TECHNICAL RESPONSE

OCEAN ACIDIFICATION

Response to Comment on "The complex effects of ocean acidification on the prominent N_2-fixing cyanobacterium *Trichodesmium*"

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Hutchins *et al*. question the validity of our results showing that under fast growth conditions, the beneficial effect of high CO_2 on *Trichodesmium* is overwhelmed by the deleterious effect of the concomitant decrease in ambient and cellular pH. The positive effect of acidification reported by Hutchins and co-workers is likely caused by culture conditions that support suboptimal growth rates.

The effects of ocean acidification on *Trichodesmium* spp., a globally important N_2-fixing cyanobacterium in oligotrophic oceans, have received considerable attention over the past decade. Paradoxically, both beneficial and deleterious effects of acidification on the rates of growth and N_2 fixation in this organism have been reported, without a mechanistic understanding of the underlying reasons. In our experiments, acidification of the medium had a clear detrimental effect on growth and N_2 fixation in *Trichodesmium erythraeum* strain IMS101 (hereafter *T. erythraeum*) (*1*). Cellular and molecular data show that this inhibition, which is amplified at low Fe concentrations, is caused by a decrease in cytosolic pH and the resulting biochemical cost of proton pumping across the thylakoid and plasma membranes.

Hutchins *et al*., who have reported a beneficial effect of acidification on *T. erythraeum* (*2*, *3*), question the validity of our results (*4*). They marshal three principal arguments: (i) They question our ability to measure growth rates and the validity of our results that show higher growth rates than in their experiments; (ii) they argue that their experiments were carried out under conditions that were essentially identical to ours; and (iii) they contend that their observation of a systematic nutrient-like response to increasing CO_2 establishes the validity of their data and conclusions. We respond to these points in order.

i) In our hands, *T. erythraeum* grows at a maximum rate of 0.56 ± 0.02 day^{-1} at 27°C and 80 μmol photons m^{-2} s^{-1} (Fig. 1A) (*1*). This growth rate is based on chlorophyll a (Chl a) measurements made every day at the same time because of the strong diel cycle exhibited by the organism. At the low biomass used in our study, Chl a and particulate organic carbon (POC) are proportional to each other (*5*). The growth rate we observed is within the range reported by several authors (μ_{max} = 0.46 to 0.7 day^{-1}) (*6*–*9*). The significantly slower growth rates [μ_{max} = 0.33 ± 0.04 day^{-1} (*2*, *3*)] obtained by Hutchins *et al*. do not militate in support of their results and interpretation.

ii) It is impossible for us to really know why Hutchins *et al*. obtained slow maximum growth rates in their experiments. Those may have been caused by some contaminant or by a component of the experimental medium. In our laboratory, *T. erythraeum* growth is strongly inhibited in the presence of 1 μM ammonium (μ_{max} = 0.37 ± 0.04 day^{-1}) (Fig. 1B), a concentration often encountered in artificial seawater (*10*). Under these conditions, acidification indeed improves rather than impairs growth and N_2 fixation, owing to the lower concentration and toxicity of membrane-permeable ammonia as the $[NH_3]/[NH_4^+]$ ratio decreases. At a concentration of ~0.5 μM ammonium, the background reported by Hutchins *et al*. for their artificial seawater, we measured no significant effect of acidification on growth or N_2 fixation (*1*). It is tempting to interpret this lack of effect as a balance between a lessening of NH_3 toxicity and the detrimental effect of low cellular pH documented in our study. The growth rate is also highly affected by low or high concentrations of the chelating agent EDTA (and hence presumably also by the background concentrations of metals that are introduced as contaminants in artificial seawater). We observed a slow maximum growth rate at 2 μM EDTA (*1*), the concentration in the YBCII recipe used by Hutchins *et al*. (*2*). And we have not been able to grow *T. erythraeum* at 100 μM EDTA, the concentration apparently used by Hutchins *et al*. (*3*) in their experiments with the Aquil medium (*11*, *12*). *T. erythraeum* is clearly quite sensitive to the chemistry of its growth medium—a situation that can easily lead to misinterpretation of experimental results.

iii) We agree with Hutchins *et al*. that high CO_2 concentrations are beneficial to *T. erythraeum*. We observed higher growth and N_2 fixation rates at high CO_2 when pH was held constant (*1*). This effect is explained by the lower material and energetic requirements to concentrate CO_2 at the site of fixation by Rubisco, as seen in the physiological and molecular data (*1*). Under conditions that promote fast growth rates, the positive effect of higher CO_2 is, however, overwhelmed by the negative effect of the lower pH when both parameters are allowed to covary as they do in natural seawater. Why the positive effect of high CO_2 may be dominant under the experimental conditions used by Hutchins *et al*. will only be understood when the reasons why they obtain slow maximum growth rates are also known.

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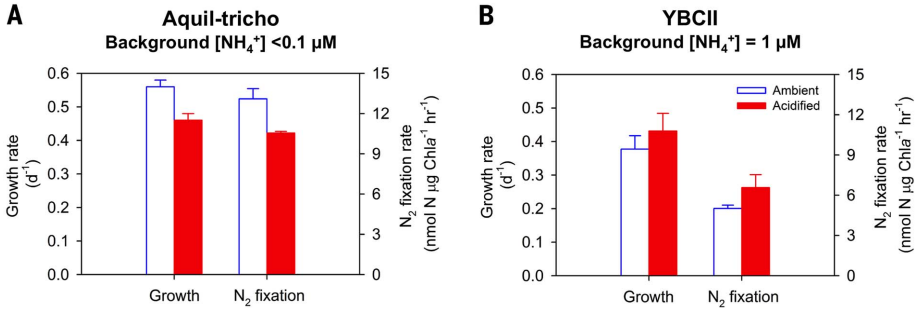

Fig. 1. The effect of ocean acidification on growth and short-term N_2 fixation of *T. erythraeum*. (**A** and **B**) Specific growth rates and short-term N_2 fixation rates of *T. erythraeum* in natural seawater Aquil-tricho medium (background $[NH_4^+]$ < 0.1 μM) (A) and in the artificial medium YBCII (background $[NH_4^+]$ = 1 μM) (B) under ambient conditions (400 μatm CO_2, pH 8.1) and acidified conditions (800 μatm CO_2, pH 7.8). Error bars denote SD of biological replicates (n = 3).

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