COMPARING CARBON FIXATION BETWEEN TWO SPECIES OF THE MARINE DIATOM *THALASSIOSIRA*

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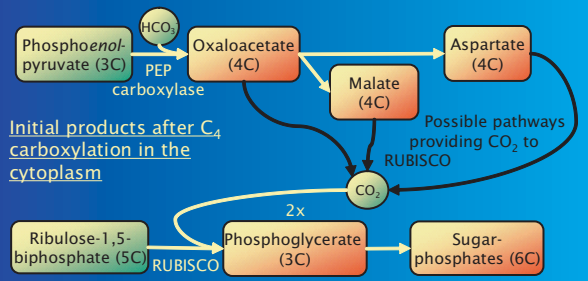
Introduction.

Marine diatoms are considered to be responsible for fixing the same amount of carbon per year as all the terrestrial rainforests, and over a quarter of the total carbon fixed by the oceans. Dissolved CO_2 is a potentially limiting factor for marine primary productivity and a great deal of research has focused on carbon-concentrating mechanisms (CCMs) in phytoplankton. In 2000, Reinfelder and co-workers provided evidence for a functional C_4 photosynthetic pathway, as well as a CCM based on active transport of inorganic carbon (or as a CCM in its own right), in the marine diatom *Thalassiosira weissflogii*[1,2]. The recently published whole genome sequence of the related *Thalassiosira pseudonana* also highlighted the presence of key enzymes of the C_4 pathway[3]. However, short-term carbon fixation and photosynthesis studies had not previously been conducted on *T. pseudonana*. In this work, we used an HPLC method to determine the short-term carbon fixation products formed by *T. weissflogii* and *T. pseudonana* under ambient (380 ppm) and low (100 ppm) CO_2 conditions.



An HPLC method for detecting ^{14}C labelling products.

Triplicate cultures were grown in artificial seawater with 2.38 mM $NaHCO_3$, at 15°C under a 12:12 hour light:dark cycle (200 µmol.m^{-2}.sec^{-1}). Cultures were maintained at ambient CO_2 levels (380 ppm) or were bubbled with air containing 100 ppm CO_2 for at least 6 days. Cells were harvested during exponential growth and concentrated in Aquil containing no $NaHCO_3$. Labelling was initiated by adding Aquil with 2.38 mM $NaH^{14}CO_3$ (27.5 µCi), and terminated by adding 2 ml boiling water. Unassimilated $NaH^{14}CO_3$ was removed from the samples by acidification. Half of each sample was then treated with Calf Intestinal Alkaline Phosphatase (CIAP) to remove any phosphate groups. Both samples were separated and visualised by HPLC (e.g. Figure 1). Peak identification was based on the co-retention of a series of ^{14}C organic acid and sugar standards.

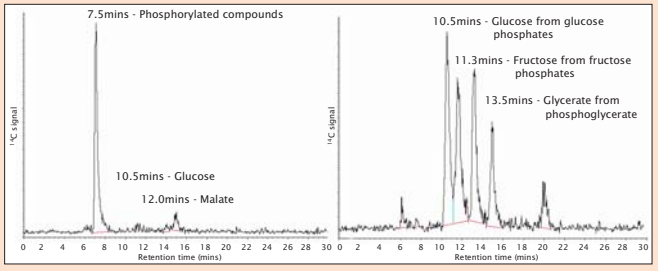
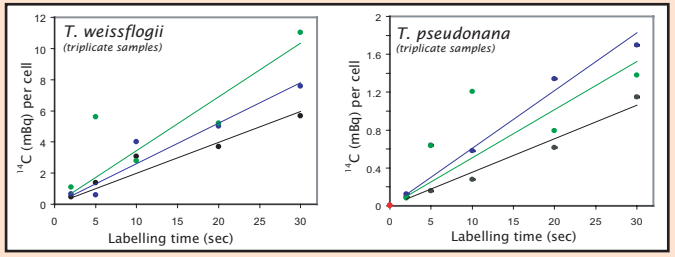
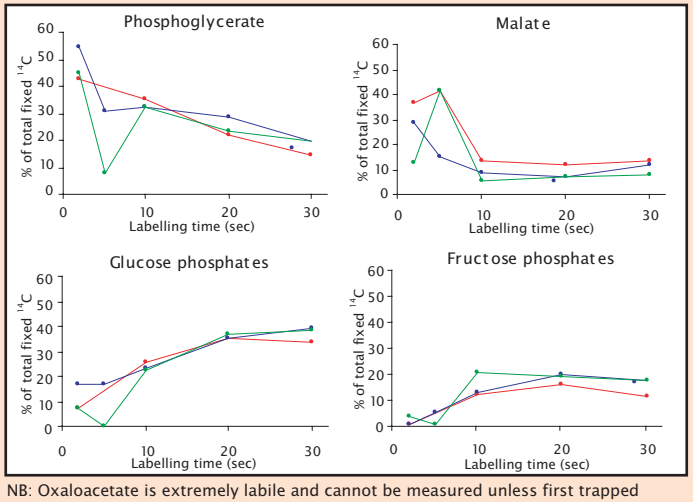


Figure 1: HPLC trace of *T. pseudonana* 10-second-labelling samples (a) before and (b) after treatment with CIAP. Peak areas correspond to compound concentration.

Exponentially-growing *T. weissflogii* cells have 7x greater volume than *T. pseudonana* and fix 6x more ^{14}C per cell after 30 seconds of labelling.



T. weissflogii cells grown under ambient (380 ppm) CO_2 conditions show large amounts of both phosphoglycerate (3C) and malate (4C) as initial products of photosynthesis. Both become less dominant with increasing time. These results suggest the use of both C_3 and C_4 photosynthetic pathways.



NB: Oxaloacetate is extremely labile and cannot be measured unless first trapped

References
1. Reinfelder *et al.* (2000) *Nature* **407**: 996-999
2. Reinfelder *et al.* (2004) *Plant Physiology* **135**:2106-2111
3. Armbrust *et al.* (2004) *Science* **306**: 79-86
4. Kaczmarska *et al.* (2006) *Journal of Phycology* **42**

Diatom images from *www.sciencedaily.com*

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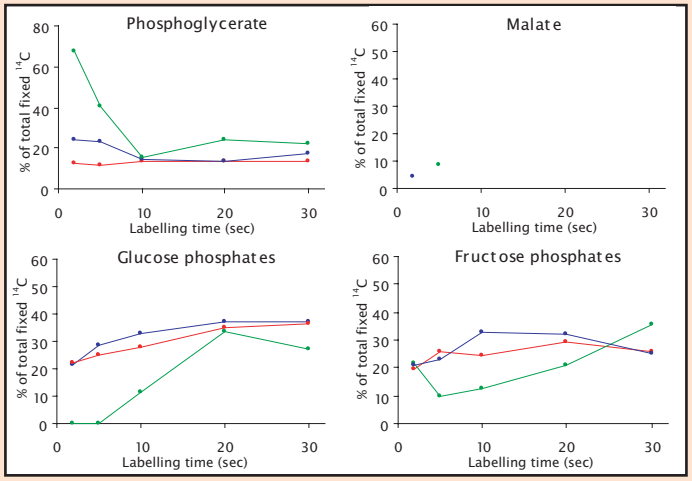
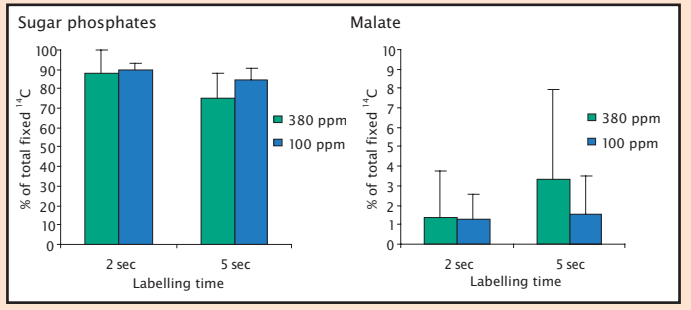
T. pseudonana cells grown under ambient (380 ppm) CO_2 conditions show virtually no short-term labelling of malate - suggesting a purely C_3 pathway



T. pseudonana cells acclimated to ambient CO_2 (380 ppm) or low CO_2 (100 ppm) produce the same short-term labelling pattern - strengthening the case for a C_3-only pathway. Malate labelling was also highly variable.



Conclusions.

Consistent with previous work[1,2], our short-term labelling studies have provided additional evidence for a functional C_4 pathway in *T. weissflogii*. Numerous studies have demonstrated the occurence of a CCM in *T. weissflogii* involving active inorganic carbon transport and carbonic anhydrase. The ability to use these two separate, but potentially complementary, carbon-acquisition pathways may enable *T. weissflogii* to out-compete other phytoplankton in areas of the ocean subject to carbon and zinc- (or cobalt-) limitation.

Despite a presence in the *T. pseudonana* genome of key enzymes in the C_4 photosynthetic pathway[3], our results have shown that *T. pseudonana* uses a purely C_3 strategy to fix inorganic carbon, after inorganic carbon accumulation as part of its CCM, even when acclimated to low CO_2. Such a fundamental difference between diatoms of the same genus may not be surprising in light of recent work suggesting a large phylogenetic distance between *T. weissflogii* and *T. pseudonana*[4].