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Modelling the dynamics of carbon–nitrogen metabolism in the unicellular diazotrophic cyanobacterium *Crocosphaera watsonii* WH8501, under variable light regimes

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ABSTRACT

A dynamical model is proposed that describes the daily dynamics of diazotrophy in a unicellular cyanobacterium, *Crocosphaera watsonii* WH8501, in regard to light limitation and obligate diazotrophy. In this model, intracellular carbon and nitrogen are both divided into a functional pool and a storage pool. An internal pool that explicitly describes the nitrogenase enzyme is also added. The various intracellular carbon and nitrogen flows between these pools lead to a complex dynamics driven by the light regime. The model is successfully validated with continuous cultures experiments of *C. watsonii* under three light regimes, indicating that the proposed mechanisms accurately reproduce the growth dynamics of this organism under various light environments. Then, a series of model simulations is run for a range of light regimes with different photoperiods and daily light doses. Results reveal how nitrogen and carbon are coupled, through the diel cycle, along with nitrogenase dynamics whose activity is constrained by the light regime. In an ecological perspective, we picture the effect of such irradiance condition on growth and on the carbon to nitrogen stoichiometry on cells. This model could prove useful to understand the latitudinal distribution of this cyanobacterium in the global ocean.

1. Introduction

Nitrogen-fixing microorganisms (diazotrophs) are considered as important actors in the tropical and (sub)tropical oceans (Karl et al., 2002), where they can support 20–40% of new production (Lee et al., 2002). The conspicuous colonial cyanobacterium *Trichodesmium* spp. (Carpenter and Romans, 1991) and the heterocystous endosymbiont *Richelia* spp. (Carpenter et al., 1999) were thought as dominant marine diazotrophs, until the importance of, unicellular N_2-fixing cyanobacteria (UCYN) was pointed out (Zehr et al., 2001; Montoya et al., 2004). UCYN, revealed through detection of gene sequences, genome amplification and flow cytometry sorting (Zehr et al., 1998; Tripp et al., 2010; Thompson et al., 2012), thrive in (sub)tropical oceans (Falcón et al., 2002; Church et al., 2008). The discovery of new players in the field of nitrogen fixation initiated a reconsideration of the role of diazotrophs in the marine nitrogen cycle. For instance, *in situ* measurements of nitrogenase activity suggest that UCYN could be responsible of 83% of total N_2-fixation in the western equatorial Pacific (Bonnet et al., 2009). For a decade now, the presence of uncultivated UCYN in the world (sub)tropical oceans, has been much better described, while their growth efficiency remains poorly understood, even if modelling approaches allowed to contour their growth rate as well as their carbon and nitrogen cell content (Goebel et al., 2007, 2008).

Among the UCYN identified to date, *Crocosphaera watsonii* WH8501 (Waterbury and Rippka, 1989) is the only free living, open ocean strain that is cultivated and has therefore received particular attention. This representative of group B diazotrophic cyanobacteria appears widely distributed in (sub)tropical oceans; it is commonly reported in the Arabian Sea (Mazard et al., 2004), the oceanic gyres of the North Atlantic (Langlois et al., 2008), North and South Pacific (Church et al., 2008; Webb et al., 2009), and the Sargasso Sea (Hewson et al., 2007). *In situ* analyses of *nifH* gene (encoding for a subunit of nitrogenase, the enzyme specific to

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nitrogen fixation) sequences indicate that *C. watsonii* abundance can be significant in the N_2 fixer community (Church et al., 2008; Langlois et al., 2008; Moisander et al., 2010). This organism shows low genomic diversity (Zehr et al., 2007) and seems to be particularly adapted to oligotrophic conditions (Church et al., 2009) but the geographical and environmental limits of its distribution in the world ocean are not clearly defined. Light dose and photoperiod strongly vary with location and play a key role for the growth of these organisms. Indeed, the nitrogenase is highly sensitive to O_2 (Robson and Postgate, 1980). Photosynthesis, leading to O_2 production, therefore cannot *a priori* occur concomitantly to nitrogen fixation. Acquisition of carbon and nitrogen is thus uncoupled in UCYN: these two processes segregate in time, N_2 fixation being mainly restricted to the dark period when oxygenic photosynthesis does not occur (Mitsui et al., 1986). Intuitively, daylight duration is ought to further affect the growth of UCYN through the direct, negative effect that oxygen evolution may exert on the nitrogenase. In this regard, characterization of the response of *C. watsonii* to photoperiod could shed light on its distribution in the global ocean.

A series of laboratory experiments was carried out with three light regimes to describe the co-variations in carbon-based growth and diazotrophy in *C. watsonii* (Dron et al., 2012b,a, 2013). These studies report how carbon and nitrogen acquisitions balance in time over the diel cycle, with an apparent tight connection to the cell cycle. Results illustrate in particular how the high energy demand for nitrogen fixation (16 ATP per N_2 fixed, Burris, 1991) is supported by consumption of carbohydrates. We thus suspect carbon reserves to be the coupling element between carbon and nitrogen metabolisms in *C. watsonii*.

On the basis of these experiments, we propose a dynamical model of diazotrophy in *C. watsonii*. We seek to explain the mechanisms underlying this 'division of labor'. Through this model, we explore the effects of photoperiod on the connection between carbon and nitrogen metabolism, and we identify conditions leading to an optimal combination for carbohydrate storage, nitrogen fixation and total biomass.

2. Material and methods

2.1. Experimental approach

The present modelling study stands on data from three experiments (Dron et al., 2012b,a, 2013) performed on the same culture device monitored by computers (Malara and Sciandra, 1991). The experimental procedure, already described in Dron et al. (2012b), is briefly resumed here to recall the information useful for the model development. Duplicate cultures (C1 and C2) of *C. watsonii* WH8501 were grown in cylindrical, 5 L continuous cultures (Bernard et al., 1996) at a constant temperature (27 °C). Cultures were grown in a modified YBCII culture medium (Chen et al., 1996), devoid of a nitrogen source. The culture medium was diluted at a fixed rate (the dilution rate D). Division rates m (h^{-1}) were estimated from cell counts according to the following relation:

$$m = \frac{1}{X}\frac{dX}{dt} + D \tag{1}$$

where X is the cell concentration (cells mL^{-1}) and D is the dilution rate (h^{-1}).

Cultures were grown under three different light:dark (LD) regimes (12:12, 16:8 and 8:16 h of light and dark, see Fig. 1). Irradiance followed a bell-shaped cycle with a maximum irradiance of 130 $\mu E/m^2/s$ at the mid light period. This light intensity is equivalent to a depth of 60 m at station ALOHA according to the 'Hawaii Ocean Time-Series – Data Organization and Graphical System' (http://hahana.soest.hawaii.edu/hot/), where *C. watsonii* usually thrives. Note that the daily light dose was different in the three LD regimes: from 2.808 $E/m^2/d$ (12:12) to 3.744 $E/m^2/d$ (16:8) and 1.872 $E/m^2/d$ (8:16).

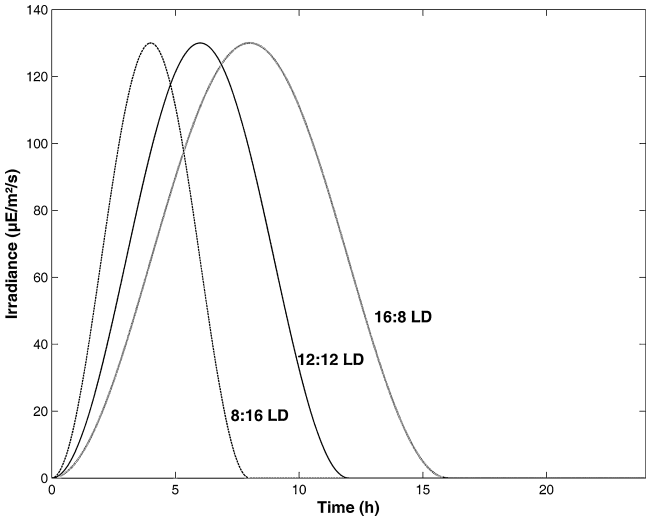

Fig. 1. Incident irradiance applied during a daily cycle, under the three tested light regimes: 16:8 (grey dashed line), 12:12 (continuous line) and 8:16 (dashed line).

Cultures were grown for 10–15 generations in their respective light conditions before starting the experiments. Once cultures reached the equilibrium, in which cell growth rate (μ) and biomass concentration show constant, daily average values, a high-frequency sampling of both duplicates was conducted for 4 days. Total particulate organic carbon (POC) and nitrogen (PON) were measured every 4 h and analyzed with a CHN analyzer (Perkin Elmer). Nitrogenase activity was monitored following the online Acetylene Reduction Assay (ARA) as described by (Staal et al., 2001). Total cellular carbohydrates were quantified from samples taken every 4 h following the method developed by Dubois et al. (1956). The present model aims at predicting the measured values for POC, PON, total cellular carbohydrates and nitrogenase activity.

2.2. Computing light distribution in the culturing device

Since the 5 L photobioreactor used for the experiments was cylindrical and illuminated from two sides, the light distribution is not straightforward. We estimated it by measuring light intensity using a waterproof 4π spherical collector (QSL-100, Biospherical Instruments) at different points and for different biomass concentrations in an additional experiment. We defined an orthonormal basis (O, x, y, z) to locate in the photobioreactor, z corresponding to the vertical axis. We identified 4 ellipsoid isolines in the plane (x, y) at a constant z for which light intensity is constant. To deal with the self-shading effect, we linked light intensity for each ellipse level to biomass and depth. To do so, we used a modified Lambert–Beer's law to calculate the light intensity $I(I_{in}(t), i(x, y), z, C_{tot})$ function of the input irradiance $I_{in}(t)$, of the ellipse i, of depth z and of carbon biomass concentration C_{tot}:

$$I(t) = I(I_{in}(t), i(x, y), z, C_{tot}) = I_{in}(t)e^{-(kC_{tot}+K_b)d_i}f(z) \tag{2}$$

where $I_{in}(t)$ is the incident light irradiance, k is the biomass specific, light extinction coefficient, K_b is the background turbidity, C_{tot} is the biomass concentration in mol-C/m^3, d_i is a distance parameter corresponding to ellipse i. Function $f(z)$ is a function linking light intensity to depth.

$$f(z) = p_1 z^2 + p_2 z + p_3 \tag{3}$$