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Interactive comment on "Flexible C : N ratio enhances metabolism of large phytoplankton when resource supply is intermittent" *by* D. Talmy et al.

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Responses to referee #1

Summary

The authors construct a new model and tune its parameters based on the measured response of a few different phytoplankton (differing in size and taxa) to changing light

and nutrient conditions. The model represents the storage of (non-functional) pools of both C and N within the cell, as a function of cell size and light and nutrient availability. They then use this model to simulate the response of phytoplankton to different light and nutrient conditions, with particular emphasis on fluctuating nutrient supply and irradiance.

The main result is that cells of larger size are predicted to have faster average growth rates under conditions of fluctuating nutrient supply and even more so under conditions of fluctuating irradiance. The latter effect is most pronounced for cells that were preacclimated to low-nutrient conditions prior to exposure to intermittent irradiance.

Overall evaluation

This is a solid contribution, using an empirically-based model to demonstrate the potential of C storage to confer an advantage to large phytoplankton in fluctuating environments. As the authors state, further studies are necessary in order to conclusively determine the extent to which this mechanism actually determines the relative dominance of large vs. small phytoplankton species in natural environments.

Finally this study also demonstrates (Fig. 9) that pre-acclimation can be particularly important to the modelled response in fluctuating environments. Using an optimality based model of photoacclimation (of quite different structure compared to the model in the present study), Wirtz and Pahlow (Mar. Ecol. Prog. Ser. 402: 81–96, 2010) similarly showed that pre-acclimation to different conditions has the potential to strongly impact the outcome of laboratory experiments, and furthermore that the acclimation timescale may be longer than typically assumed for the set-up of continuous culture

experiments. Taking this a step further, I would like to suggest that for future studies there may be a good reason to try some optimality-based approaches for studying the effect of C storage capacity. Although I understand and appreciate the value of the authors' approach of grounding their model formulation firmly in the response measured by controlled experiments, the results of this study and of Wirtz and Pahlow (2011) suggest that there is some danger in extrapolating from quite limited experimental results, which may themselves include the un-appreciated effects of pre-acclimation. In other words, if pre-acclimation did impact the experimental results, would this not be cause for questioning the predictions of any model, which does not account for such pre-acclimation in its formulation or tuning of parameter values?

RESPONSE: We agree that optimality concepts are a useful tool for exploring adaptation in algae. Optimality models provide predictions of the responses of organisms to environmental forcing based on the costs and benefits of different behaviours. In the case of phytoplankton growth, most models have sought to maximize the rate of biomass production by finding the optimal allocation of C, N and/or P amongst different functional and structural pools. Defining the costs and benefits of changes in allocation is key to the successful application of optimality principles to phytoplankton growth models.

Some costs are easier calculate than others. For example, the capital cost of constructing enzymes, pigments and other components can be obtained from flux balance analysis (FBA) (see Steuer et al., 2012), and the benefits can be quantified from the catalytic capacity of these components (e.g., the forward and reverse rate constants of an enzymatic reaction or the light absorption cross section of a pigment molecule). In addition to capital costs are running costs and opportunity costs. Some running costs such as the energy required to convert nutrients into biomass can also be calculated from FBA (Steuer et al., 2012). Other running costs can be calculated from knowledge of damage and repair processes, for example the cost of photoinhibition (Raven 2011).

However, some other running costs have not yet been fully quantified, for example the net cost of repairing damage caused by UV or photoxidative stress. Capital and running costs can be calculated from knowledge of cellular physiology and biochemistry.

A more difficult category of costs to evaluate is that of "opportunity costs." As discussed by (Geider et al., 2009), an opportunity cost is the value of a future opportunity that is not realised because of the resources that have been devoted towards an alternative option. For example, diversion of resources from Calvin cycle enzymes to the light-harvesting pigments in low-light environments would increase growth in a chronically low-light environment, but would reduce the opportunity to exploit intermittent exposure to high light in a light-limited deeply mixed layer. This issue was examined by Talmy et al. (2013), who combined optimality concepts with hypothetical descriptions of natural variability. They concluded that photoacclimation state is a consequence of optimization of resource allocation to the set of environmental parameters that define not only the mean light environment but also its variability: these parameters included surface irradiance, depth of mixing, and light attenuation.

By combining optimality concepts with hypothetical descriptions of natural variability, Talmy et al. (2013) attempted to predict organism acclimation state, irrespective of the laboratory environment. Talmy et al. (2013) found that hypothetical cells optimally adapted to contrasting environments had very different traits with regard to carbon fixation capacity. Interestingly, when the modelled optimality rules were used to predict organism acclimation state, the results were consistent with observed differences amongst species adapted to analagously contrasting environments (e.g. diatoms adapted to very variable light environments vs. Prochlorococcus adapted to relatively stable gyres).

We interpret the results of Talmy et al. (2013) as reassurance that, despite the possibility of organism adaptation to laboratory conditions, there is a signature in the observed response that is consistent with what might be expected, if the organism retains some fingerprint of adaptation to naturally varying conditions.

We appreciate the suggestion by this reviewer, that optimality arguments may help guide future investigations of photoacclimation and cell growth. Indeed, earlier iterations of the model presented here used optimality arguments similar to Talmy et al. (2013). Unfortunately, for the context here, we were somewhat restricted by data availability, and we felt that the lack of fully appropriate data would add unessecary uncertainty to the main predictions. We nonetheless support future studies that combine the size constraints presented here, with optimality concepts, to explore organism adaptation to the natural environment.

Minor Comments

p. 5186, just below eq. 12
The statement, '. . .because we do not follow individual cell quotas explicitly, we assume a fixed cell carbon content, . . .' needs to be revised. It is particularly confusing given that the model simulates not only the whole-cell C:N ratio but also the pools of functional and non-functional C and N, respectively. The C:N ratio of the functional components is assumed constant, but the non-functional components (and hence the whole-cell C:N) are explicitly modelled. Thus, the sentence as stated does constitute any clear reason for assumption of a fixed cell carbon content, and as best I understand it this C content per cell is NOT fixed in this model.

RESPONSE: We understand the confusion here. Calculations of the package effect require knowledge of the carbon per cell. However, all carbon based variables in the model have units nmol C m^{-3} - i.e. they are density units and do not keep track of individual cells. To keep track of a dynamic C per cell, we would also need to keep track of population cell count. Keeping track of cell count is not difficult. However, there is a very small range in the package effect for individual cells undergoing changes in carbon content. The largest influence of the package effect is between organisms

of very different size, and the model is able to account for those differences by just choosing a fixed value for the C cell^{-1}.

We thank this reviewer for requesting this clarification and, should a revised manuscript be requested, we will clarify in more detail what we mean.

p. 5195, line 5-7
'Their subsequent assimilation into proteins must lead to a net gain in at least one additional elemental quota.' This overstates the case, because the overall net effect will of course depend on the environmental conditions. I would suggest something like: 'Their subsequent assimilation into proteins must lead to an increase in the capacity for assimilating C, N, or both.'

We appreciate this suggestion and will make this correction, should a revised manuscript be requested

Responses to referee #2

General comments

The present article explores the physiology of phytoplanktonic cells of different sizes using a mechanistic model with flexible C:N ratio and allometric relationships. The manuscript is clear and well written. It addresses a relevant scientific question within the scope of BG, since phytoplankton plays a major role in aquatic ecosystems and global ocean biogeochemical cycles.

Indeed, organism size plays a major role in structuring plankton community but its impact on the phytoplankton metabolism and photophysiology still remains poorly known. Since the impact of phytoplankton size may be important under intermittent light and nitrogen supply, the authors have developed an original mechanistic model for phytoplankton physiology. This model takes into account photoacclimation and energy storage using a flexible C:N ratio. It is empirically constrained from allometric relationships and tuned against previous experimental data. In the Methods section, a brief overview of the model is given, before going into more details. The model equations are very clearly described and numerous references are given. The model is tuned against available experimental data sets for different species.

The comparison of the model outputs with the available data comfort the modeling approach adopted by the authors. Their results give new insight on "how energy stored in carbohydrate and lipid influences phytoplankton growth rate in environments with ephemeral" photon flux density (PFD). However, the choice of this ephemeral PFD is not clearly justified and it needs to be. Besides, it would be even more interesting to present different scenarios of intermittent PFD. This should not require too much

work, so I suggest to the authors to test the impact of different scenarios of intermittent PFD and to better justify them and discuss them. This would also help the authors to build their discussion on the distribution of phytoplankton in the ocean on more solid grounds.

Finally, I am a bit disappointed by the Discussion section, since the authors finish it with two sentences that should be developed in two paragraphs (fluctuations in C:N ratio and impact on trophic interactions and biogeochemical cycles). Indeed these aspects are of huge importance of the audience of Biogeosciences.

For these reasons, and given the very good quality of this manuscript, which presents very interesting results obtained from well-applied methodology, I recommend minor revisions in order to 1) test and justify different intermittent light scenarios, 2) discuss in more details the implications of their work. This should easily be improved, especially since the authors mention in their conclusion that "the model presented may be combined with more detailed descriptions of PFD variability" (Page 5195, Line 24-25). You will find more details bellow.

RESPONSE: In response to this reviewers suggestion, we have devised several additional sets of experiments to explore how size dependent differences in growth rate are sensitive to different assumptions about light variability. Should a revised manuscript be requested, the following text would constitute an additional subsection in 'methods':

Parameterizing resource variability

In the ocean, surface wind and temperature forcing cause vertical transport of phyto-

plankton due, for example, to deep convection (Backhaus et al., 2003), and turbulent mixing (Huisman et al., 1999). Due to the attenuation of light by water and floating material, cells that undergo such vertical motions experience variation in ambient photon flux density, sometimes over several orders of magnitude. Consequently, the effective 'photophase' (i.e. the time period in which cells are in the light) may in some conditions be extremely short. For example, in the North Atlantic, transport due to deep convection may result in cells completing 800m vertical loops on the order of a day (Backhaus et al., 2003). Assuming a constant average vertical velocity and a euphotic depth of approximately 100m, cells in such a system would be in the dark for roughly 21 hours.

We mimicked the effect of vertical transport on phytoplankton exposure to light by conducting simulations in which hypothetical, model organisms were exposed to intermittent photon flux doses within a 24 hour period:

$$E(t) = \{ 2\,00 \qquad if \qquad 0 < t \leq \rho, \qquad\qquad 0 \qquad otherwise$$

when $\rho = 0$, the cells are exposed to complete darkness for the whole day. When $\rho = 1$, the cells are exposed to saturating irradiance for 24 hours. By varying ρ within the range $[0, 1]$, we were able to mimic changes in average photon flux density and photosynthesis, due to changes in photophase. Note that the value 200 was chosen to completely saturate photosynthetic rates within the euphotic zone.

In addition to light, phytoplankton cells may experience variability in nutrient concentration by passing in and out of small scale nutrient 'patches' (Seymour et al., 2009). We accounted for the possibility that organisms may pass in and out of small scale nutrient patches by applying similar, idealized step changes in the ambient substrate

concentration:

$$S(t) = \{ 1 \qquad if \qquad 0 < t \leq \rho, \qquad\qquad 0 \qquad otherwise$$

In order to test the sensitivity of our results to different assumptions regarding PFD and nutrient variability, we also tested two additional scenarios (see supporting information). One additional set of experiments mimicked multiple visits to the euphotic zone or nutrient patch by allowing multiple intermittence phases within a single 24 hour period. The other allowed for much slower transport by testing intermittence phases on the order of a week.

To test the combined effect of nutrient starvation and intermittent light on organism metabolic state, a repeat of all scenarios was performed in which model organisms were pre-acclimated to very low nutrient conditions. The results of these experiments were contrasted against the main set of experiments, in which there was an initial spin-up time involving exposure to resource replete conditions.

The resource sufficient spin-up was imposed by setting the ambient nutrient concentration far higher than the Michaelis-Menten half saturation constant for nutrient uptake ($S >> K_S$), and the ambient irradiance to be significantly greater than $E_k = P_m/\alpha$, the saturation point of photosynthesis ($E >> E_k$). In contrast, experiments that tested the combined effect of nutrient starvation and intermittent light on organism metabolic state, imposed a very low ambient nutrient concentration (i.e. $S << K_S$), and a saturating PFD ($E >> E_k$), during the model spin-up.

Specific comments

Main comments regarding the intermittent forcing:

- Page 5190, Lines 9-11: "modeled organisms were exposed to an intermittent PFD with constant nitrogen supply, and constant PFD with intermittent nitrogen supply". Please mention here the frequencies you used/tested and justify these choices.

RESPONSE: The above text provides the information requested by this referee

- Page 5192, Line 5: Why did you use this light frequency? How relevant is it for phytoplankton in the global ocean? As indicated above, this needs to be mentioned and justified in the Methods section, and it will need to be discussed in the Discussion section.

RESPONSE: As discussed above, we used this light frequency to mimic the effect of deep mixing and nutrient patchiness on growth rate. If a revised manuscript is requested, we will be clear about this in the discussion section

- Page 5193, Line 1-2: " We used a model to understand how energy stored in carbohydrate and lipid influences phytoplankton growth rate in environments with ephemeral PFD". There is still a need to justify the frequency of PFD you are looking at...

RESPONSE: please see the above text

- Page 5195, Line 24-25: "the model presented may be combined with more detailed

descriptions of PFD variability". As mentioned above, this should be done in the present study (at least two simplistic cases/scenarios should be presented as in Figure 8). Otherwise, your article might just focus on the description of a new model and the comparison with experimental data, but without any implications for phytoplankton distribution at global scale, but it would then be much less interesting for Biogeosciences (whereas the quality of your work and of your manuscript are in agreement with the high standards of this journal).

RESPONSE: please see the above text and supporting information

Main comments regarding the discussion section:

-Page 5180, Line 17-19: " We suggest this mechanism is a significant constraint on phytoplankton C : N variability and cell size distribution in different oceanic regimes": these two aspects should be discussed with much more details, for instance in respect to some recent articles dealing with phytoplankton stoichiometry and/or size. You could for instance refer to the recently published articles (and references therein) listed at the end of this review.

RESPONSE: we will insert the following paragraphs after the line ending 'may also be a vital ecological strategy when growth maximization determines fitness', on page 5181, line 26

Storage of carbon in the form of carbohydrate and lipid has a significant influence on the phytoplankton C:N ratio (Geider and La Roche 2002). Eukaryotic autotrophs such as diatoms and coccolithophores often accumulate significant C reserves under N or P stress, when the ability to fix carbon (and thus to store energy), exceeds rates of

protein synthesis (Geider and La Roche 2002). It is not uncommon for eukaryotes to possess C:N ratios more than double the Redfield C:N (Caperon and Meyer 1972). Bloom forming eukaryotes with flexible C:N are extremely prevalent at high latitude, where resource supply is relatively variable (Fig. 1).

Small cell size has been emphasized as a factor contributing to dominance of picoplankton in oligotrophic waters, because small cells with high surface area to volume ratios have reduced transport-limitation of nutrient uptake, (Clark et al 2013). Yet, small cell size may also prevent the accumulation of large carbon reserves. If small cell size places a limit on the capacity of organisms to store carbon, they may have relatively narrow ranges of C:N. However, this may not be a problem given the less variable conditions in stratified oligotrophic waters, where the build-up and mobilization of carbon reserves may be less of a constraint on growth rate.

- Page 5195, Lines 10-14: " Accumulation of storage compounds is nonetheless responsible for large fluctuations in the C:N ratio, so is intimately connected with ocean biogeochemical cycles. Predator–prey interactions are thought to be modulated by cell stoichiometry (Mitra, 2006), so C:N dynamics described here may also influence foodweb interactions.": These aspects should be developed, especially in a journal as Biogeosciences.

RESPONSE: we thank this reviewer for his/her suggestion. If a revised manuscript is requested, we will close the sentence after 'responsible for large fluctuations in the C:N ratio.', delete the words ', so is intimately connected with ocean biogeochemical cycles.' and insert the following text:

How do these size dependent constraints on stoichiometry influence large scale patterns in C:N? A compilation of existing data by Martiny et al. (2013), suggests nutrient

poor, high light environments have relatively high ratios of particulate organic carbon (POC), to particulate organic nitrogen (PON) (i.e. high POC:PON). By contrast, darker, nutrient rich waters have lower POC:PON. How can these observations be reconciled with the suggestion here that large cells more likely to dominate at high latitude can have the highest C:N? We hypothesize that, even if more temperate environments favor large cells with the propensity for high C:N, large cells only obtain such high values transiently, when the ability to fix carbon exceeds the rate at which nitrogen may be assimilated. It may therefore not come as a surprise that a compilation of data taken over a large spatio-temporal range indicates that, on average, light limited, nutrient rich environments have relatively low C:N. Cyanobacteria that dominate in the gyres may not have the capacity to accumulate such large carbon reserves, but may well maintain C:N ratios close to their maximum limit in direct response to the local environment.

Phytoplankton stoichiometry is also likely to influence foodweb dynamics (Loladze et al., 2000). Phytoplankton cells with high carbon relative to other main constituents (N,P), are often less palatable to herbivores (Urabe and Waki, 2009), although these effects may be offset when predators are able to graze upon multiple food types (Urabe et al., 2009). The manner in which prey stoichiometry influences herbivore growth is likely to influence rates of export production (Anderson et al 2013). We suggest that the model presented here is a useful tool for further investigations of the influence of phytoplankton C:N on ecosystem function.

We have focused here on the benefit of a large carbon reserve to organism growth rates. We nonetheless do not exclude to possibility that 'excess' C may be excreted from the cell, forming a protective polysaccharide layer (Wotton 2004). Furthermore, using reserve carbon to fuel respiratory costs associated with the maintenance of buoyancy (Waite et al., 1997), may also be a valuable survival mechanism when cells are vulnerable to rapid sinking away from the euphotic zone. We anticipate that the model of intracellular C:N dynamics presented here may in future be expanded to include multiple ecological benefits of a large carbon reservoir.

- Page 5189, Line 12: Some parameter tuning may have been better if you had use an optimization algorithm, even for a small number of parameters only.

RESPONSE: we do not believe that an optimization algorithm is necessary here. We will nonetheless do some additional manual tuning to ensure the model fits look neater

- Page 5184 and following: it may be useful to write $V_n(S, N_R)$ instead of only Vn. Same comment for $V_m(N_R)$, $P_n(N_{LH}, N_F, E, C_R)$, $P_m(C_R)$.

RESPONSE: we thank this reviewer for the suggestion, and will update the manuscrupt accordingly

Comments on the Figures and Tables

The legends of the Figures and Tables are very detailed and self-explained. However, I have a few comments on them.

- Table 4: Indicated the parameter that have been tuned and the ones obtained from previous studies.

RESPONSE: we thank this reviewer for the suggestion, and will update the manuscrupt accordingly

- Figure 1: The labels a and b should be more visible. Add a layer with the continents on panel a because it seems a bit weird like this... The continent line should look like

the same as in panel b.

RESPONSE: we thank this reviewer for the suggestion, and will update the manuscrupt accordingly

- Figure 3: This Figure should rather appear as a supplementary figure, especially because it is not described nor discussed in the manuscript. Indeed, if I understood correctly, you just want to compare your relationships (black line simulated by the model) with (only?) three experimental points to justify your parameter values?

RESPONSE: we will add this to the supplementary information

- Figure 5: The text on the figures seems very small.

RESPONSE: we will enlarge the text

- Figure 8: " Shaded regions correspond to complete darkness, whereas light regions correspond to 1000 μmol photons $m^{-2}s^{-1}$": this should be mentioned in the Methods section, with an indication on the frequency of the light/dark cycle. Again, I am not convinced by the appropriateness of this light/dark cycle since you did not give any justification.

RESPONSE: the above text gives a more detailed description of this. Note that, we use a different irradiance value here (1000 μmol photons $m^{-2}s^{-1}$ as opposed to 200 μmol photons $m^{-2}s^{-1}$. This figure is only really for illustrative purposes and that number gave the most visually interpretable results

- Figure 9: The text on this figure is much too small. The frequency of nutrient supply and light and dark cycle is not clear.

RESPONSE: we will enlarge this text and add additional axes to make the information more legible

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