Journal of Experimental Marine Biology and Ecology 497 (2017) 188–196

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Photosynthesis enhanced dark respiration in three marine phytoplankton species

Mustafa Mantikci*, Jørgen L.S. Hansen, Stiig Markager

Department of Bioscience, Aarhus University, Frederiksborgvej 399, 4000 Roskilde, Denmark

ARTICLE INFO

Keywords:
Dark respiration
Photosynthesis
Phytoplankton
Light dose
Growth phase
Optode

ABSTRACT

The effects of previous photosynthetic activity on dark respiration rates were investigated in *Heterocapsa rotundata* (Dinophyceae), *Rhodomonas salina* (Cryptophyceae) and *Thalassiosira weissflogii* (Coscinodiscophyceae). Dark respiration rates were measured after 1 to 3 h of light exposure in 12 bottles where incubation irradiances ranging from 0 to 600 μmol photons m^{-2} s^{-1}. The results showed that the dark respiration increases with increasing light exposure (both irradiance and duration) in all exponentially growing cultures but not in two experiments with cultures in stationary phase (*T. weissflogii*). This relationship could be described by a linear model for all species, relating respiration to the previous accumulated photosynthetic product with a slope of about 0.035 h^{-1}, indicating a growth-related respiration of 3.5% per hour of the newly fixed carbon. The maximum respiration rate (R_{max}) was estimated from both light dose and accumulated photosynthetic product using a non-linear model. Although R_{max} and light-saturated gross photosynthesis (P^g_{max}) varied between the three species, a similar R_{max}: P^g_{max} ratio of ~0.12 was found. The maintenance respiration rates which are comparable with maintenance respiration rates from literature, varied from 0.004 to 0.02 mol O_2 g^{-1} Chl h^{-1} for the three species. Overall, this study suggests a diel periodicity of phytoplankton respiration driven by accumulation of newly fixed carbon in light. The consequence is a decreasing respiration rate during the night or during long dark incubations.

1. Introduction

Respiration is a key process in pelagic systems and the main sink for organic matter produced by phytoplankton. The net microbial community production is gross of primary production minus respiration by microbes and determines the organic matter surplus available for higher trophic levels or export by for example sedimentation. Pelagic community respiration also determines the oxygen consumption in the water column and the carbon dioxide produced affects the global carbon cycle (del Giorgio and Duarte, 2002). Furthermore, parameterization of respiration is essential for modelling primary productivity of marine systems (Platt et al., 1991). Community respiration is composed of two components; respiration by autotrophs (phytoplankton) and by heterotrophs (zooplankton and bacteria), although mixotrophy can complicate this distinction. The substrate for the two components are different as phytoplankton respiration (PR) rely on internal substrates accumulated from photosynthesis (Markager et al., 1992; Markager and Sand-Jensen, 1989; Weger et al., 1989) and heterotrophic respiration (mainly heterotrophic bacteria) is using dissolved organic matter (DOM) (Robinson, 2008) which can originated directly from phytoplankton photosynthesis or from external sources e.g. freshwater inflow. Diel variation of photosynthetic substrates in phytoplankton has been shown to affect community respiration rates both when phytoplankton (Markager and Sand-Jensen, 1989) and bacteria (Sadro et al., 2011) are dominating the pelagic community. PR often constitutes about 50% of the community respiration (del Giorgio and Williams, 2005), therefore, more detailed knowledge about interactions between PR and photosynthesis is needed to increase our understanding of pelagic respiration and net primary productivity of pelagic systems as well as concepts like critical depth (Platt et al., 1991; Sverdrup, 1953). Since PR cannot be measured in natural samples where autotrophic and heterotrophic communities are combined, it has been deduced from laboratory cultures (Falkowski et al., 1985b) or from other kinds of measurements with many assumptions (Marra and Barber, 2004; Williams and Lefèvre, 2008).

The relationship between photosynthesis and light has long been well established from incubations across a light gradient (e.g. Jassby and Platt, 1976), but there are only a few studies showing the relationship between phytoplankton dark respiration (R_D) and previous light history (Beardall et al., 1994; Ekelund, 2000; Falkowski et al.,

* Corresponding author at: TUBITAK Marmara Research Center, Kocaeli, Turkey.
E-mail address: mustafa.mantikci@tubitak.gov.tr (M. Mantikci).

http://dx.doi.org/10.1016/j.jembe.2017.09.015
Received 26 May 2017; Accepted 23 September 2017

1985a; Falkowski and Owens, 1978; Weger et al., 1989). To obtain daily production rates, R_D has typically been assumed to be constant during night, even though several studies have shown higher respiration rates just after sunset than before dawn in aquatic systems (Markager et al., 1992; Markager and Sand-Jensen, 1989; Sadro et al., 2011). It is therefore likely that the previous light history as well as photosynthesis play a key role in determining R_D. To the authors' knowledge, the accumulated photosynthetic production, as a proxy for the available substrate pool for R_D, has not yet been related to observation of R_D.

The ratio of maximum dark respiration to light-saturated gross photosynthesis (R_{max}:P^g_{max}) has been used in water column production models (Langdon, 1993; Platt et al., 1991) because it provides an estimate of respiration rates in the absence of direct measurements. R_{max}:P^g_{max} is often assumed to be 0.10 although it varies between phytoplankton species. R_{max} has previously been defined as a function of the gross photosynthesis rate at maximum growth (Langdon, 1993) since R_D has been assumed to be a function of growth rate (Geider and Osborne, 1989; Langdon, 1988). In addition, enhanced dark respiration after light exposure has been suggested to be a function of the intracellular content of substrate (Beardall et al., 1994; Markager et al., 1992). In order to understand and model pelagic primary production and carbon cycling, there is a need for experimental studies with focus on how phytoplankton respiration is related to the previous photosynthetic activity.

The experiments reported here were designed to determine the effects of the previous light history and photosynthesis on R_D, and to develop a model for R_{max}. This was done by continuous measurements of oxygen concentration in light-dark cycles with varying intensity and duration of irradiance. In order to describe the variability between taxa, three different phytoplankton groups (Dinophyceae, Cryptophyceae, Coscinodiscophyceae) were investigated. The experiments test the hypothesis that phytoplankton dark respiration is a function of the intracellular pool of recently fixed carbon, which in turn, is related to the previous cumulated photosynthetic activity and thereby light dose.

2. Material and method

2.1. Organisms and growth conditions

Heterocapsa rotundata SCCAP K-0483 (Dinophyceae) was obtained from the culture collection at University of Copenhagen, Denmark and *Rhodomonas salina* (Cryptophyceae) and *Thalassiosira weissflogii* (Coscinodiscophyceae) from DTU AQUA, Denmark. Phytoplankton stock cultures were maintained in 100 ml Erlenmeyer flasks with L media (Guillard and Hargraves, 1993) prepared with pasteurized seawater (salinity 30) under low irradiance (c 10 µmol photons m^{-2} s^{-1}) at a L:D cycle of 16:8 at 16 °C. Cultures were re-stocked to fresh seawater with L media every month. Prior to the experiments, cultures were grown under cool white fluorescent tubes (150 µmol photons m^{-2} s^{-1}). *R. salina* and *T. weissflogii* were bubbled with air during cultivation. Cultures were maintained in exponential growth phase by regular dilution. In addition, experiments with *T. weissflogii* were performed in both stationary phase (exp. 1–2) and exponential growth (exp. 3–4) phase in order to observe possible effects of the growth rate on dark respiration and photosynthesis. Replicate experiments (two to three) were done for all cultures.

2.2. Chlorophyll, POC and PON

Chlorophyll (Chl) was determined spectrophotometrically by filtering 50 ml sample onto 25 mm filters (Advantec GF75), extracted in 10 ml 96% ethanol and stored in the dark at 4 °C overnight. For the determination of particulate organic carbon (POC) and nitrogen (PON), 50 ml samples were filtered onto pre-combusted (3 h, 450 °C) filters and stored at − 20 °C until analysis. Filters were dried for 24 h at 40 °C and analyzed on a Perkin Elmer 2400 CHNS analyzer.

2.3. Incubation procedures and experimental setups

2 L freshly diluted culture was prepared and kept in darkness for at least 16 h prior to each experiment. During this dark-adaptation, cultures were stirred with a magnet to keep cells in suspension. In order to minimize oversaturation with oxygen (O_2) and bubble formation prior to the incubations, the O_2 concentration in the cultures was reduced to ~50% air saturation by bubbling with N_2 gas for 2 min. Bubble formation during incubation is an error source that tends to increase the measured respiration as some oxygen will move from the oversaturated water into the bubbles. In some experiments, where the water became highly oversaturated with oxygen (> 160%), the corresponding respiration rates were erroneously high and not included in the analyses (see e.g. Fig. 5). Incubations were conducted at 16 °C, corresponding to the growth conditions

2.4. Light-dark cycles

Net photosynthesis (P) and dark respiration (R_D) were measured as changes in O_2 concentration in light and darkness, respectively. P and R_D rates were estimated by linear regression of O_2 concentration versus incubation time. Dark-adapted culture samples were gently dispensed into 12 glass BOD bottles (60 ml) and placed into a custom-built light/dark incubator. Light was supplied by light-emitting diodes (LEDs) and intensities independently modified for each bottle ranging from 8 to 600 µmol photons m^{-2} s^{-1} using neutral density filters. One dark bottle was covered with aluminum foil during experiments. Prior to each experiment, bottles were allowed to equilibrate in darkness for 45 min in the incubator.

Three different light periods (1, 2 and 3 h) were applied during the incubations (Fig. 1). The duration of the experiment was chosen such that the light periods did not exceed the 16:8 h light:dark cycles to which the algae were adapted and such that accumulation of O_2 did not interfere with the measurements. On the other hand, it was necessary to have long enough light periods to approach saturation of intracellular storage capacity of photosynthetic products. Photosynthesis-irradiance (PE) curves were fitted for each light period to calculate photosynthetic parameters (P_{max}, α, *r*). Following each light exposure, a 45 min dark period was applied. Each experiment lasted for 9 h in total.

The O_2 concentration was measured continuously with optodes (FirestingO_2-PyroScience) with sensor spots fixed to the inner wall of 60 ml glass bottles. Sensor spots were optically isolated in order to minimize the effect of colored samples and ambient light during

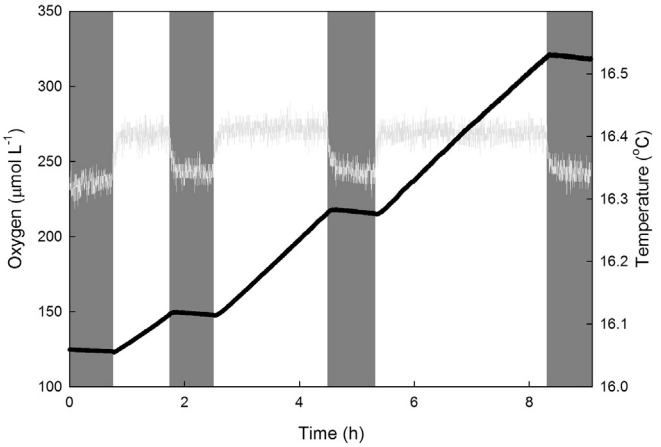

Fig. 1. An example of an experiment showing light and dark cycles with white and grey backgrounds, respectively, and the oxygen concentration (black dots). The temperature inside the bottles are shown as a grey line.