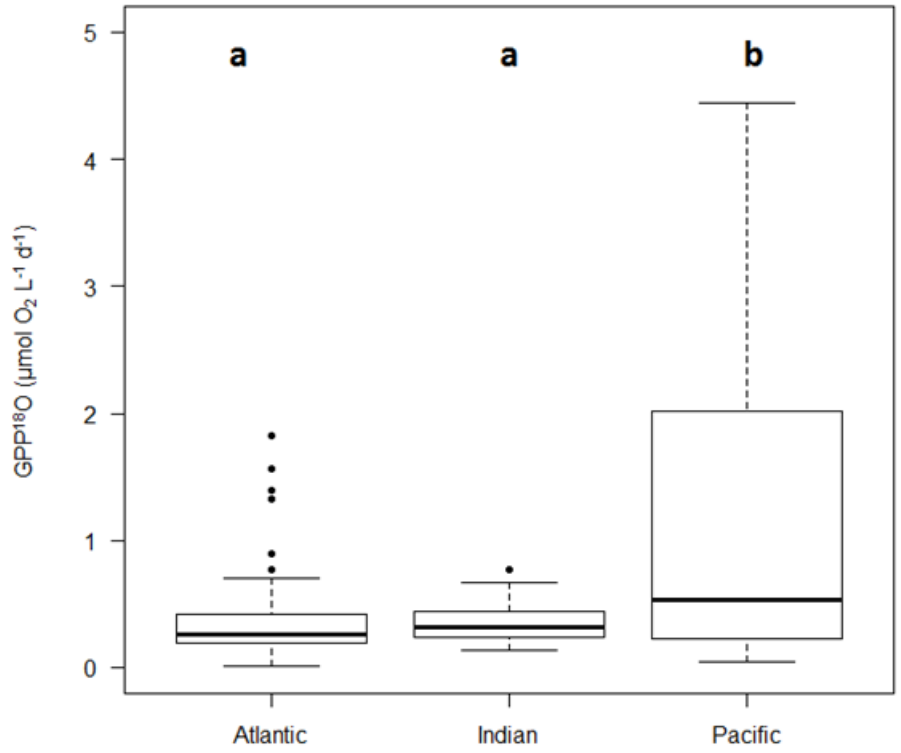

Fig. 1.3. Distribution of GPP^{18}O (µmol O_2 L^{-1} d^{-1}) per ocean. Rates were not significantly different for oceans with the same letter (Tukey HSD test). The boxes show the median rate plus the lower (25%) and upper (75%) quartiles, the whiskers indicate 1.5 times the interquartile range, extreme rates are shown as closed circles.

A paper reported results from a dataset [*Regaudie-de-Gioux and Duarte*, 2013b] compiling plankton metabolism rates for the euphotic zone derived from different methods, measured between 1981 and 2011 [*Regaudie-de-Gioux and Duarte*, 2013a]. Most estimates of GPP (83%) in their data set had been derived using the bulk O_2 dark-light method, with few estimates evaluated by the other techniques and an even more limited geographic coverage. Their GPP database consisted mainly of rates coming from chl *a*-rich oceans and North Atlantic waters, with much fewer rates in the Indian and South Pacific Oceans. Hence, the report of GPP rates for 87 stations sampled in the tropical and subtropical ocean provided here represents a significant contribution to expand and balance, in terms of geographic distribution and productivity regimes, our knowledge of gross primary production rates in the global ocean. In

particular, the rates here are characterized by very low values, suggesting that mean values inferred from previously existing data did not characterize adequately the global ocean, as was already acknowledged [*Regaudie-de-Gioux and Duarte,* 2013a]. The ^{18}O technique is the most precise way to assess GPP, with minimum number of assumptions necessary to estimate the rate [*Regaudie-de-Gioux et al.*, 2014]. The results presented depict much of the tropical and subtropical ocean as highly unproductive, where low GPP rates impose an important constraint to the organic carbon available to the food web across this vast ocean domain. .

Acknowledgements

This study is a contribution to the Malaspina 2010 Circumnavigation Expedition. We thank the captains and crews of R/V *Hespérides* and R/V *Sarmiento de Gamboa* for help throughout the expedition; Eugenio Fraile and Verónica Benítez for providing and processing CTD data; Marta Estrada and colleagues for chlorophyll a data, X. Antón Álvarez-Salgado, Antonio Bode and Luana Pinho for the classification of stations in Longhurst provinces; and the Malaspina participants for the oxygen analysis.

CHAPTER 2

Planktonic gross primary production in the European Arctic Sector

Planktonic gross primary production in the European Arctic sector

Abstract

We assessed planktonic gross primary production using the ^{18}O method ($GPP^{18}O$) in the Arctic Ocean, the first estimates by this method. Five cruises were conducted in the spring/summer period of 2012, 2013 and 2014 off the west margin of Svalbard. Mean (± SE) volumetric $GPP^{18}O$ was 14.00 ± 1.49 µmol O_2 L^{-1} d^{-1} (range: 0.19 - 69.15). Mean integrated rate was 236.71 ± 45.54 mmol O_2 m^{-2} d^{-1} (range: 10.84 – 866.91 mmol O_2 m^{-2} d^{-1}). Seasonal variability in $GPP^{18}O$ was strong, with May having the maximum mean monthly rate (26.60 ± 2.89 µmol O_2 L^{-1} d^{-1}) due to the spring bloom, and August having the lowest mean $GPP^{18}O$ rate (2.25 ± 0.32 µmol O_2 L^{-1} d^{-1}). The data proved consistent with estimates by the dark-light method ($GPPO_2$), as the mean rate from both methods did not differ significantly.

Introduction

The shelf regions of the Arctic Ocean belong to the most productive regions of the world, supporting a food web including abundant marine megafauna, such as whales and polar bears. Arctic primary production is characterized by a spring bloom contributing much of annual production, with much lower rates through the rest of the year when recycling processes dominate [*Wassmann et al.*, 2006b; *Vaquer-Sunyer et al.*, 2013]. Ecosystem processes are constrained by the energy available to support this process, which is determined by gross primary production [GPP, *Duarte et al.*, 2011], rendering the evaluation of GPP an important goal in characterizing marine ecosystems [e.g., *Robinson et al.*, 2009; *Quay et al.*, 2010].

Marine primary production rates can be assessed with different methods, such as the triple oxygen method [e.g. *Luz et al.*, 2000; *Juranek and Quay*, 2013], additions of ^{14}C, ^{13}C or ^{18}O, and bulk oxygen production [cf. *Regaudie-de-Gioux et al.*, 2014]. The use of different techniques results in differences in estimates, which has fueled an ongoing debate over the suitability, limitations and advantages of different methods [*Quay et al.*, 2010; *Marra*, 2012; *Regaudie-de-Gioux et al.*, 2014]. The ^{18}O method, first introduced by Bender and coauthors in 1987 [*Bender et al.*, 1987], has been used in very few studies. In this technique, GPP is assessed measuring the ^{18}O in the oxygen produced via photosynthesis [*Bender et al.*, 1987]. The estimates involve adding ^{18}O-labelled water ($H_2{}^{18}O$) to the sample water and then evaluating

the enrichment in ^{18}O in the dissolved oxygen pool derived by the release of ^{18}O from the hydrolysis of $H_2{}^{18}O$ during photosynthesis, thus providing an estimate of GPP without assuming equal respiration in the light and in the dark, or possible losses of GPP due to respiratory processes. This method is believed to provide the most precise assessesment of GPP with minimum assumptions necessary to measure the rate [*Duarte et al.,* 2013; *Regaudie-de-Gioux et al.,* 2014]. For comparison, the other techniques evaluating GPP are the Winkler bulk oxygen mass balance [$GPPO_2$, *Regaudie-de-Gioux et al.,* 2014] and the triple oxygen method [e.g. *Luz and Barkan,* 2000; *Juranek and Quay,* 2013]. However, both methods are based on assumptions. The Winkler bulk oxygen mass balance, in contrast to the ^{18}O method, does not evaluate GPP directly, but GPP is calculated as community respiration plus net community production, evaluated from the rate of O_2 consumption in communities incubated in the dark. The triple oxygen method assumes oxygen to be in steady state in the mixed layer, with no vertical mixing with deeper water [*Luz and Barkan,* 2000]. Hence, errors and assumptions in these estimates affects the final GPP estimates.

Despite the superiority of the ^{18}O method to evaluate GPP [*Duarte et al.,* 2013; *Regaudie-de-Gioux et al.,* 2014], less than twenty studies worldwide, to the best of our knowledge, have used this technique so far. In particular, there are no such estimates in the Arctic Ocean, where GPP has been estimated exclusively by the Winkler bulk oxygen mass balance [cf. *Vaquer-Sunyer et al.,* 2013]. The assumption of equal respiration in the light and in the dark is especially critical for the high Arctic in spring and summer, when there is an extended period of sustained daylight.

Here, we provide the first assessment of GPP in the Arctic using the ^{18}O method. We do so on the basis of five cruises conducted in the spring/summer period of 2012, 2013 and 2014 off the west margin of Svalbard Islands. We also compare these estimates with GPP evaluations by the dark-light method, to determine the consistency between estimates derived using both methods.

Methods

Five cruises were conducted to the west of the Svalbard region up to 80° 52' N, sampling a total of 34 stations (Fig. 2.1). The cruises, undertaken with R/V *Helmer Hanssen* (Univ. Tromsø), took place in 2012 (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). Hence, the rates reported here correspond to spring or summer, when plankton grows under a 24-hour photoperiod.

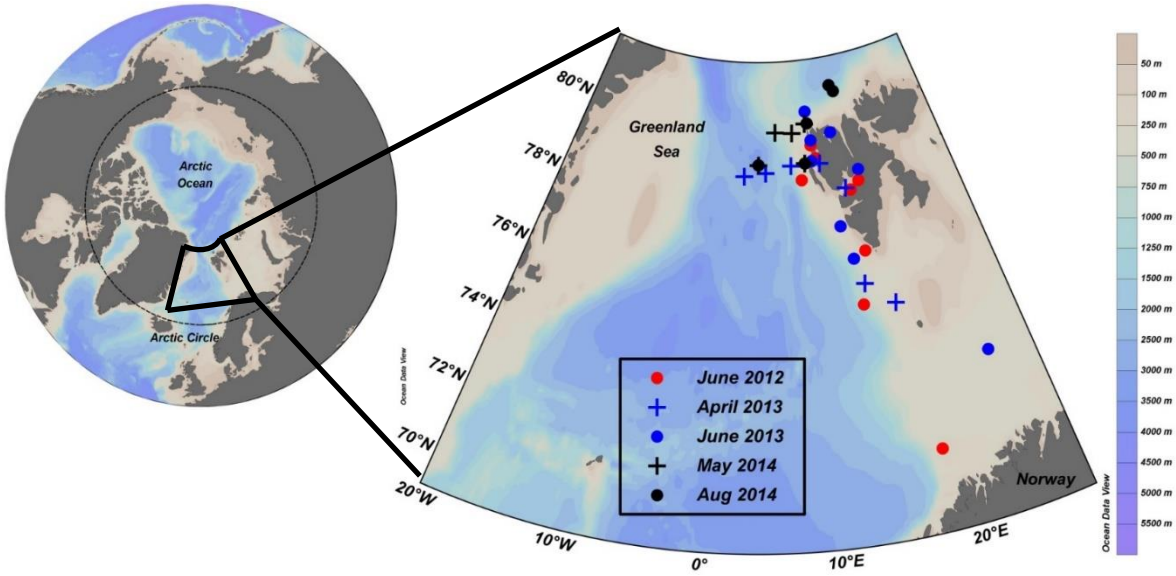

Fig. 2.1. Location of the stations sampled in the different cruises.

A Rosette sampler system, consisting of 10-L Nisking bottles and a calibrated Seabird CTD, was used to collect water samples at three different depths: surface (2.12 ± 0.13 m), depth of chlorophyll maximum (DCM, 24.56 ± 1.63 m), receiving 1% of surface irradiance on average, and at an intermediate depth (13.56 ± 0.93 m) between surface and DCM, reached by 20% of the incident irradiance.

Eight 12-ml glass vials were filled with water carefully siphoned from the Niskin bottles at each depth for the assessment of gross primary production ($GPP^{18}O$). Four replicate samples were immediately fixed (biological activity stopped) with 80 µl of saturated $HgCl_2$ solution for later analysis of initial $\delta^{18}O$ (O_2) values, and stored in darkness upside down. The other four vials, containing glass beads inside to ensure mixing, were spiked with 80 µl of 98% $H_2{}^{18}O$ and immediately shaken, to ensure that $H_2{}^{18}O$ was homogeneously distributed inside the vial. These vials were incubated inside tubes on deck, with continuous water flow. Filters were used

to simulate the light *in situ* and temperature was set to be the same as *in situ*. After the 24-hour incubation, vials were fixed with 80 µl of saturated $HgCl_2$ solution and stored in darkness upside down.

Back at the laboratory, vials were flushed with a helium flow, preventing atmospheric oxygen from contaminating samples at any time, and creating a 4 ml headspace. Samples were left at room temperature to equilibrate and after 24 hours, the $\delta^{18}O$ of dissolved oxygen in the headspace was measured in a Finnigan GasBench II conected to a Finnigan DeltaPlusXP isotope ratio mass spectrometer. Precision was better than 0.1 ‰. A liquid nitrogen trap was set before GasBench II, to remove water vapor from the flow. A Molecular Sieve 5Å chromatographic column separated nitrogen and oxygen. Data, corrected with atmospheric air, are reported as $\delta^{18}O$ value (‰) relative to V-SMOW (Vienna Standard Mean Ocean Water) standard.

A liquid water isotope analyzer (Los Gatos Research) was used to measure the $\delta^{18}O(H_2O)$ composition of spiked samples, with precision of 0.1 ‰. In order to avoid contamination of the analyzer with highly ^{18}O-enriched H_2O (≈ 3000 ‰), the samples were diluted with a laboratory standard of known isotopic composition.

$GPP^{18}O$ was determined with the equation of [*Bender et al.,* 1999]:

$$GPP^{18}O = [(\delta^{18}O_{final} - \delta^{18}O_{initial})/(\delta^{18}O_{water} - \delta^{18}O_{initial})] \times [O_2]_{initial}$$

where $\delta^{18}O_{initial}$ and $\delta^{18}O_{final}$ are the initial and final $\delta^{18}O$ of dissolved O_2 (‰), respectively; $\delta^{18}O_{water}$ is the $\delta^{18}O$ of the spiked sea water (‰); and $[O_2]_{initial}$ is the initial O_2 concentration (µmol O_2 L^{-1}) measured by high-precision Winkler titration. Rates are reported as the mean and standard error of replicate samples (µmol O_2 L^{-1} d^{-1}). $GPP^{18}O$ was integrated down to 20 m following *Vaquer-Sunyer et al.* [2013], close to the chlorophyll *a* maximum layer (24.56 ± 1.63 m). Integrated rates are expressed in mmol O_2 m^{-2} d^{-1}.

For the evaluation of $GPPO_2$, borosilicate Winkler bottles were carefully filled from the Niskin bottles. Sets of seven replicates per each depth were used to evaluate initial oxygen concentration, and to measure the oxygen concentration after a 24 h incubation in the "light" and in the "dark". Incubations were done on deck, in the same water baths as the $GPP^{18}O$ samples. Oxygen concentrations were measured with automated high-precision Winkler titration [*Carpenter,* 1965; *Carrit and Carpenter,* 1966], holding a potentiometric electrode

and via automated endpoint detection [*Oudot et al.,* 1988]. $GPPO_2$ was calculated as the final oxygen concentration of the "light" bottles minus the final oxygen concentration of the "dark" bottles [*Carpenter,* 1965; *Carrit and Carpenter,* 1966].

To determine the chlorophyll a concentration, 200 mL of water were filtered through Whatman GF/F filters, and chlorophyll a was extracted in 90% acetone for 24 hours. As specified by *Parsons et al.* [1984], chlorophyll a concentration was determined in a Shimadzu RF-5301PC spectrofluorometer.

Results and Discussion

Volumetric $GPP^{18}O$ rates ranged from 0.19 to 69.15 µmol O_2 L^{-1} d^{-1}, with a mean value (± SE) of 14.00 ± 1.49 µmol O_2 L^{-1} d^{-1}. $GPP^{18}O$ varied significantly along the productive season (ANOVA, $P < 0.001$), with maximum mean monthly rates observed in May, when the spring bloom occurs (26.60 ± 2.89 µmol O_2 L^{-1} d^{-1}) and the lowest mean GPP rate (2.25 ± 0.32 µmol O_2 L^{-1} d^{-1}) found in August (Fig. 2.2). April had the lowest number of estimates of all months ($N = 12$) but the highest variability in rates (range: 3.04 – 45.67 µmol O_2 L^{-1} d^{-1}), as the plankton spring bloom typically occurs in late April to May. $GPP^{18}O$ was strongly and linearly correlated with chlorophyll *a* concentrations (Fig. 2.3), indicating that the $GPP^{18}O$ yield per unit chlorophyll *a* did not vary significantly from sparse to dense phytoplankton communities.

Integrated $GPP^{18}O$ rates were high, ranging from 10.84 to 866.91 mmol O_2 m^{-2} d^{-1} (Fig. 2.4), with a mean integrated $GPP^{18}O$ rate of 236.71 ± 45.54 mmol O_2 m^{-2} d^{-1}. Half of the total number of stations (17 out of 34) supported an integrated $GPP^{18}O$ exceeding 100 mmol O_2 m^{-2} d^{-1}. There were no consistent differences in integrated $GPP^{18}O$ rates between fjord and open-ocean stations, as high and low rates occurred in both areas (Fig. 2.4).

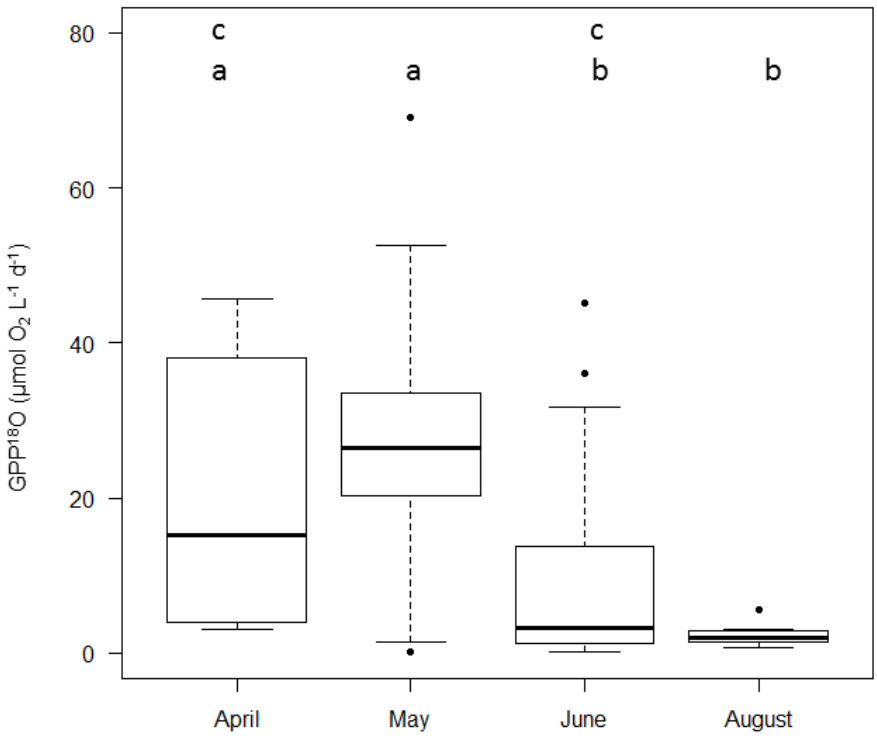

Fig. 2.2. Boxplot showing the distribution of $GPP^{18}O$ (µmol O_2 L^{-1} d^{-1}) for the different months. Letters indicate the results for a Tukey HSD test, whereby the $GPP^{18}O$ did not differ significantly for months with the same letter.

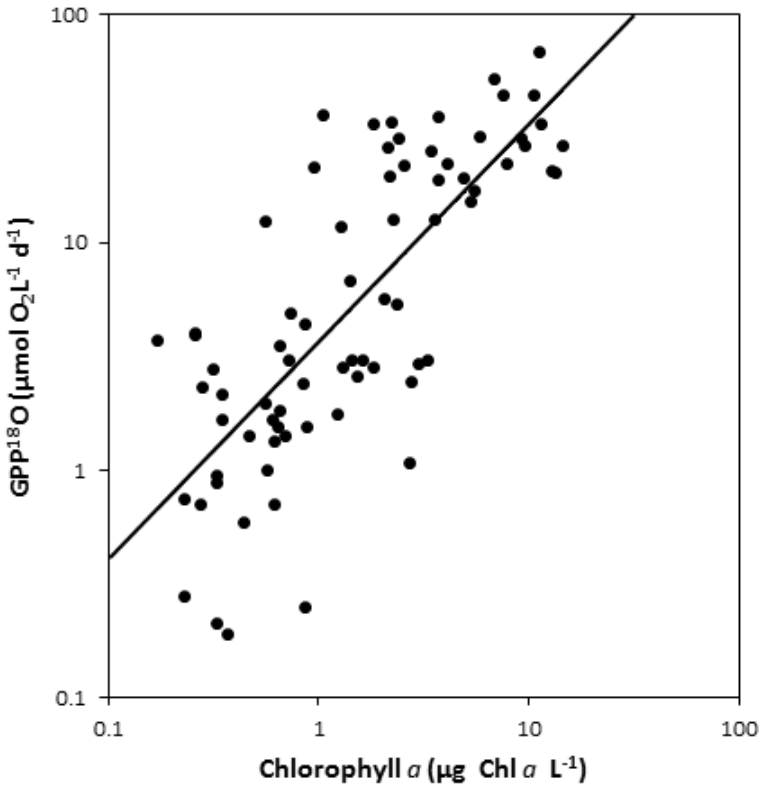

Fig. 2.3. Relationship between $GPP^{18}O$ (µmol O_2 L^{-1} d^{-1}) and concentration of chlorophyll *a* (µg L^{-1}). The solid line shows the fitted regression equation: $y = 3.60x^{0.96\ (\pm\ 0.35)}$ ($R^2 = 0.58$, $p < 0.0001$, $N = 75$) .

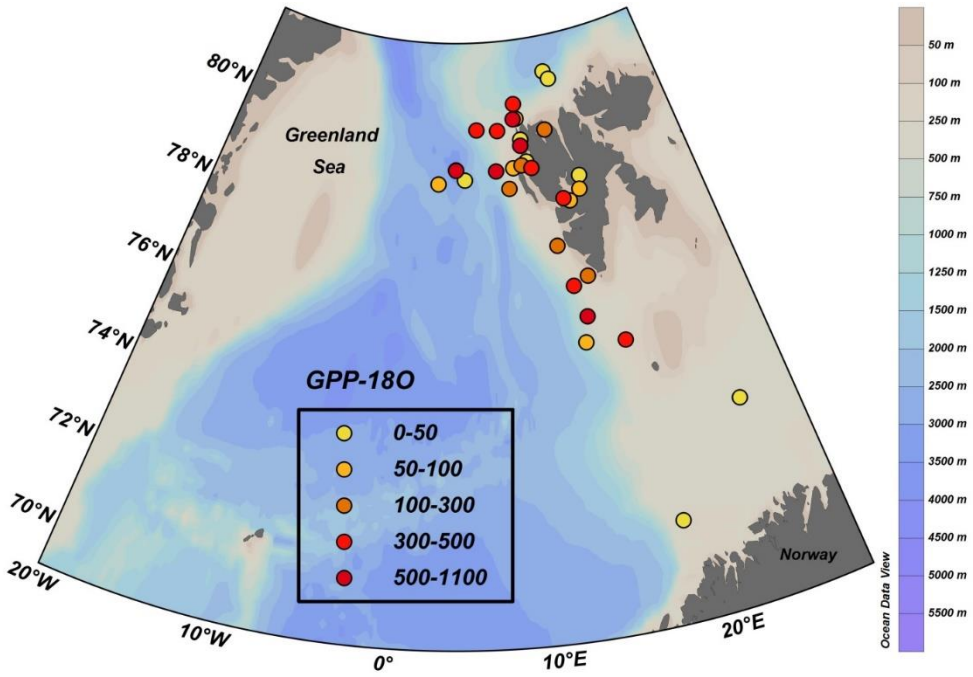

Fig. 2.4. Integrated $GPP^{18}O$ (mmol O_2 m^{-2} d^{-1}) for the stations sampled.

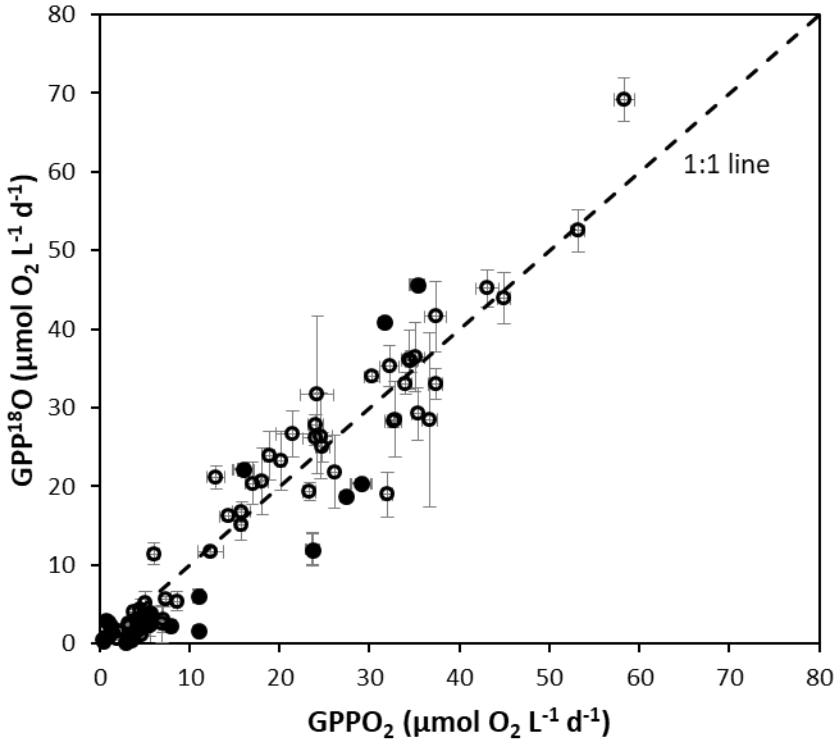

Fig. 2.5. Relationship between $GPP^{18}O$ (µmol O_2 L^{-1} d^{-1}) and $GPPO_2$ (µmol O_2 L^{-1} d^{-1}). Fitted regression equation: y = 1.03 (± 0.03) x - 0.54 (± 0.81) (R^2 = 0.94, p < 0.0001, N = 79). Full and open symbols denote observations where the 95% confidence limits of $GPPO_2$ and $GPP^{18}O$ do not overlap and do overlap, respectively.

$GPP^{18}O$ rates were strongly and linearly correlated with $GPPO_2$ rates, closely conforming to the 1:1 line (Fig. 2.5). The two methods gave similar (t-test, $P > 0.05$) estimates in 75% of estimates (59 out of 79). As a result, mean $GPP^{18}O$ and $GPPO_2$ rates for the entire data sets did not differ significantly (paired t-test, t=0.03, df=116, p=0.98).

No GPP estimates had been reported using the ^{18}O method for the Arctic Ocean thus far, which renders our $GPP^{18}O$ rates the first to be reported for this region. Comparing our $GPP^{18}O$ rates to those published to date with this method for other systems, the mean volumetric $GPP^{18}O$ rate (14.00 ± 1.49 µmol O_2 L^{-1} d^{-1}) in our study is higher than that reported in previous assessments of $GPP^{18}O$, except for those reported by *Robinson et al.* [2009], in the Celtic Sea (mean 35.95 ± 7.59 µmol O_2 L^{-1} d^{-1}) and *Goldman et al.* [2015], in the Western Antarctic Peninsula (mean 32.03 ± 10.70 µmol O_2 L^{-1} d^{-1}). The mean integrated rate (236.71 ± 45.54 mmol O_2 m^{-2} d^{-1}) obtained in our study is also above all previous reports except those reported by *Robinson et al.* [2009], in the Celtic Sea (mean 1278.48 ± 552.59 mmol O_2 m^{-2} d^{-1}) and *Bender et al.* [1999], in the Equatorial Pacific Ocean (mean 286.14 ± 23.52 mmol O_2 m^{-2} d^{-1}). $GPP^{18}O$ rates reported in the present study are higher than those measured by *Gazeau et al.* [2007] in eutrophic estuaries (3.1 ± 0.6 µmol O_2 L^{-1} d^{-1}) and *Ostrom et al.* [2005] in Lake Erie (range 4.56 – 24.72 µmol O_2 L^{-1} d^{-1}), and comparable to those reported by *Luz et al.* [2002] for Lake Kinneret (0.26 ± 0.03 mol O_2 m^{-2} d^{-1}).

$GPPO_2$ rates were reported for a set of cruises between 2006 and 2011 in the same region as our study [*Vaquer-Sunyer et al.,* 2013]. Their $GPPO_2$ rates reported for all spring and summer cruises were in the range of 0 to 80.0 ± 1.7 µmol O_2 L^{-1} d^{-1}, which is very similar to our $GPP^{18}O$ values (range 0.19 - 69.15 µmol O_2 L^{-1} d^{-1}). Similarly, they report a strong relationship between gross primary production and chlorophyll a concentrations [*Vaquer-Sunyer et al.,* 2013]. They also reported maximum GPP rates in the spring, declining toward the summer, consistent with the seasonal variability reported here [*Vaquer-Sunyer et al.,* 2013]. GPP was evaluated with the triple oxygen method in the Beaufort Gyre region of the Canada Basin, in August 2011 and 2012 [*Stanley et al.,* 2015]. Although this Arctic region is different from the one in our study, their mean basin-wide GPP for August 2012, with record-low ice conditions, was 38 ± 3 mmol O_2 m^{-2} d^{-1}, which is similar to our mean $GPP^{18}O$ rate for August (only five stations, in 2014), 37.27 ± 6.60 mmol O_2 m^{-2} d^{-1}.

$GPP^{18}O$ rates reached the maximum monthly mean in May and the lowest in August. The spring bloom in southern regions of the Arctic Ocean occurs mainly in late April and May,

as submarine PAR increases with increasing incoming radiation and melting of seasonal ice, and has been reported to represent 40% of the total annual primary production [*Lavoie et al.,* 2009].

The estimates reported here do not include the contribution of ice algae to GPP, usually reported to account, in shelf areas, for 5 to 10% of total primary production [*Horner and Schrader,* 1982; *Gosselin et al.,* 1997; *Lavoie et al.,* 2009]. In the Beaufort Sea, the production of ice algae can average 36 mg C m^{-2} d^{-1}, having a peak of 62 mg C m^{-2} d^{-1} in May [*Horner and Schrader,* 1982]. For the ice-covered region of the Barents Sea, maximum annual ice algal production has been reported to be 5.3 g C m^{-2} [*Hegseth,* 1998].

Arctic GPP rates had only been estimated using the triple oxygen method and the high-precision Winkler method (i.e. $GPPO_2$ rates) thus far. Yet, the triple oxygen method assumes a mixed layer without vertical mixing with deeper water and where oxygen is in steady state. The $GPPO_2$ method assumes that respiration in the light is the same as in the dark [*Regaudie-de-Gioux et al.,* 2014], which may be questionable for the sustained 24 h daylight period in the high Arctic in spring and summer. Yet, our results showed a remarkable good correspondence between $GPP^{18}O$ and $GPPO_2$ rates, which therefore provide an unbiased estimate of gross primary production rates in the European Arctic sector. The mean ± SE ratio $GPP^{18}O/GPPO_2$ in our study was 1.03 ± 0.09. In contrast, $GPPO_2$ rates have been shown to be, on average, around 50% lower than $GPP^{18}O$ rates (mean ± SE of the ratio $GPP^{18}O/GPPO_2$, 1.9 ± 0.2) [*Regaudie-de-Gioux et al.,* 2014].

In summary, we report here the first estimates of Arctic GPP evaluated using the ^{18}O method. The rates obtained are comparable, in magnitude and seasonal pattern, to those reported earlier using the high-precision Winkler method, and were characterized by high rates during the spring bloom in late April and May, with declining rates throughout the summer. The rates evaluated using the ^{18}O method compared well with those derived using the high-precision Winkler method. The estimates reported here portray the European sector of the Arctic Ocean as ranging amongst the most productive areas of the ocean.

Acknowledgements

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CHAPTER 3

Primary production in a Northeast Greenland fjord: assessment with the ^{18}O method

Primary production in a Northeast Greenland fjord: assessment with the ^{18}O method

Abstract

We assessed planktonic gross primary production with the ^{18}O method ($GPP^{18}O$) in a high-arctic marine ecosystem in North East Greenland (Young Sound fjord) during the ice-free period of 2014. Four stations were sampled along the fjord three times each, in three different campaigns, from 1 August to 2 October 2014. Mean (± SE) $GPP^{18}O$ was low, 0.123 ± 0.026 mmol O_2 m^{-3} d^{-1} (range 0.001 – 0.330), which are by far the lowest estimates reported with this method to date. Correspondingly, mean chlorophyll *a* concentration was also low, 0.62 ± 0.11 µg L^{-1} (range 0.10 – 2.32). Specific $GPP^{18}O$ rates were significantly correlated with Photosynthetic Active Radiation and water temperature. The cold East Greenland Current and the input of freshwater and runoff might be important drivers causing this low production in Young Sound.

Introduction

Gross primary production (GPP) constraints the supply of the organic matter and energy required to support all components and processes in marine ecosystems, from microbial processes to the activity of top predators [*Duarte et al.,* 2011]. Hence, it is surprising that GPP has received limited attention in the Arctic Ocean [e.g. *Vaquer-Sunyer et al.,* 2013].

The few available estimates of GPP for the Arctic Ocean [*Regaudie-de-Gioux et al.,* 2010; *Vaquer-Sunyer et al.,* 2013; *Sejr et al.,* 2014; *Holding et al.,* 2015; *Stanley et al.,* 2015], are so high, particularly during the spring bloom, as to identify this ecosystem as one of the most productive regions in the ocean, sustaining large apical species, such as whales and polar bears. The very high GPP during the spring bloom is sustained over a limited time window, with much lower GPP rates through the rest of the year, when recycling processes dominate [*Vaquer-Sunyer et al.,* 2013; *Sejr et al.,* 2014; *Holding et al.,* 2015]. In Greenland, in particular, there is only one report of planktonic GPP, published recently for the plankton community in the subarctic Kobbefjord, at 64.10 ^{o}N [*Sejr et al.,* 2014]. This paper reports maximum GPP values of about 20 mmol O_2 m^{-3} d^{-1} in the spring, comparable to those reported for the spring bloom in the Svalbard region [*Wassmann et al.,* 2006; *Vaquer-Sunyer et al.,* 2013].

Primary production rates have been resolved through a number of methods, including ^{14}C and ^{13}C additions, bulk oxygen production and ^{18}O additions, providing different estimates [see *Regaudie-de-Gioux et al.,* 2014]. The differences in estimates resulting from the application of different methods has fostered an ongoing debate over the suitability of different estimates and what each method actually measures [*Quay et al.*, 2010; *Marra,* 2012; *Regaudie-de-Gioux et al.,* 2014; *Hancke et al.,* 2015]. One of the least applied techniques is the ^{18}O method, first introduced by Bender and coauthors in 1987, which measures the GPP using the stable isotope ^{18}O as a tracer of molecular oxygen production through photosynthesis [*Bender et al.,* 1987]. The sample water is enriched in ^{18}O derived by the photosynthetic release of ^{18}O from the hydrolysis of $H_2{}^{18}O$ during photosynthesis, and thus provides an estimate of GPP free of assumptions on the effect of light on respiration or possible losses of GPP due to respiratory processes, providing the most accurate measure of GPP with the fewest assumptions required during estimate of the rate [*Duarte et al.,* 2013; *Regaudie-de-Gioux et al.,* 2014]. It must be noted, however, that some of the O_2 produced is consumed during photosynthesis by the Mehler reaction, photorespiration, and plastoquinol terminal oxidase activity [e.g. *Eisenstadt et al.,* 2010], which would lead to ^{18}O estimates somewhat underestimating GPP. The only other incubation method providing an estimate of GPP is the Winkler bulk oxygen mass balance [*Regaudie-de-Gioux et al.,* 2014]. However, unlike the ^{18}O method, this method does not provide a direct estimate of GPP, but GPP is calculated as net community production plus community respiration. So, error in each of these estimates as well as assumptions, such as that respiration in the light equals that on the dark (specially relevant in the longer photoperiod of the spring/summer period of the Arctic), compounds on the resulting GPP estimates. While incubation-free methods, such as the triple oxygen method [e.g. *Luz et al.,* 1999; *Luz and Barkan,* 2000; *Juranek and Quay,* 2013] can also elucidate GPP, they require that the photic layer is encompassed within an actively-mixing mixed layer, a condition that is often not met in the Arctic, where ice cover and/or shallow pycnoclines derived from ice melting prevent mixing of the water column [*Randelhoff et al.,* 2014]. Despite the superiority of the ^{18}O method to estimate GPP [*Duarte et al.,* 2013; *Regaudie-de-Gioux et al.,* 2014], less than twenty studies, to the best of our knowledge, have used this method to date, with only estimates derived from an experiment assessing responses to CO_2 additions, reported in the Arctic Ocean [*Holding et al.,* 2015].

Here we assess planktonic GPP with the ^{18}O method ($GPP^{18}O$) in Young Sound fjord during the ice-free period of 2014.

Few studies have been carried out in Northeast Greenland, where Young Sound (74 °N) is the first fjord to be fully surveyed [*Rysgaard et al.,* 2003]. The water masses in this high-arctic fjord come from both the Greenland Sea and the Greenland Ice Sheet (by direct meltwater flux), which makes Young Sound an appropiate study area to monitor the effects of climate change [*Rysgaard and Nielsen,* 2006]. This led to the establishment of ZERO (Zackenberg Ecological Research Operations) station. Previous research on planktonic GPP in Greenland fjords has been limited to the subarctic Kobbefjord in west Greenland [*Sejr et al.*, 2014], so the results presented here from a high-arctic east Greenlandic fjord are novel.

Methods

The study was conducted in Young Sound, a high-arctic (74 °N) marine ecosystem in NE Greenland (Fig. 3.1). The fjord is 2-7 km wide and ≈ 90 km long, covering an area of 390 km^2, with an average depth of ≈ 100 m [*Rysgaard et al.,* 2003]. Depth varies along Young Sound, with a maximum of 360 m in the deep inner fjord, between 140 and 150 m depth in the shallower outer fjord, and a 50 m deep sill at its mouth that reduces the water exchange between the Greenland Sea and the fjord [*Kirillov et al.,* 2015]. Ice regulates the processes occurring in the fjord throughout the year. The breakage of fast ice in the fjord in July, together with melting snow and ice on land, result in freshwater inputs to the system, which strongly affect the hydrographic conditions in Young Sound during summer [*Rysgaard et al.,* 1999, 2003; *Bendtsen et al.,* 2014]. New ice, beginning to form in September, can grow to 10-15 cm thick by early October [*Rysgaard et al.,* 2003].

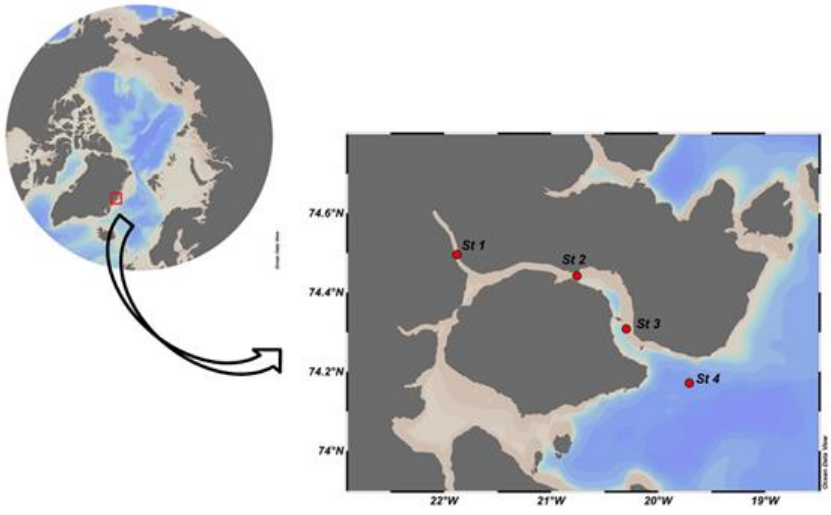

Fig. 3.1. Location of the 4 stations sampled along the fjord

Four stations were sampled in each of three different campaigns from 1 August to 2 October 2014, during the ice-free period of the fjord (Fig. 3.1). The ice had started to break the 15th July. Water samples were collected at 1 m depth and at the depth of the deep chlorophyll maximum (DCM, 21.82 ± 1.96 m), which receives, on average, 1% of the incident radiation on the surface. Surface waters were sampled manually with a 5 L Niskin bottle, while the DCM waters were sampled using a Rosette sampler system fitted with 1.2 L Niskin bottles and a SeaBird CTD probe. For the assessment of gross primary production (GPP^{18}O), eight 12-ml glass vials (Exetainer, Labco, UK) were filled for each depth, after screening the seawater through 180 µm mesh to remove large zooplankton. Four replicate samples were immediately fixed (biological activity stopped) with 50 µl of saturated $HgCl_2$ solution for later analysis of initial $\delta^{18}O(O_2)$ values, and stored upside down in darkness. The other four vials, containing glass balls inside, were spiked with 200 µl of 98% $H_2{}^{18}O$. After being closed, these spiked vials were immediately agitated, to ensure that $H_2{}^{18}O$ was homogeneously distributed inside the vial. Eight borosilicate glass narrow-mouth Winkler bottles were filled and fixated from each depth, for later determination of dissolved oxygen concentration using Winkler titration [*Hansen,* 1999]. The spiked samples were incubated in situ for 48 hours, deploying a buoy from the deck of the ship, and anchoring it to the bottom. After the incubation, the vials were fixed with 50 µl of saturated $HgCl_2$ solution and stored upside down in darkness.

In the laboratory, a 4-ml headspace was generated in each vial, by flushing with a helium flow. Special attention was paid not to contaminate samples with atmospheric oxygen at any time. The vials were left for equilibration for 24 hours at room temperature, so that the dissolved gases in the water equilibrated with the headspace, originally 100% helium. After 24 hours, the $\delta^{18}O$ 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 ‰. The flow was passed through a liquid nitrogen trap before entering GasBench II to retain water vapour. Oxygen and nitrogen were separated in a Molecular Sieve 5Å chromatographic column. Data, which were corrected using atmospheric air as reference, are reported as $\delta^{18}O$ value (‰) relative to V-SMOW (Vienna Standard Mean Ocean Water) standard.

$\delta^{18}O(H_2O)$ of spiked samples was measured in a liquid water isotope analyzer (Los Gatos Research), with precision of 0.1 ‰. In order to avoid contamination of the analyzer with highly ^{18}O-enriched H_2O, the spiked water was diluted (approximately 1:30) with a laboratory standard of known isotopic composition.

$GPP^{18}O$ in 24 h (mmol O_2 m^{-3} d^{-1}) was calculated using the equation of *Bender et al.* [1999]:

$$GPP^{18}O = [(\delta^{18}O_{final} - \delta^{18}O_{initial})/(\delta^{18}O_{water} - \delta^{18}O_{initial})] \times [O_2]_{initial}$$

where $\delta^{18}O_{initial}$ and $\delta^{18}O_{final}$ are the initial and final $\delta^{18}O$ of dissolved O_2 (‰), respectively; $\delta^{18}O_{water}$ is the $\delta^{18}O$ of the spiked sea water (‰); and $[O_2]_{initial}$ is the initial O_2 concentration (mmol O_2 m^{-3}) measured by the Winkler method. Rates are expressed as the mean and standard error of replicate samples (mmol O_2 m^{-3} d^{-1}).

Chlorophyll a was extracted in 5 ml 96% ethanol for 12-24 hours and analysed on a Turner Design fluorometer calibrated against a chlorophyll a standard before and after HCl addition [*Jespersen and Christoffersen*, 1987]. Photosynthetic active radiation (PAR) was measured at each depth with a spherical 4 Pi sensor (Biospherical QSP2000), and is expressed in μE cm^{-2} s^{-1}.

Results

Water temperature for the communities sampled ranged from -1.26 °C to 7.31 °C, both recorded in the August campaign, in stations 4 and 2, respectively, with a mean water temperature of 1.48 ± 0.51 °C across the study. For each campaign, water temperature decreased from the inner to the outter fjord by, on average, 2.95 °C and 1.97 °C for surface and DCM depths, respectively. In general, water temperature also decreased with depth from surface to DCM, except in the October campaign, when it increased in 3 out of the 4 stations sampled. The mean water temperature decreased along the study, from 2.25 °C, 1.96 °C and 0.41 °C in August, September and October, respectively.

The DCM was located at an average depth of 21.82 ± 1.96 m, and varied from a mean of 28.67 ± 1.63 m in the August campaign to 19.25 ± 4.41 m and 19.25 ± 0.87 m in September and October campaigns. The waters at Young Sound supported a low mean chlorophyll *a* concentration of 0.62 ± 0.11 μg Chl *a* L^{-1}, ranging from minimum values of 0.10 μg Chl *a* L^{-1} –observed at 1 m depth in station 2 to a maximum value of 2.32 μg Chl *a* L^{-1} observed at 30 m depth in station 4.

Volumetric $GPP^{18}O$ rates were low, ranging from 0.001 observed at 20 m depth in station 3 to a maximum value of 0.330 mmol O_2 m^{-3} d^{-1} observed at 1 m depth in station 2, with a mean value (± SE) of 0.123 ± 0.026 mmol O_2 m^{-3} d^{-1}. $GPP^{18}O$ rates were significantly higher

(t-test, t = -3.59, df = 17, p = 0.002) at the surface (mean 0.19 ± 0.03 mmol O_2 m^{-3} d^{-1}) than at the DCM (mean 0.05 ± 0.02 mmol O_2 m^{-3} d^{-1}). There was no significant correlation between $GPP^{18}O$ rates and chlorophyll *a* concentration. The Chl *a*-specific $GPP^{18}O$ rates increased significantly with PAR (Fig. 3.2b) and with water temperature (Fig. 3.3b).

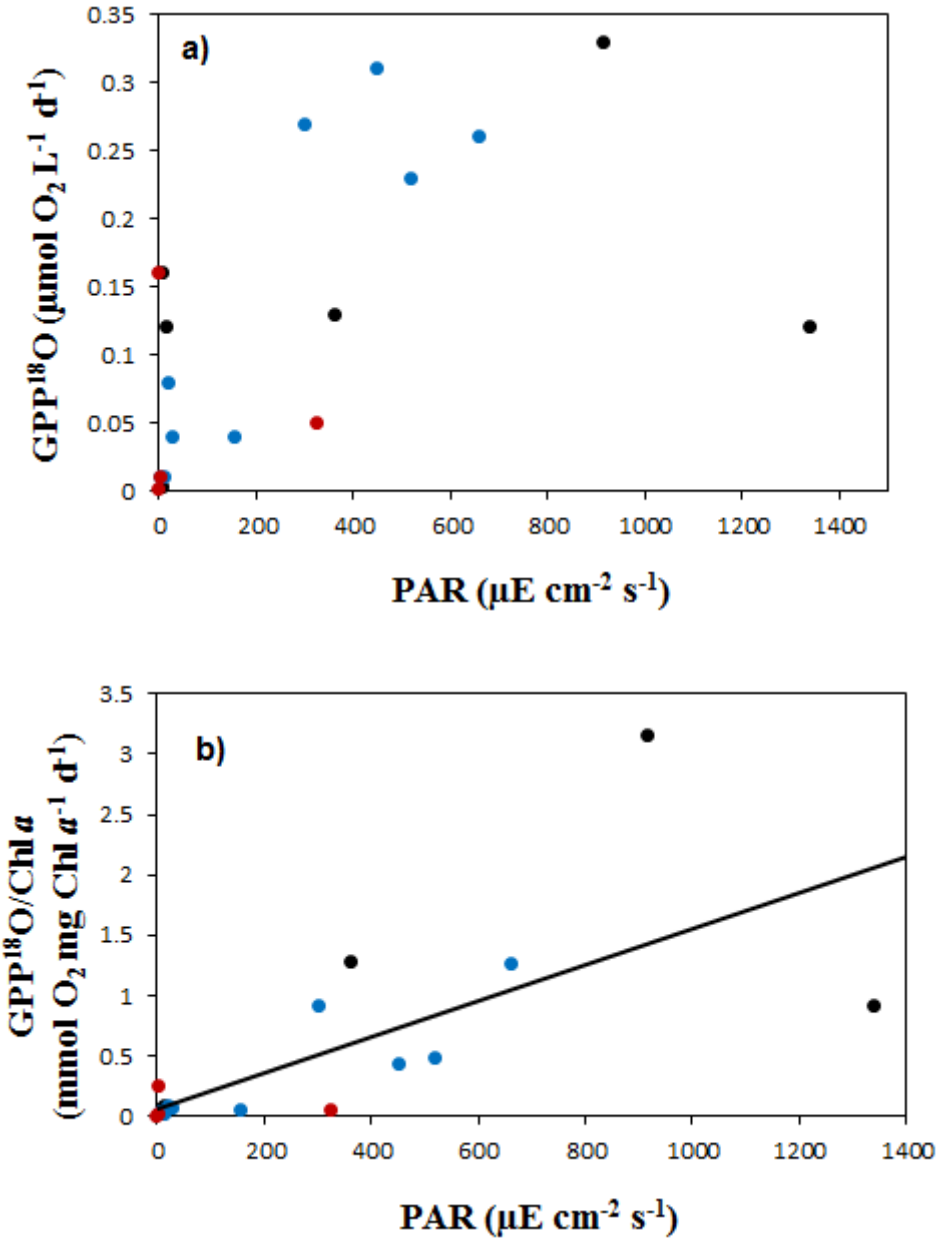

Fig. 3.2. Relationship between photosynthetic active radiation (PAR, µE cm^{-2} s^{-1}) and a) $GPP^{18}O$ (µmol O_2 L^{-1} d^{-1}), b) $GPP^{18}O$/Chl *a* (mmol O_2 mg Chl a^{-1} d^{-1}). Fitted regression equation: y = 0.0015 (± 0.0003) x + 0.06 (± 0.14) (R^2 = 0.52, p < 0.001, N = 22). Black/blue/red dots: estimates in August/September/October, respectively.

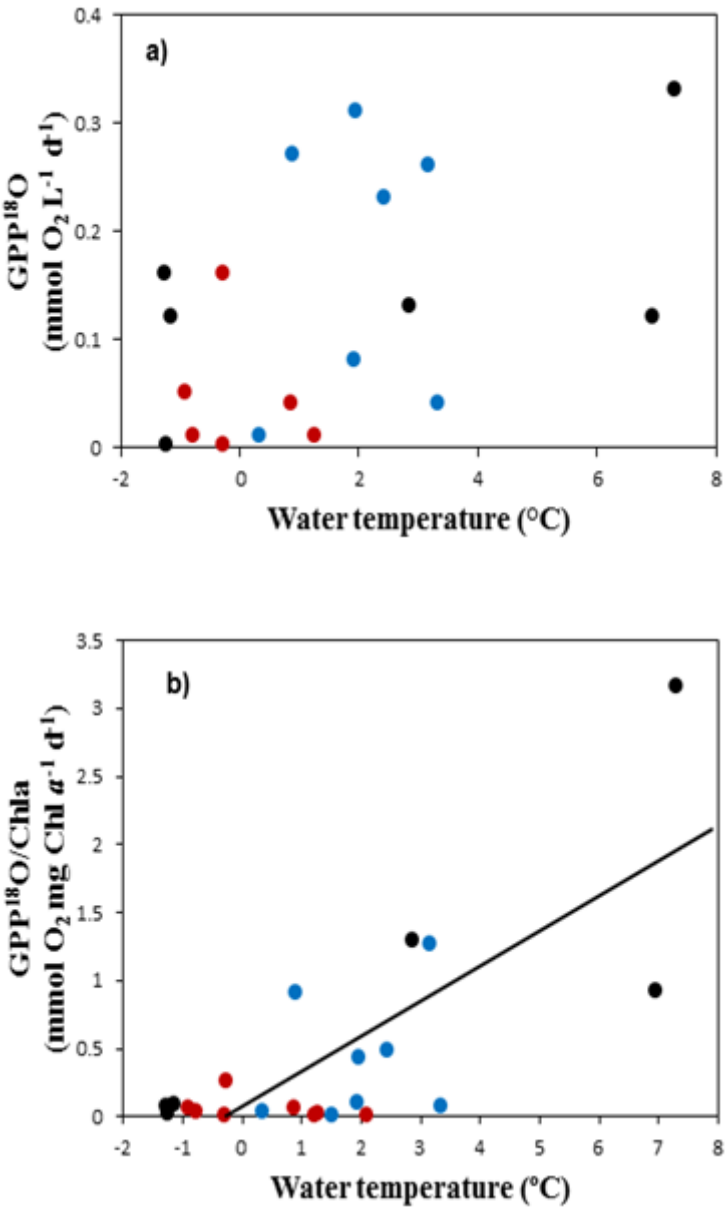

Fig. 3.3. Relationship between water temperature (°C) and a) GPP^{18}O (mmol O_2 L^{-1} d^{-1}), b) GPP^{18}O/Chl *a* (mmol O_2 mg Chl a^{-1} d^{-1}). Fitted regression equation: y = 0.23x (± 0.05) + 0.07 (± 0.13), R^2 = 0.53, p < 0.001, N = 22). Black/blue/red dots: estimates in August/September/October, respectively.

Discussion

The GPP^{18}O rates measured in Young Sound (mean 0.123 ± 0.026 mmol O_2 m^{-3} d^{-1}) are by far the lowest estimates assessed by this method to date. *Juranek and Quay* [2005] reported the lowest mean volumetric rates so far, 0.78 ± 0.10 mmol O_2 m^{-3} d^{-1}, in the oligotrophic waters of the subtropical Pacific Ocean.

Estimates of GPP are very scarce in Greenland coastal waters, particularly along the east coast. High rates of primary production have been measured along West Greenland, mainly using the ^{14}C tracer method [*Steemann Nielsen,* 1952]. *Jensen et al.* [1999] reported an overall primary production for the studied area of 5.58 to 267.25 mmol O_2 m^{-2} d^{-1} (mean ± SD = 28.42 ± 61.92 mmol O_2 m^{-2} d^{-1}). For the period between 2005 and 2012, *Juul-Pedersen et al.* [2015] reported a total annual primary production between 7050 and 11592 mmol O_2 m^{-2} yr^{-1}, for sub-Arctic Kobbefjord fjord. *Sejr et al.* [2014], using the Winkler method, reported GPP rates between 0.5 and 20 mmol O_2 m^{-3} d^{-1} in May and September for sub-Arctic Kobbefjord fjord, much higher than the rates reported here for Young Sound. The only primary production estimates available for Young Sound, derived using the ^{14}C method, are from *Rysgaard et al.* [1996, 1999]. *Rysgaard et al.* [1996] measured primary production during the early summer thaw (first two weeks of July 1994) in a sampling site close to station 3 in our study, but they do not give a volumetric estimate: they only report a constant primary production rate of 5.3 mmol O_2 m^{-2} d^{-1} during the study period. Their reported constant Chl *a* concentration, 4.1 µg L^{-1}, is higher than our mean value for station 3, 0.7 ± 0.2 µg L^{-1}. Our measurements covered a different period than theirs, as ours started two weeks after the ice broke and continued up to 2 October 2014, which might explain the difference. *Rysgaard et al.* [1999] performed 11 measurements in a similar location, from 20 June to 25 August 1996. When the sea ice broke, they measured a primary production rate of 1 mmol O_2 m^{-3} d^{-1} in a subsurface bloom, a higher value than our first measurement in station 3 (on 7 August 2014), which was 0.12 mmol O_2 m^{-3} d^{-1} for both surface and DCM, but this was three weeks after the sea ice started to break. These results indicate that the waters in Young Sound are colder and less productive than those in fjord systems studied in the West coast of Greenland.

Different hydrological conditions might explain the higher annual production on the west coast of Greenland than that on the east coast. On the west coast, the warmer water of the Irminger Current controls the sea ice coverage, conditioning the length of the productive ice-free period [*Rysgaard et al.,* 1999], which is much longer than that in the East Greenland coast. Along the East Greenland coast, the East Greenland Current, which transports cold, often < 0 °C Arctic water southward along, is the main regulating factor [*Rysgaard et al.,* 1999]. These contrasting currents, transporting warm water poleward along West Greenland and cold Arctic water toward the south along the East coast of Greenland, result in highly contrasting thermal patterns as well as duration of the ice-free season. Thus, temperature might be indirectly conditioning the annual production rate by controlling sea ice cover and therefore, the duration

of the productive open water period [*Rysgaard et al.,* 1999]. Yet, the strong positive relationship between the Chl *a*-specific GPP and water temperature and between Chl *a*-specific GPP and underwater irradiance reported here, shows that temperature and underwater irradiance may be controlling specific primary production rates.

Other factors have been suggested to affect the low production in Young Sound, including the input of freshwater with land runoff. Runoff entering the fjord comes from land areas around Young Sound, local glaciers and the Greenland Ice sheet. The freshwater influx stratifies the surface layer strongly, preventing turbulent mixing of nutrients from deeper water into the photic zone, and consequently limiting annual primary production [*Sejr et al.,* 2011]. The low GPP^{18}O rates measured in our study agree well with former research in the outer region of Young Sound suggesting that mineralization is higher than primary production. Therefore, the system is net heterotrophic, depending on additional carbon input to balance mineralization [*Glud et al.*, 2000, 2002b]. Hence, the combined effects of cold temperature (due to the influence of the cold East Greenland Current) and extensive freshwater input, render the East Greenland coastal systems highly unproductive when compared with the productive, warmer, West Greenland coastal fjords. It is not clear how future warming of the East Greenland coast will affect the annual primary production, as both an overall increase or decrease might be possible depending on the magnitude of the multiple effects. A longer ice-free period would result in higher light availability, which would be expected to increase primary production [*Murray et al.,* 2015]. On the other hand, higher freshwater runoff would cause stronger stratification, leading to less vertical mixing of nutrients and consequently, lower primary production [*Tremblay et al.,* 2012].

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CHAPTER 4

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

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

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Abstract

Plankton respiration rate is a major component of global CO_2 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_{light} : R_{dark} ratio increased from an average value of 1.37 at the median R_{light} measured here (3.62 µmol O_2 L^{-1} d^{-1}) to an average value of 17.56 at the highest R_{light} measured here (15.8 µmol O_2 L^{-1} d^{-1}). The role of respiratory processes as a source of CO_2 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.

Introduction

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 ecosystem [*Duarte et al.,* 2011]. Oceanic respiration, estimated to release 66 Gt C $year^{-1}$ globally, is one of the main elements of the carbon flux in the biosphere [*Del Giorgio and Duarte*, 2002], but remains the least constrained term in most models of metabolism, gas exchange and carbon mass balance in the ocean [*Balkanski et al.,* 1999; *Williams and Del Giorgio,* 2005].

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 (R_{dark}) to be equivalent to that in the light (R_{light}) [*Williams and Del Giorgio,* 2005]. However, published reports suggest that respiration in the light might be higher than that in the dark [*Harris and Lott,* 1973; *Bender et al.,* 2000; *Robinson et al.,* 2009], 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 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 [*Vaquer-Sunyer et al.,* 2010; *Duarte et al.,* 2012; *Holding et al.,* 2013]. 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 ($GPP^{18}O$), evaluated with the ^{18}O method and net community production (NCP), evaluated from bulk oxygen mass balance, of communities incubated under ambient incoming irradiance.

Methods

Plankton community respiration 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. 4.1). Respiration 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.

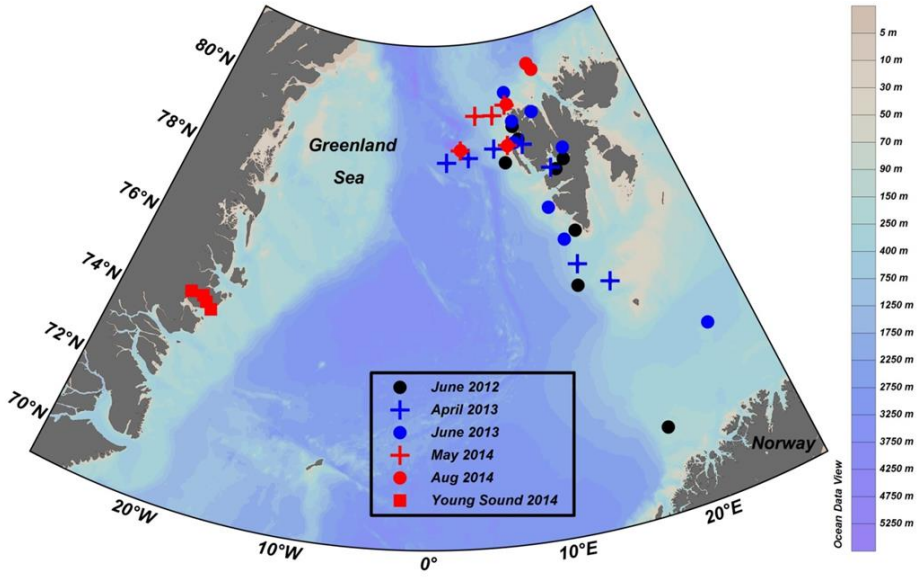

Fig. 4.1. Location of the stations sampled. Map created with Ocean Data View software (version 4.6.3, http://odv.awi.de/)

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), depth of chlorophyll maximum, 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-precission Winkler titration [*Carpenter,* 1965; *Carrit and Carpenter,* 1966] in Svalbard cruises and by visual end-point detection [*Grasshoff,* 1983] in Young Sound, and (2) respiration in the light (R_{light}) was assessed as the difference between gross primary production ($GPP^{18}O$), evaluated using $H_2{}^{18}O$ additions [*Bender et al.,* 1987], and net community production (NCP), evaluated from oxygen changes resolved using high-precision Winkler titration [*Carpenter,* 1965; *Carrit and Carpenter,* 1966] in Svalbard and using visual end-point detection [*Grasshoff,* 1983] in Young Sound, of 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 disolved 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 titrator 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, ± 1°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 Titrando [*Oudot et al.,* 1988] 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 μmol O_2 L^{-1} d^{-1} and standard errors were calculated using error propagation. In order to compare the R_{light}:R_{dark} ratios obtained here with those observed in the past, we surveyed the literature for results reported in the past [*Bender et al.,* 1987; *Grande et al.,* 1989a; *Bender et al.,* 2000; *Hitchcock et al.,* 2000; *Dickson and Orchardo,* 2001; *Robinson et al.,*

2009]. An extreme value reported by one of the studies [*Robinson et al.,* 2009] (ratio R_{light}:R_{dark} = 19), 8-fold higher than the rest, was excluded from the comparison.

For evaluation of $GPP^{18}O$, eight 12-ml glass vials were filled per depth. Four replicate samples were immediately fixed (biological activity stopped) with 80 µl of saturated $HgCl_2$ solution for later analysis of initial $\delta^{18}O(O_2)$ values, and stored upside down in darkness. The other four vials, containing beads inside to ensure mixing, were spiked with 80 µl and 200 µl 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 µl 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 $\delta^{18}O$ 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 ‰. $\delta^{18}O$-H_2O of spiked samples was measured in a liquid water isotope analyzer (Los Gatos Research), with precision of 0.1 ‰, and $GPP^{18}O$ was calculated [*Bender et al.,* 1999].

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.

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 4.1). Mean monthly community respiration rates in the Svalbard region were highest in April and lowest in August (Fig. 4.2), 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. 4.2), 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. 4.3A, 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 DCM, and surface samples having the minimum mean respiration rate in the light among the three depths (Table 4.1). 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 4.2, Fig. 4.3C, D).

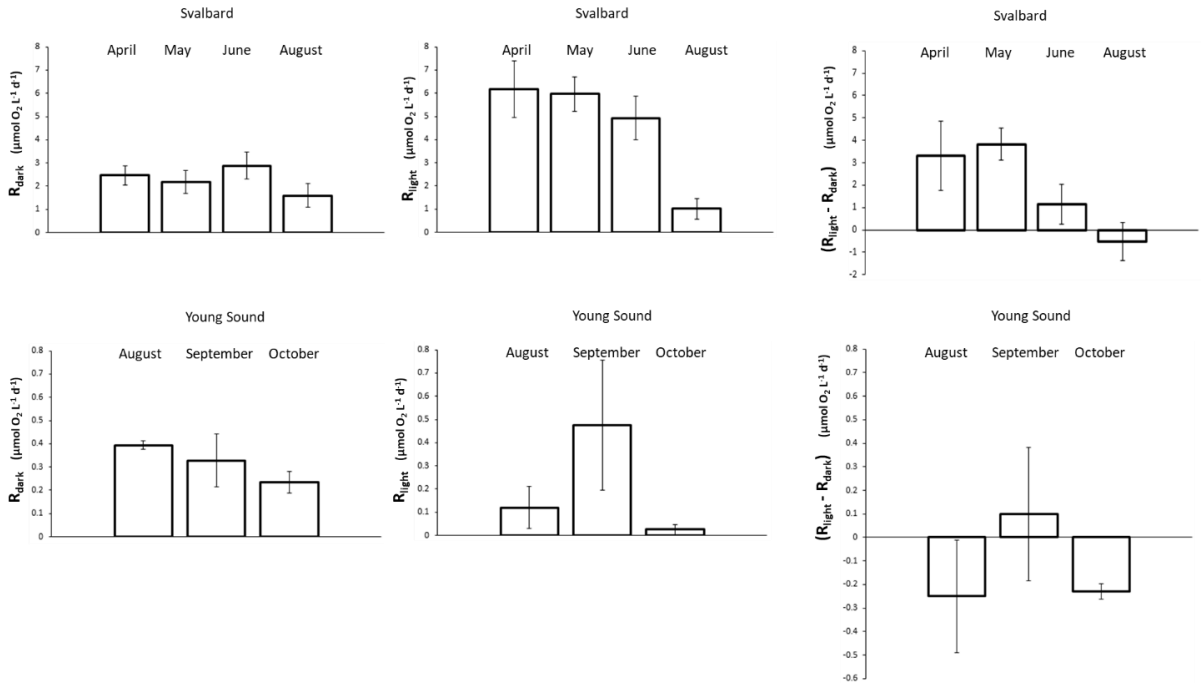

Fig. 4.2. Mean (± SE) monthly respiration in the dark (Rdark), respiration in the light (Rlight), and the difference between both (Rlight – Rdark) in the Svalbard region and Young Sound.

	Svalbard														
	R_{dark}					R_{light}					$R_{light}:R_{dark}$				
	mean ± SE	**median**	**min**	**max**	**N**	**mean ± SE**	**median**	**min**	**max**	**N**	**mean ± SE**	**median**	**min**	**max**	**N**
Surface	2.50 ± 0.42	2.06	0.21	8.25	28	3.18 ± 0.64	2.68	0.09	9.56	20	2.87 ± 1.08	1.40	0.03	17.88	18
20% PAR	2.68 ± 0.72	1.66	0.12	17.69	25	6.67 ± 0.93	6.59	0.76	13.35	19	7.66 ± 3.31	2.06	0.57	52.51	18
DCM	2.40 ± 0.49	1.64	0.10	11.52	28	5.65 ± 0.92	4.01	0.44	15.80	27	5.56 ± 1.84	2.48	0.24	39.80	24
Overall	2.52 ± 0.31	1.76	0.10	17.69	81	5.20 ± 0.52	3.97	0.09	15.80	66	5.38 ± 1.26	1.85	0.03	52.51	60

	Young Sound														
	R_{dark}					R_{light}					$R_{light}:R_{dark}$				
	mean ± SE	**median**	**min**	**max**	**N**	**mean ± SE**	**median**	**min**	**max**	**N**	**mean ± SE**	**median**	**min**	**max**	**N**
Surface	0.30 ± 0.09	0.25	0.03	0.89	9	0.51 ± 0.24	0.68	0.12	0.73	3	2.27 ± 2.09	0.82	0.32	5.67	3
20% PAR	-----	-----	-----	-----	----	-----	-----	-----	-----	----	-----	-----	-----	-----	----
DCM	0.34 ± 0.10	0.29	0.06	0.77	7	0.02 ± 0.01	0.02	0.01	0.04	3	0.12 ± 0.04	0.14	0.05	0.17	3
Overall	0.32 ± 0.06	0.27	0.03	0.89	16	0.27 ± 0.15	0.08	0.01	0.73	6	1.20 ± 0.99	0.25	0.05	5.67	6

	Overall														
	R_{dark}					R_{light}					$R_{light}:R_{dark}$				
	mean ± SE	**median**	**min**	**max**	**N**	**mean ± SE**	**median**	**min**	**max**	**N**	**mean ± SE**	**median**	**min**	**max**	**N**
Surface	1.96 ± 0.35	1.22	0.03	8.25	37	2.83 ± 0.59	2.55	0.09	9.56	23	2.78 ± 0.94	1.37	0.03	17.88	21
20% PAR	2.68 ± 0.72	1.66	0.12	17.69	25	6.67 ± 0.93	6.59	0.76	13.35	19	7.66 ± 3.31	2.06	0.57	52.51	18
DCM	1.99 ± 0.41	1.26	0.06	11.52	35	5.09 ± 0.88	3.97	0.01	15.80	30	4.96 ± 1.66	1.18	0.05	39.80	27
Overall	2.16 ± 0.27	1.47	0.03	17.69	97	4.78 ± 0.50	3.62	0.01	15.80	72	5.00 ± 1.15	1.57	0.03	52.51	66

Table 4.1. Mean ± standard error (SE), median, range and number of estimates (N) for volumetric (μmol O_2 L^{-1} d^{-1}) rates (respiration in the dark and in the light), and for the ratio between both.

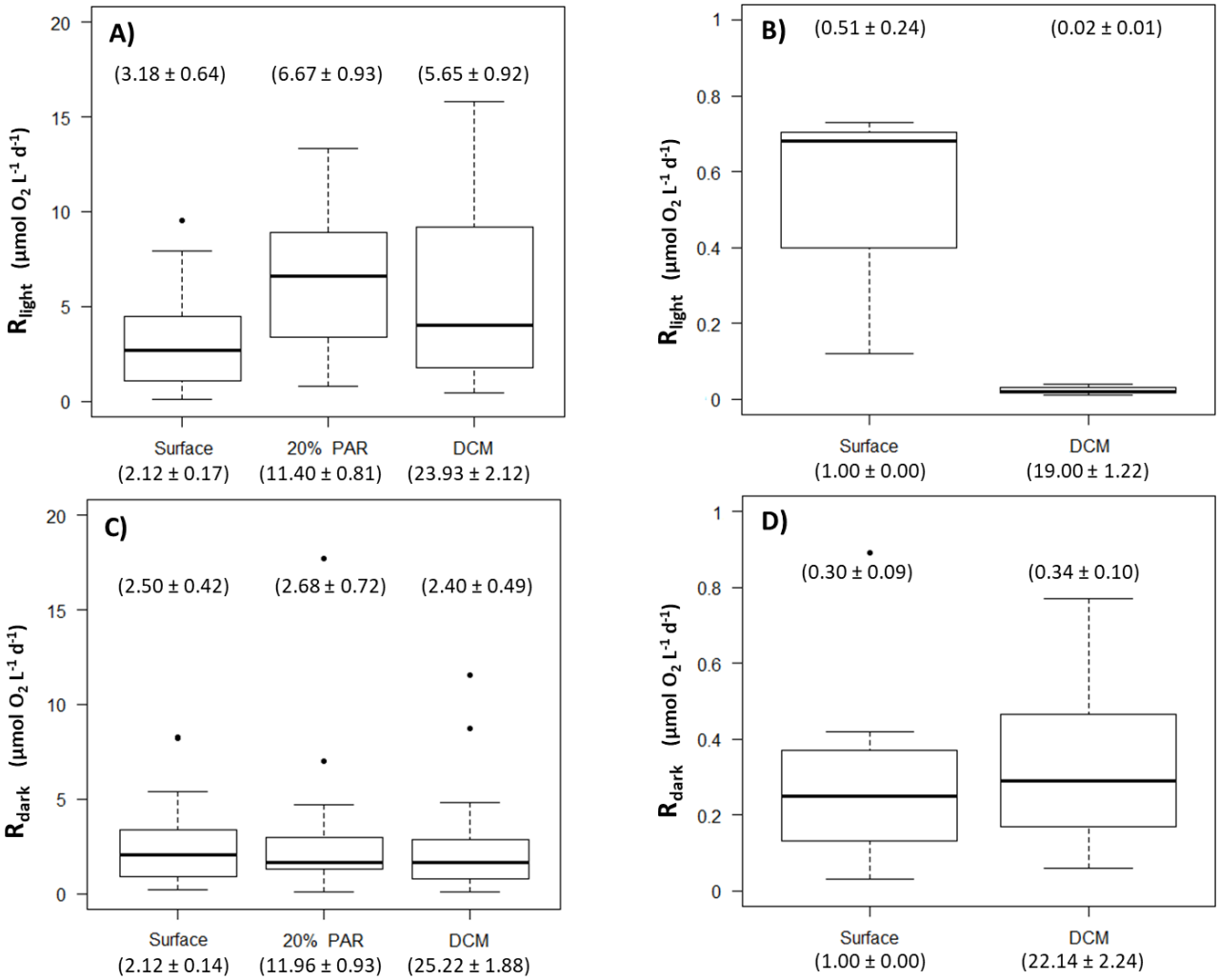

Fig. 4.3. Box-and-Whisker plots showing the distribution of community respiration A) in the light in Svalbard, B) in the light in Young Sound, C) in the dark in Svalbard and D) in the dark in Young Sound, for the depths sampled (mean depth ± SE in parentheses). The boxes show the median rate plus the 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.

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. 4.4A). 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. 4.2, 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_{light}:R_{dark} varied three orders of magnitude across communities (Table 4.1), and increased significantly ($R^2 = 0.50$, $P < 0.001$) in communities showing high respiration in the light (Fig. 4.4B). The fitted regression equation showed that the ratio R_{light}:R_{dark} was scaled to the 2/3 power of R_{light} (Fig. 4.4B), so that the R_{light} and R_{dark} were similar for R_{light} of the order of 1 µmol O_2 L^{-1} d^{-1}, but R_{light} was four-fold greater than R_{dark} for high R_{light} rates of the order of 10 µmol O_2 L^{-1} d^{-1} (Fig. 4.4B).

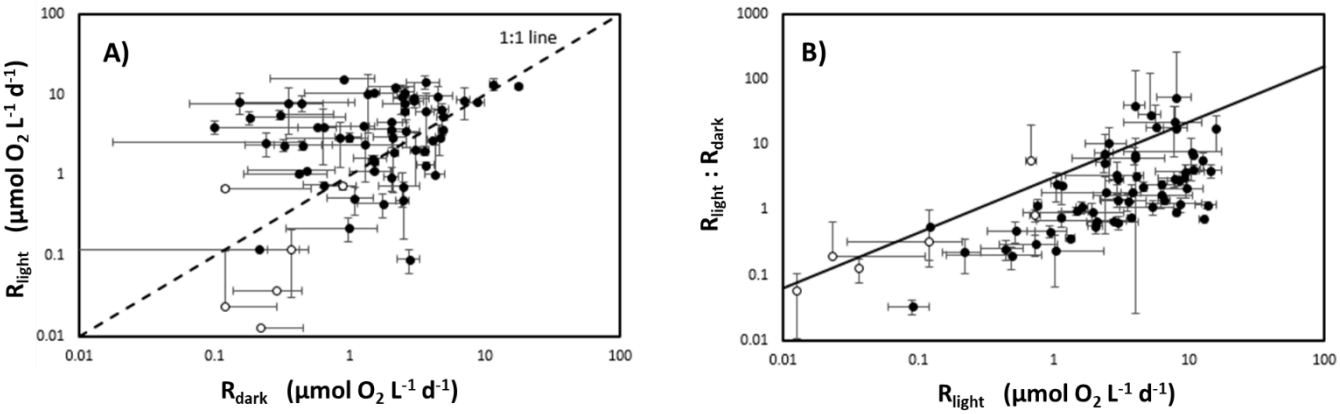

Fig. 4.4. The relationship between A) respiration in the dark (R_{dark}) and that in the light (R_{light}), and B) respiration in the light (R_{light}) and the ratio between respiration in the light and that in the dark (R_{light} : R_{dark}) in the Svalbard region (black symbols) and in Young Sound (white symbols). The solid line in B) shows the fitted regression line Ln (R_{light}:R_{dark}) = -0.02 + 0.68 * Ln (R_{light}) (µmol O_2 L^{-1} d^{-1}), $R^2 = 0.50$, $P < 0.001$. Error bars are the SE.

The difference between community respiration rates evaluated in the light and in the dark increased significantly with increasing $GPP^{18}O$ rates (Fig. 4.5), with no significant difference in community respiration rates at $GPP^{18}O$ rates < 10 µmol O_2 L^{-1} d^{-1} (Fig. 4.5).

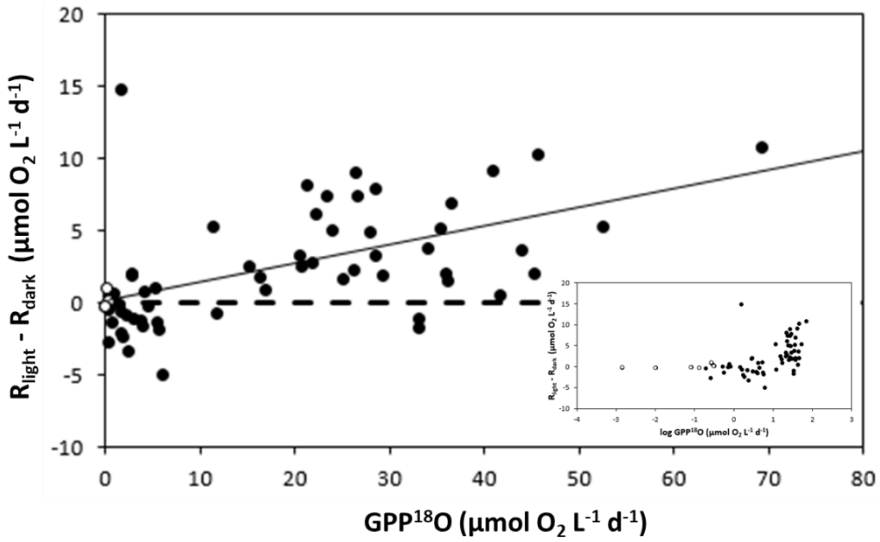

Fig. 4.5. The relationship between gross primary production ($GPP^{18}O$) and the difference between community respiration in the light and that in the dark (R_{light} - R_{dark}) in the Svalbard region (black symbols) and in Young Sound (white symbols). The insert shows the same figure with log-transformed gross primary production (log $GPP^{18}O$), to allow examination of the values at low $GPP^{18}O$ values.

Discussion

The respiration of plankton communities is a major component of the carbon budget of the oceans [*Del Giorgio and Duarte*, 2002]. Yet, estimates of community respiration rates are much less frequent than those of primary production, particularly in the Arctic Ocean (white symbols) [*Sherr and Sherr*, 2003; *Cottrell et al.*, 2006; *Regaudie-de-Gioux and Duarte*, 2010; *Vaquer-Sunyer et al.*, 2013; *Sejr et al.*, 2014]. 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 [*Grande et al.*, 1989a; *Hitchcock et al.*, 2000; *Bender et al.*, 2000; *Dickson and Orchardo*, 2001; *Robinson et al.*, 2009], involving all, except two [*Marra and Barber*, 2004; *González et al.*, 2008], 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 [*Bender et al.*, 2000; *Hitchcock et al.*, 2000]. These studies also concluded that

respiration in the light tends to be greater than that in the dark. The mean vertically-integrated R_{light} : R_{dark} ratio was reported to be 1.95 in a summer cruise around 76 °S in the Ross Sea [*Bender et al.,* 2000]; 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 region [*Hitchcock et al.,* 2000]. The median R_{light} : R_{dark} ratio in our study was 1.57, within the range of values reported for Southern Ocean plankton communities [*Bender et al.,* 2000; *Hitchcock et al.,* 2000]. We found, however, that the R_{light} : R_{dark} ratio increased as the 2/3 power of R_{light} so that the R_{light} : R_{dark} increased from an average value of 1.37 at the median R_{light} measured here (3.62 µmol O_2 L^{-1} d^{-1}) to an average value of 17.56 at the highest R_{light} measured here (15.8 µmol O_2 L^{-1} 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 of grow primary production thus far available for the Arctic Ocean [*Sherr and Sherr,* 2003; *Cottrell et al.,* 2006; *Regaudie-de-Gioux and Duarte,* 2010; *Vaquer-Sunyer et al.,* 2013; *Sejr et al.,* 2014]. This was interpreted to indicate that R_{light} tends to be higher than R_{dark} [*Regaudie-de-Gioux et al.,* 2014], 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_{ligth}:R_{dark} ratio predicted from the regression equation in Fig. 4.4, the NCP predicted as the difference between GPP^{18}O and this corrected R estimate is strongly consistent with the observed NCP (Fig. 4.6). Hence, whereas reported NCP for plankton communities in the Arctic Ocean [*Sherr and Sherr,* 2003; *Cottrell et al.,* 2006; *Regaudie-de-Gioux and Duarte,* 2010; *Vaquer-Sunyer et al.,* 2013; *Sejr et al.,* 2014] 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.

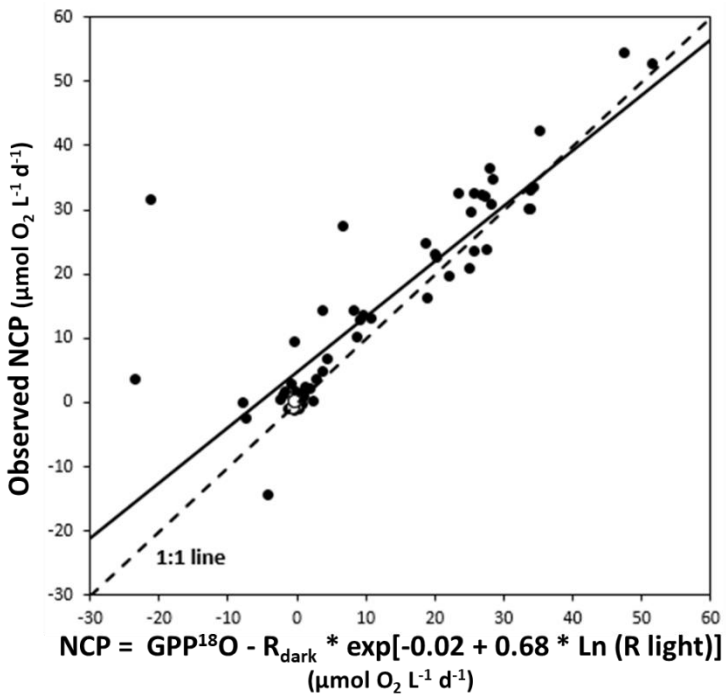

Fig. 4.6. The relationship between NCP calculated as $GPP^{18}O$ - R_{dark} * exp[-0.02 + 0.68 * Ln (R light)] and observed net community production (NCP) in the Svalbard region (black symbols) and in Young Sound (white symbols). The solid line shows the fitted regression equation: y = 0.86 (± 0.06) x + 4.81 (± 1.19) (R^2 = 0.74, p < 0.001, N = 66) .

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 reaction [*Bender et al.*, 1999]. Autotrophic respiration has also been proposed to dominate community R during the pre-bloom and bloom phases of the seasonal cycle in the Southern Ocean [*Goldman et al.*, 2015]. These observations are consistent with the observation that the difference between R_{light} and R_{dark} estimates increased with increasing gross primary production (Fig. 4.5). Figure 4.5 also shows that for $GPP^{18}O$ < 10 µmol O_2 L^{-1} d^{-1}, most R_{dark} tend to be higher than R_{light} , reflecting that metabolic processes supporting R_{light} may be specially enhanced over a $GPP^{18}O$ threshold of 10 µmol O_2 L^{-1} 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 Ocean [*Vaquer-Sunyer et al.*, 2013]. Estimates of net community production in the Arctic [*Vaquer-Sunyer et*

al., 2013; *Sejr et al.*, 2014], which are derived from incubations in the light, are not affected by the bias introduced by dark incubations to estimate respiration rates. However, 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.

Acknowledgements

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CHAPTER 5

Dissolved oxygen in Arctic waters: insights from $\delta^{18}O(O_2)$

Dissolved oxygen in Arctic waters: insights from $\delta^{18}O(O_2)$

Abstract

We measured $\delta^{18}O(O_2)$ and concentration of dissolved oxygen in Arctic waters during the spring-summer period of 2012, 2013 and 2014. Data were collected off the west margin of the Svalbard islands and in the Young Sound fjord, in North-East Greenland. Oxygen concentration (mean ± SE, 360.98 ± 2.59 µmol L^{-1}; range 303.80 - 431.39 µmol L^{-1}) correlated well with $\delta^{18}O(O_2)$ values (mean 23.91 ± 0.12 ‰, range 21.17 - 25.83 ‰). We suggest this correlation might be explained by gross primary production (GPP), respiration (R) and ice melting. $\delta^{18}O(O_2)$ residuals (‰) showed correlation with salinity values up to 33 ‰. The mean $\delta^{18}O(O_2)$ values for April, May, June, and August were 23.96 ± 0.31, 22.59 ± 0.16, 23.79 ± 0.19, and 25.31 ± 0.07 ‰, respectively.

Introduction

The concentration of $O_2(aq)$ is a fundamental property of aquatic environments, and is, therefore, one of the most commonly measured parameters in marine and freshwater systems. However, the isotopic composition of dissolved O_2, $\delta^{18}O(O_2)$, is far less measured but can provide detailed insight into photosynthesis, respiration (R), and the dissolution of atmospheric gases in aquatic systems [*Barth et al.,* 2004], and is a required parameter in calculations of gross primary production (GPP) using ^{18}O and in the triple oxygen isotope method [e.g. *Luz and Barkan,* 2000; *Juranek and Quay,* 2013]. A key reason for the paucity of studies on the isotopic composition of O_2 is the complexity of analyzing O_2 isotopes with traditional methods [*Roberts et al.,* 2000].

Like its concentration, the $\delta^{18}O(O_2)$ of oxygen dissolved in seawater is affected by air-water gas exchange, respiration, photosynthesis and mixing. Dissolved oxygen (DO) in equilibrium with the atmosphere has a stable isotope value of 23.88‰ [*Barkan and Luz,* 2005]. When gas exchange dominates over metabolic processes, including photosynthesis and respiration, as is the case in the surface ocean, dissolved oxygen is close to saturation [*Quay et al.,* 1993] and the $\delta^{18}O(O_2)$ has been estimated to be ~23.7‰ for the surface ocean [*Luz and Barkan,* 2011]. When photosynthesis and respiration are significant compared to air-sea

exchange, dissolved oxygen can present significantly lower and higher $\delta^{18}O(O_2)$ values, respectively [*Wissel et al.*, 2008]. $\delta^{18}O(O_2)$ has been used, together with oxygen measurements, to evaluate the ratio of GPP to R [*Bender and Grande*, 1987; *Quiñones-Rivera et al.*, 2007], to trace water masses [*Bender*, 1990; *Levine et al.*, 2009], or as a tool to study diurnal cycles in the coastal ocean [*Rafelski et al.*, 2015].

The Arctic Ocean is one of the most metabolically-intense marine ecosystems on Earth, acting as one of the most intense sinks for CO_2 [*Bates and Mathis*, 2009], particularly during the spring bloom when primary production reaches very high levels and respiratory activity is rather low [*Vaquer-Sunyer et al.*, 2013; *Sejr et al.*, 2014]. Moreover, cold waters together with intense photosynthetic activity by ice algae and plankton lead to some of the highest O_2 concentration in the ocean, although air-sea exchange is often impeded by ice cover and the presence of shallow pycnoclines derived from sea-ice melt [*Pabi et al.*, 2008]. Hence, the surface Arctic Ocean is likely to exhibit strong deviations from the expected $\delta^{18}O(O_2)$ of oxygen dissolved in seawater of 23.7‰. Unfortunately, this possibility has not yet been examined as no studies have quantified the distribution of $\delta^{18}O(O_2)$ values in the Arctic Ocean so far.

Here we examine the relationship between the variability in $\delta^{18}O(O_2)$ and the concentration of dissolved oxygen in Arctic waters during the spring-summer period of 2012, 2013 and 2014. $\delta^{18}O(O_2)$ was assessed based on samples collected off the west margin of the Svalbard Islands and Young Sound, a high-arctic marine ecosystem in NE Greenland (Fig. 5.1). We considered salinity, a proxy of freshwater inputs, as a possible factor affecting the relationship between $\delta^{18}O(O_2)$ and the concentration of dissolved oxygen, and examined the variability in the distribution of $\delta^{18}O(O_2)$ values during the months of April to August.

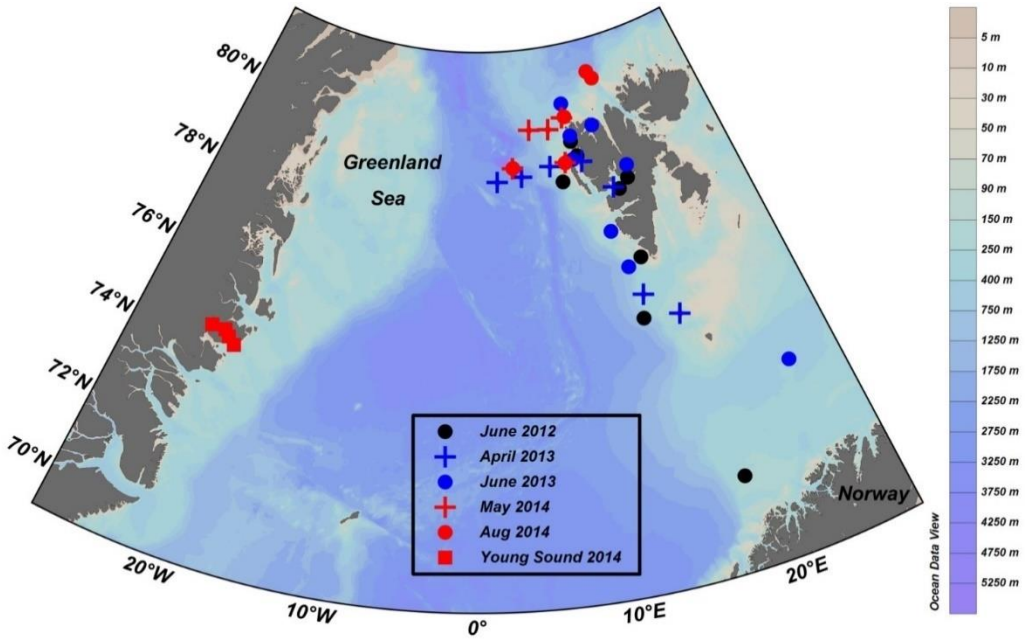

Fig. 5.1. Location of the stations sampled.

Methods

Samples were collected using a Rosette sampler system fitted with a calibrated CTD, from which salinity values were obtained. For the stations sampled in the Svalbard Island, three different depths were sampled: surface (2.12 ± 0.13 m), depth of chlorophyll maximum (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. In Young Sound stations, samples were collected at 1 meter depth and at the depth of chlorophyll maximum (DCM, 21.82 ± 1.96). At each depth, seven 100-mL narrow-mouth Winkler bottles were filled and fixed, and four 12-ml glass vials were filled and fixed with saturated $HgCl_2$ solution, for later determination of dissolved oxygen concentration and $\delta^{18}O(O_2)$ values, respectively.

The concentration of dissolved oxygen (µmol O_2 L^{-1}) was determined by automated high-precision Winkler titration [*Carpenter*, 1965; *Carrit and Carpenter*, 1966] in the case of Svalbard data. Measurements were performed with a Metrohm 888 Titrando using a potentiometric electrode and automated endpoint detection [*Oudot et al.*, 1988]. For Young Sound data, the dissolved oxygen concentration was determined using visual end-point detection [*Grasshoff*, 1983], as a titrator was not available.

$\delta^{18}O(O_2)$ values were determined after 24-hour equilibration with gases in the previously generated headspace of the vial. They were measured in a Finnigan GasBench II attached to a Finnigan DeltaPlusXP isotope ratio mass spectrometer, with precision better than 0.1 ‰. Data are reported as $\delta^{18}O(O_2)$ value (‰) relative to V-SMOW (Vienna Standard Mean Ocean Water) standard.

Results

Oxygen concentration ranged from 303.80 to 431.39 µmol O_2 L^{-1}, with a mean (± SE) concentration of 360.98 ± 2.59 µmol L^{-1}. Out of the total of 124 samples analyzed, 94 (75.8 %) were supersaturated and 30 (24.2%) were undersaturated. The corresponding $\delta^{18}O(O_2)$ values ranged broadly, from 21.17 ‰ to 25.83 ‰, with a mean (± SE) value of 23.91 ± 0.12 ‰, which was not significantly different from the expected value of 23.7 ‰. The $\delta^{18}O(O_2)$ values were significantly, and negatively correlated with the oxygen concentration ($R^2 = 0.27$; $p < 0.0001$), with the mean $\delta^{18}O(O_2)$ value at 100 % O_2 saturation being 23.88 ‰, the $\delta^{18}O(O_2)$ at atmospheric equilibrium in the absence of fractionation (Fig. 5.2). The residuals of the relationship between $\delta^{18}O(O_2)$ (‰) and oxygen concentration increased with salinity values up to 33 ‰ (Fig. 5.3). The distribution of $\delta^{18}O(O_2)$ values per month varied significantly (Kruskal-Wallis test, $P < 0.001$), with August having the highest monthly mean $\delta^{18}O(O_2)$ (± SE) value, 25.21 ± 0.06 ‰, and May having the lowest monthly mean, 22.59 ± 0.16 (Fig. 5.4).

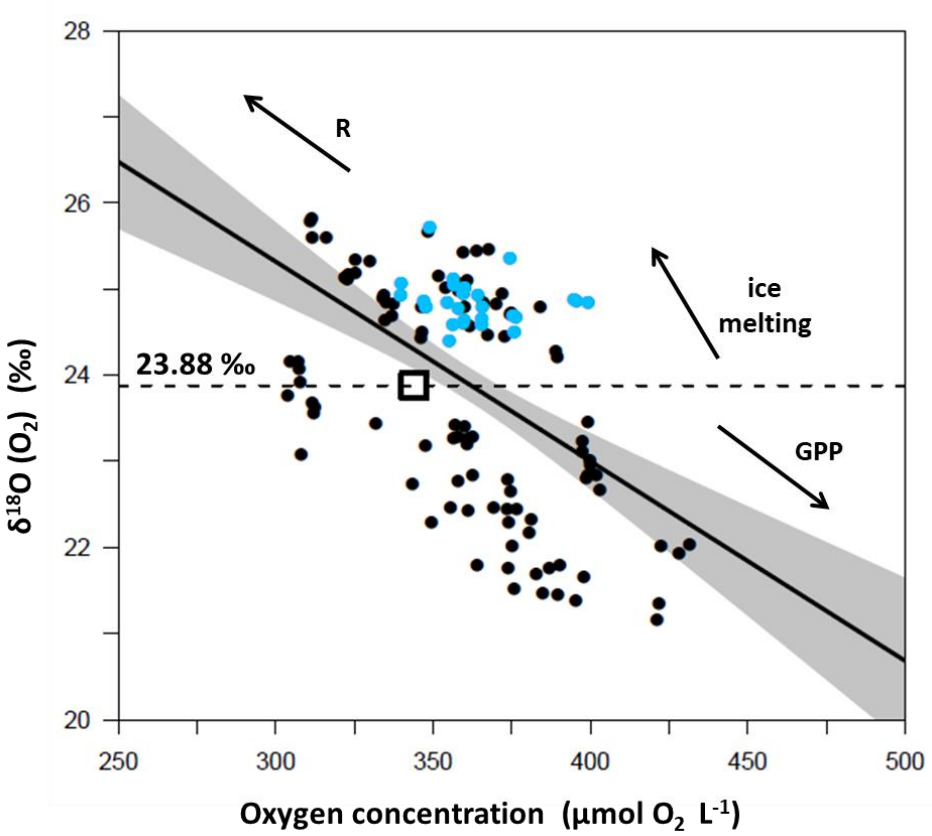

Fig. 5.2. Relationship between oxygen concentration (µmol O_2 L^{-1}) and $\delta^{18}O$ (O_2) (‰). Fitted regression equation: y = -0.023 (± 0.003) x + 32.272 (± 1.243) (N = 124; R^2 = 0.27; p < 0.0001). The shaded area encloses the 95% confidence limits of the regression slope. Black points: salinity > 33 ‰. Blue points: salinity < 33 ‰. Square : mean oxygen concentration at saturation, 343.98 ± 1.98 µmol O_2 L^{-1}.

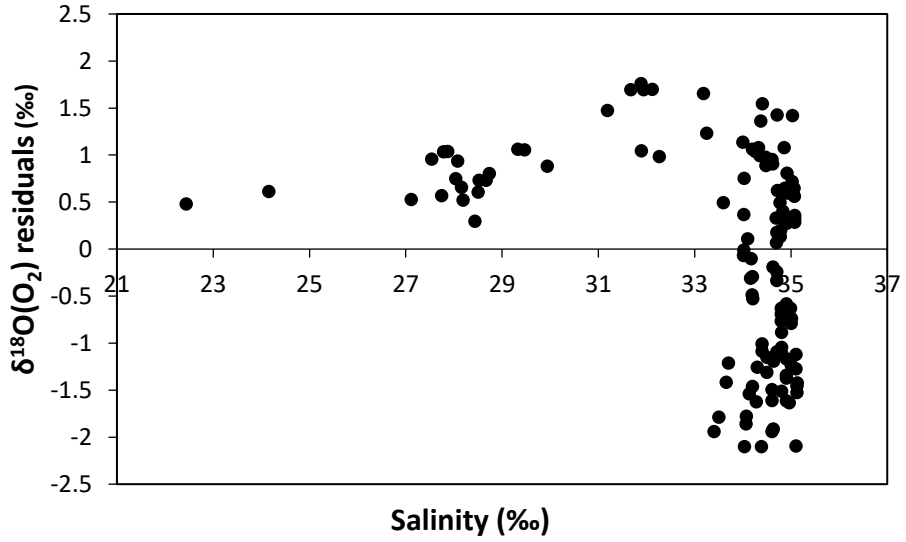

Fig. 5.3. Relationship between salinity (‰) and $\delta^{18}O(O_2)$ residuals (‰).

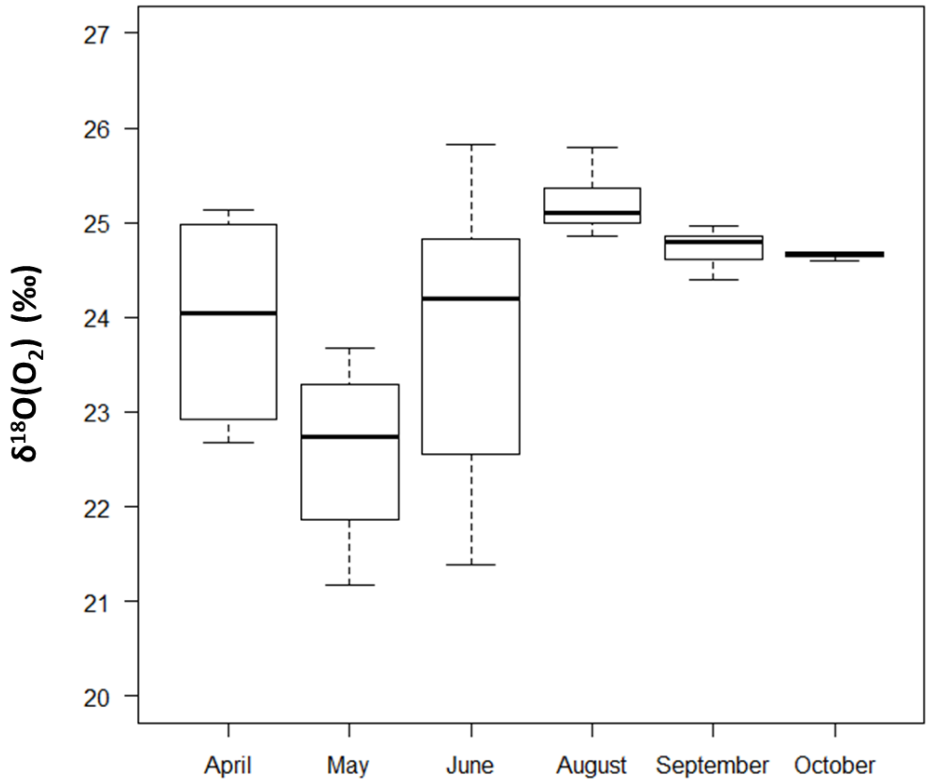

Fig. 5.4. Boxplot showing the distribution of $\delta^{18}O(O_2)$ (‰) per month. The central lines represent the median, the boxes encompass the lower (25%) and upper (75%) quartiles, and the whiskers extend to 1.5 times the interquartile range. The mean ± SE $\delta^{18}O(O_2)$ (‰) for April, May, June, August, September and October was 23.96 ± 0.31, 22.59 ± 0.16, 23.79 ± 0.19, 25.21 ± 0.06, 24.73 ± 0.05 and 24.66 ± 0.02, respectively.

Discussion

The concentration and isotopic composition of dissolved O_2 in seawater vary with air-sea gas exchange, photosynthesis, respiration and mixing [e.g., *Hendricks et al.*, 2005; *Levine et al.*, 2009]. Ventilation drives $[O_2]$ towards saturation and $\delta^{18}O(O_2)$ towards the stable isotope value of dissolved oxygen in equilibrium with the atmosphere, 23.88‰ [*Barkan and Luz*, 2005]. The difference between the $\delta^{18}O$ of air O_2 and that of seawater oxygen is known as the Dole Effect (DE), which *Bender et al.* [1994] defined as consisting of a terrestrial and a marine component- the effect that would result from O_2 exchange by photosynthesis and respiration on land alone or in the ocean alone. *Luz and Barkan* [2011] reevaluated the components of the DE and concluded that the marine and the terrestrial DE were of similar magnitude (23.5 ± 1.8 ‰ and 23.5 ± 2.3 ‰, respectively).

Gross primary production (GPP) decreases $\delta^{18}O(O_2)$ values because the oxygen that photosynthesis adds to the DO pool is isotopically depleted, as it comes from the source

seawater [*Guy et al.,* 1986; 1993], whose $\delta^{18}O$ composition is 0‰ [*International Atomic Energy Agency*, 2006]. In contrast, respiration increases the $\delta^{18}O(O_2)$ of the residual oxygen pool because it removes light oxygen preferentially, with a large fractionation factor of -15‰ to -25‰ [*Kroopnick,* 1975; *Quay et al.,* 1995; *Hendricks et al.,* 2004]. However, for the same amount of GPP and R, GPP decreases the $\delta^{18}O(O_2)$ more than R increases it [*Bender and Grande,* 1987; *Quay et al.,* 1993], and consequently, even if there is no net production, $\delta^{18}O(O_2)$ changes when O_2 is biologically used [*Bender et al.,* 2000]. These effects are shown in the distribution of $\delta^{18}O(O_2)$ values per month for our data (Fig. 5.4). May is the month when the effect of GPP is strongest, reaching a minimum $\delta^{18}O(O_2)$ value of 21.17 ± 0.07‰, most likely due to the combined effects of the ice-algae and pelagic plankton blooms in spring.

Our data show a very significant decrease in $\delta^{18}O(O_2)$ values as oxygen concentration increases, reflecting the important role of GPP, which tends to increase oxygen concentration and decrease $\delta^{18}O(O_2)$ values. The covariation between $\delta^{18}O(O_2)$ and O_2 concentration and how it is affected by photosynthesis and respiration, has been explored previously for surface waters of the Subarctic Pacific [*Quay et al.,* 1993]; for the Ross Sea in the Antarctic Ocean [*Bender et al.,* 2000]; in a eutrophic coastal ecosystem [*Quiñones-Rivera et al.,* 2007] and in the Southern California coastal ocean [*Rafelski et al.,* 2015].

We hypothesize that the addition of freshwater from Arctic ice melting might also be affecting $\delta^{18}O(O_2)$ values. Ice expels oxygen during freezing [*Top et al.,* 1985] and isotopic fractionation could occur if ^{16}O atoms were preferentially excluded, leading to ice enriched in $^{18}O(O_2)$ atoms. To the best of our knowledge, this has not been reported in the literature so far. Ice melting would then increase the $\delta^{18}O(O_2)$ values of the dissolved oxygen pool. This is consistent with our results, which showed $\delta^{18}O(O_2) > 23.88$ ‰ for all waters with salinity values < 33 ‰, therefore receiving freshwater inputs (Figs. 5.2, 5.3). All points with salinity < 33 ‰ have a $\delta^{18}O(O_2)$ above 23.88 ‰ (Fig. 5.2), and the $\delta^{18}O(O_2)$ residuals appear to be related to salinity up to a salinity value of 33 ‰ (Fig. 5.3). However, the $\delta^{18}O(O_2)$ residuals would be expected to decrease as salinity increases (assuming that $\delta^{18}O(O_2)$ increases with freshwater coming from sea ice melting), but what we find is the opposite tendency (Fig. 5.3). This might be explained by the fact that all samples with salinity < 33 ‰ correspond to Young Sound fjord, where the freshwater input comes not only from melting of sea ice, but also as runoff from land-terminating glaciers during June-September [*Rysgaard et al.,* 2003; *Bendtsen et al.,* 2014]. Indeed, the contribution of glacial rivers to surface freshwater has been estimated to be higher than 50% in a model for the inner fjord [*Bendtsen et al.,* 2014].

These results suggest that freshwater inputs are the dominant factor driving $\delta^{18}O(O_2)$ values in surface waters of the Arctic Ocean for salinity values < 33 ‰, while metabolic processes, either photosynthesis or respiration, drive $\delta^{18}O(O_2)$ values at higher salinity values. To the best of our knowledge, these are the first $\delta^{18}O(O_2)$ values reported for the Arctic Ocean, and the possible influence of ice melting in increasing the $\delta^{18}O(O_2)$ of the DO pool has not been reported in the literature before.

Acknowledgements

This study is a contribution to projects ARCTICMET and ATOS, funded by the Spanish Ministry of Economy and Competitiveness (CTM2011-15792-E and POL2006-00550/CTM, respectively), the ATP project funded by the FP-7 of the EU (CTM2009-07781-E), and the CarbonBridge project funded by the Norwegian Research Council. Work in Young Sound was funded by the Greenland Ecosystem Monitoring (GEM) program. We gratefully acknowledge the contributions from the Arctic Research Centre, Aarhus University. We thank the crew of R/V *Helmer Hanssen* and Young Sound participants for support, and L.G.C. and P.C. for help with oxygen determinations. This work is a contribution to the Arctic Science Partnership (ASP) asp-net.org.

GENERAL DISCUSSION

General discussion

This thesis is a significant step towards a better understanding of planktonic metabolism in the global ocean. It adds 124 stations to the 33 stations available to date (Fig D.1), so we are increasing the database almost four-fold (only twelve published studies have assessed GPP with the ^{18}O technique in the ocean thus far). In particular, this thesis provides data for underrepresented areas, such as the Pacific and Indian Oceans, including the southern subtropical gyres of the ocean. Moreover, we are reporting the first GPP^{18}O estimates for the Arctic Ocean: in the European Arctic Ocean, GPP had been evaluated only by the bulk oxygen method; and for Young Sound fjord in East Greenland, primary production had been measured exclusively with the ^{14}C method. Therefore, we are getting closer to a representative global picture of GPP^{18}O estimates.

We have also evaluated, for the first time, respiration under *in situ* solar radiation in the 24-hour photoperiod of the Arctic Ocean, demonstrating that respiration in the light is higher than that in the dark, contrary to the commonly used assumption of equal respiration in both conditions. Finally, we have explored the concentration and $\delta^{18}O$ of dissolved oxygen in Arctic waters, providing insights on the influence of planktonic metabolism (gross primary production and respiration) and ice melting on these $\delta^{18}O$ values, the first ones reported for Arctic waters. These are the first $\delta^{18}O$ values reported for Arctic waters.

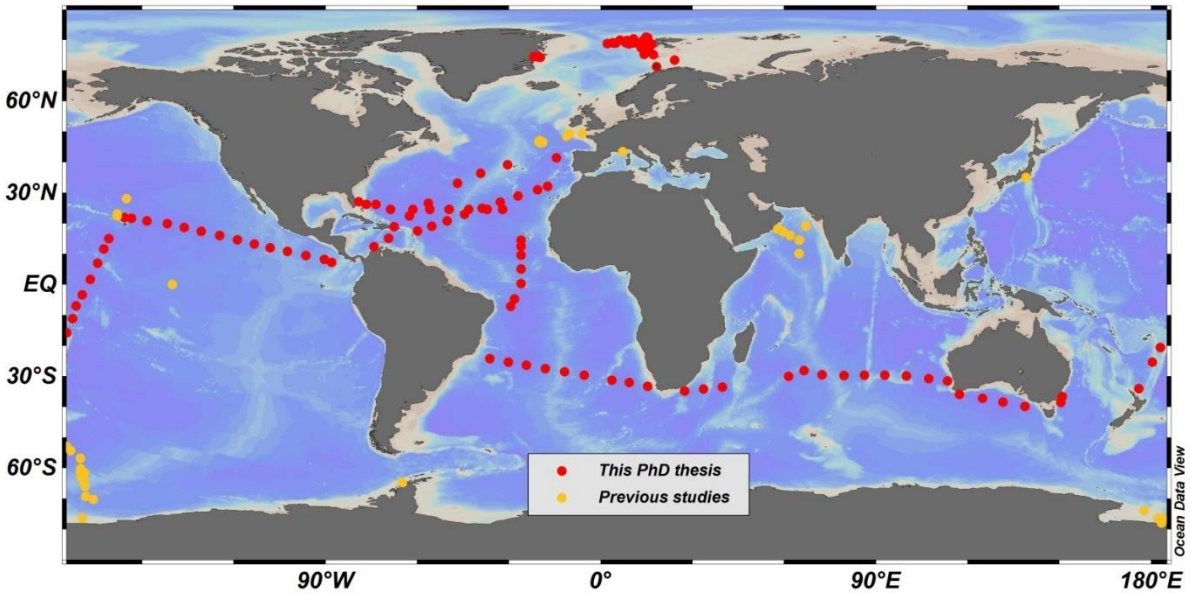

Fig D.1. Stations where GPP^{18}O was evaluated in this PhD thesis and in former published studies.

GENERAL CONCLUSIONS

General conclusions

I. The mean (± SE) $GPP^{18}O$ rate for the stations evaluated in the Malaspina Expedition was 0.60 ± 0.06 µmol O_2 L^{-1} d^{-1} (range 0.01 - 11.49). The study occupied four of the five subtropical gyres, being the first one to report $GPP^{18}O$ rates in these low chlorophyll areas, which explains that our mean rate is lower than previously published estimates. This suggests that mean values previously inferred from existing data did not characterize the global ocean adequately.

II. The mean $GPP^{18}O$ rate for the European Arctic Sector was 14.00 ± 1.49 µmol O_2 L^{-1} d^{-1} (range: 0.19 - 69.15). The seasonal variability in $GPP^{18}O$ was strong, with May having the maximum mean monthly rate (26.60 ± 2.89 µmol O_2 L^{-1} d^{-1}) due to the spring bloom, and August having the lowest mean $GPP^{18}O$ rate (2.25 ± 0.32 µmol O_2 L^{-1} d^{-1}). The data proved consistent with estimates by the dark-light method ($GPPO_2$), as the mean rate from both methods did not differ significantly.

III. Mean $GPP^{18}O$ for Young Sound fjord was 0.123 ± 0.026 µmol O_2 L^{-1} d^{-1} (range 0.001 – 0.330), which are by far the lowest estimates reported with the ^{18}O method to date. These low $GPP^{18}O$ rates agree well with earlier investigations from the outer region of Young Sound suggesting that mineralization exceeds primary production, and that as such, the system is net heterotrophic, requiring additional carbon input to balance mineralization.

IV. For the planktonic communities in the Arctic Ocean (in a 24-h photoperiod during summer), mean R light (4.78 ± 0.50 µmol O_2 L^{-1} d^{-1}, range 0.01 - 15.80), was significantly higher than mean R dark (2.16 ± 0.27 µmol O_2 L^{-1} d^{-1}, range 0.03 - 17.69), showing that the common assumption of equal R light and R dark is incorrect. The mean ratio R_{light}:R_{dark} (5.00 ± 1.15) was higher than the one reported to date (2.72 ± 0.75) in studies outside the Arctic.

V. For Arctic waters, the mean oxygen concentration (360.98 ± 2.59 µmol L^{-1}; range 303.80 - 431.39 µmol L^{-1}) correlated well with $\delta^{18}O$ (O_2) values (mean 23.91 ± 0.12 ‰, range 21.17 - 25.83 ‰). This correlation might be explained by gross primary production, respiration and ice melting.

Conclusiones generales

I. La $GPP^{18}O$ media (± SE) para las estaciones evaluadas en la Expedición Malaspina fue 0.60 ± 0.06 µmol O_2 L^{-1} d^{-1} (rango 0.01 - 11.49). El estudio ocupó cuatro de los cinco giros subtropicales, siendo el primero en evaluar tasas de $GPP^{18}O$ en estas zonas de baja clorofila, lo cual explica que nuestra tasa media sea más baja que las estimas publicadas hasta ahora. Esto sugiere que los valores previamente inferidos de los datos existentes no caracterizaban el océano global adecuadamente.

II. La $GPP^{18}O$ media para el sector europeo del Ártico fue 14.00 ± 1.49 µmol O_2 L^{-1} d^{-1} (rango: 0.19 - 69.15). La variabilidad estacional en la $GPP^{18}O$ fue fuerte, teniendo mayo la máxima tasa mensual media (26.60 ± 2.89 µmol O_2 L^{-1} d^{-1}) debido al bloom de primavera, y agosto la tasa $GPP^{18}O$ media más baja (2.25 ± 0.32 µmol O_2 L^{-1} d^{-1}). Los datos fueron consistentes con las estimas del método de incubaciones en luz-oscuridad ($GPPO_2$), ya que la tasa media obtenida de ambos métodos no difirió significativamente.

III. La $GPP^{18}O$ media para el fiordo Young Sound fue 0.123 ± 0.026 µmol O_2 L^{-1} d^{-1} (rango 0.001 – 0.330), siendo éstas las estimas más bajas, con diferencia, publicadas hasta la fecha con el método ^{18}O. Estas bajas tasas de $GPP^{18}O$ son coherentes con investigaciones previas en la región externa de Young Sound, sugiriendo que la mineralización es mayor que la producción primaria, y que por tanto, el sistema es netamente heterotrófico, requiriendo una entrada adicional de carbono para equilibrar la mineralización.

IV. Para las comunidades planktónicas del océano Ártico (con un fotoperiodo de 24 horas durante el verano), la R media en la luz (4.78 ± 0.50 µmol O_2 L^{-1} d^{-1}, rango 0.01 - 15.80), fue significativamente más alta que la R media en la oscuridad (2.16 ± 0.27 µmol O_2 L^{-1} d^{-1}, rango 0.03 - 17.69), demostrando que la asunción general de que la R es igual en luz que en oscuridad, es incorrecta. El ratio R_{luz}:R_{oscuro} medio (5.00 ± 1.15) fue mayor que el publicado hasta la fecha (2.72 ± 0.75) para estudios fuera del Ártico.

V. Para las aguas del Ártico, la concentración de oxígeno media (360.98 ± 2.59 µmol L^{-1}; rango 303.80 - 431.39 µmol L^{-1}) tuvo una buena correlación con los valores de $\delta^{18}O$ (O_2) (media 23.91 ± 0.12 ‰, rango 21.17 - 25.83 ‰). Esta correlación podría venir explicada por la producción primaria bruta, la respiración y la fusión de hielo.

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