

Interactive comment on “Physiological constraints on the global distribution of *Trichodesmium* – effect of temperature on diazotrophy” by E. Breitbarth et al.

Anonymous Referee #1

Received and published: 19 August 2006

General comments: The authors examine the effects of temperature on rates of N₂ fixation, growth rates and photosynthetic response in cultures of *Trichodesmium* IMS101. This is important because to date, physiological studies in culture systems have examined only a relatively narrow temperature range (24 to 28.5°C) and the observed distribution of this genus in nature is much broader (20 - 36 °C). Further, temperature is used to constrain and predict N₂ fixation by *Trichodesmium* (the dominant identified N₂ fixer) in models and temperature is projected to change significantly over the next century. This is a valuable data set because it examines C and N₂ fixation by *Trichodesmium* under a wider range of temperatures than has been previously examined. It is certainly worth publishing after the following specific comments are addressed.

Specific comments (most of these might be addressed through clarifications in the methods section): I think that based on recent results, we can say that *Trichodesmium* do occur at temperatures higher than 30°C (e.g., North of Australia, Indian Ocean, Gulf of Mexico, etc.). Not too important since the range of temperatures examined went to 36°C.

In the materials and methods sections there were a few things that were unclear. Page 782, line 10-12, why were the cultures transferred and which measurements were made on these unacclimated cultures?

For the N₂ fixation measurements, the acetylene additions were small as was the volume of headspace, was diffusion of the gas adequate within the incubation vials? The next line indicates that ARA's were carried out for 3 individual times on semi-continuously growing cultures? I didn't understand what this meant. Were the assays in triplicate, where they started at 3 different times over the light cycle? What were the semi-continuous cultures? These were not described in section 2.1 (just batch cultures were indicated there). Were acetylene reduction assays only 4 hours in duration? Which 4 hours of the light cycle? Rates really vary over the course of the light cycle. Ideally, the hourly rates presented are an average over the 12 hour light cycles and a daily rate can be calculated by multiplying the hourly rate by 12. This is important because if N₂ fixation was NOT evaluated over the 12 hour light cycle, then the differences among temperature treatments may simply reflect a shift in the timing of peak rates of N₂ fixation. This happens both in nature and cultures.

In the results section, on page 784, lines 24 -26, I do not understand what the authors mean, this is probably because I didn't understand the portion in the methods section where cultures were transferred to different temperatures before experiments were done. Weren't the cultures acclimated? If there were no differences in N-based growth rates, there should be little difference in net N₂ fixation (so long as no N was added to the media) and any change in N₂ fixation would seem to indicate release. I think that I am just not understanding something because changes in growth rates are indicated

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in Fig. 1.

Page 785, lines 1-2. Growth rates were optimum I would extend this from 24oC - 32oC based on looking at Fig. 1. The rates at 24 are nearly the same as those at 32oC. Looking at the figure, rates are lower in cultures growing at about 21oC and 34oC so anything between that could be optimum.

Were cultures grown at different temperatures all in the same growth phase (e.g., exponential) when N2 fixation rate measurements were made?

In Figure 1, I would recommend separating that into 2 figures because the bell curve distracts from looking at the C-specific N2 fixation rates, which appear to increase with temperature up to some point between 29 and 34oC (there is no data between those temperatures unfortunately to describe a break-point) after which it is inhibited. Photosynthetic efficiency was high up to 34oC arguing for a higher range to the optimum range of temperatures for growth. This also suggests that cells were viable (but would argue for higher POC:PON ratios if there was increased C incorporation).

In Fig. 1, why are there so many gaps? It seems that the growth rates and N2 fixation rates were measured on different cultures and at different temperatures. This may explain some of the variability.

In Fig. 2, why are the number of sampling points so different between temperature treatments? That may explain much of the variability in the slopes. Also, the authors indicate that data are pooled from measurements made over the entire growth period at each temperature even though they acknowledge that the ratios varied over the growth cycle. So, I don't understand why the data are pooled as they are in Fig. 2. Is Fig. 3 then a reflection of the slopes of the regressions from Fig. 2? Comparing ratios during a single common growth phase may be more appropriate.

In the discussion on page 787, lines 6-10, do the authors mean this for surface waters? Most of the ocean is cold and nitrate replete.

In general, I would argue that the authors have no data points between 29 and 34 oC and so I don't understand why 30oC was set as the upper limit to optimum growth. That seems quite arbitrary to me.

Page 788, lines 4-5, the statement that at lower temperatures that POC:PON ratios were reduced seems inaccurate based on Fig. 3b (two were lower and one was higher than the peak around 25oC and then ratios decreased again).

Page 789, in the discussion of photosynthetic physiology, it would be useful to know during which 4 hours the measurements were made and whether the timing of the photosynthetic maximum shifts during the day depending on the growth conditions. Maximum rates of photosynthesis and N₂ fixation for *Trichodesmium* have been observed at different times of the day for different populations and ideally, because these are both light dependent processes in *Trichodesmium*, there should be measurements throughout the light cycle. It is beyond the scope to redo the experiments but, modifying the methods and discussion sections to address the timing of measurements would be useful for the reader.

Finally, in the discussion of global temperature changes, the authors might wish to modify this to reflect a wider temperature range optimum - perhaps suggest multiple scenarios.

Technical corrections:

Some awkward English that can be corrected later.

Interactive comment on Biogeosciences Discuss., 3, 779, 2006.

BGD

3, S381–S384, 2006

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