



Preliminary evidences of CCM operation and its down regulation in relation to increasing CO₂ levels in natural phytoplankton assemblages from the coastal waters of Bay of Bengal

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Bay of Bengal (BoB), a low productive part of the North Indian Ocean, often possesses low CO₂ levels in its surface water and diatoms dominate the phytoplankton communities. Virtually no studies are available from this area reporting how this diatom dominated phytoplankton community would respond any increase in dissolved CO₂ levels either naturally or anthropogenically. In most of the marine phytoplankton, the inefficiency of the sole carbon fixing enzyme Rubisco necessitates the need of concentrating dissolved inorganic carbon (DIC) (mostly as HCO₃⁻) inside the cell in excess of the ambient water concentrations in order to maintain high rate of photosynthesis under low CO₂ levels through an energy consuming carbon concentration mechanisms (CCMs). The ubiquitous enzyme carbonic anhydrase (CA) plays a vital role in CCMs by converting HCO₃⁻ to CO₂ and usually utilizes the trace metal zinc (Zn) as a cofactor. However, it is evident in many marine phytoplankton species that with increasing external CO₂ levels, CCMs can be down-regulated leading to energetic savings which can be reallocated to growth; although exceptions occur. Hence, in order to predict their responses to the projected changes, it is imperative to understand their carbon metabolism patterns.

We have conducted a series of incubation experiments in microcosms with natural phytoplankton communities from the coastal waters of BoB under different CO₂ levels. Our results revealed that the rate of net photosynthetic oxygen evolution and biomass build-up increased in response to increasing CO₂ levels. The depletion in $\delta^{13}\text{C}_{\text{POM}}$ values were more in the high CO₂ treatments relative to the low CO₂ treated cells (control), indicating that dissolved CO₂ uptake was higher when CO₂ levels were increased. When additional Zn was added to the low CO₂ treated cells, net photosynthetic oxygen evolution rate was increased significantly than that of the untreated control. It is likely that upon the supply of Zn under low CO₂ levels, CA activity was enhanced and accelerated DIC transport and photosynthetic rate. Moreover, $\delta^{13}\text{C}_{\text{POM}}$ values of low CO₂ samples (both Zn treated and untreated) were almost identical, though the rate of photosynthesis was higher in response to Zn addition. This could be because of the fact that under low CO₂ levels, DIC was possibly transported as HCO₃⁻ and an active HCO₃⁻ transport can contribute to low discrimination of ¹³C compared to diffusive CO₂ uptake leading to unaltered values of $\delta^{13}\text{C}_{\text{POM}}$. Furthermore, under low CO₂ treatments, the need of nitrogen resource can be higher to maintain an active CCM (to build-up required proteins, Rubisco and CCM components) and our results showed higher values of $\delta^{15}\text{N}_{\text{POM}}$ under low CO₂ levels relative to the high CO₂ treatments suggesting higher nitrogen utilization efficiency in the former case. These observations strengthen the possibility of operating an active CCM under low CO₂ levels.

HPLC pigment analysis revealed the occurrences of diatoxanthin (DT) [indicator of non-photo-chemical quenching (NPQ)] and high values of photoprotective carotenoid to light harvesting carotenoid ratios (PPC/LHC) in the low CO₂ treated cells indicating light stress. This is likely that, when CO₂, the only substrate for Rubisco, is low, absorbed light energy within the cell can be surplus leading to photo-damage and to protect the cell from potential damage, DT was produced by energy dissipation via NPQ and PPC were synthesized in excess of LHC. Conversely, in Zn and high CO₂ treated cells, the absence of DT and reduced values of PPC/LHC indirectly indicates reduced light stress which was possibly because of enhanced supply of Rubisco substrate either via active bicarbonate transport or diffusive CO₂ supply. Thus, we infer that the diatom dominated phytoplankton communities from the study area perform CCMs under low CO₂ conditions and the same can be down regulated upon the increasing levels of CO₂ and the community may benefit from the increasing CO₂ levels followed by increased rate of carbon fixation. These can have large biogeochemical significance.