

year age classes (so sex-ratios of offspring of 'toy boys' and 'sugar daddies' are identifiable). For cells with standard errors of less than 0.01, the sex ratios varied within the range 0.51 to 0.53. The sex ratios of offspring of 'toy boys' and 'sugar daddies' do not differ significantly from one another nor from that of the remaining births. Hence the result of Manning *et al.* is specific to the population of Liverpool schoolchildren they studied, and is probably dependent on sampling error.

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I have used the birth records for Illinois from 1989 to 1995 to compare with the report on parental age differences and the sex ratio of first-born children¹. These contain 326,207 single, live first births with no reported anomalies. Couples in which the fathers were at least 5 years older than the mothers had 37,487 first-born sons and 36,173 first-born daughters, a sex ratio of 1.0363. Couples in which the mothers were 1–9 years older than the fathers had 27,816 first-born sons and 26,571 first-born daughters, a sex ratio of 1.0469. This small difference is in the opposite direction to the effect reported by Manning *et al.*, but is well short of statistical significance. Hence the effect that Manning *et al.* reported arises from some factor other than the relative ages of the parents.

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Active uptake of bicarbonate by diatoms

Marine diatoms play a predominant role in the biological carbon pump¹ transferring carbon dioxide from surface to deep waters. Laboratory studies show that a number of species take up HCO_3^- and concentrate inorganic carbon intracellularly allowing rapid growth despite low CO_2 availability^{2,3}. In contrast, many oceanographers, particularly when interpreting carbon isotope data^{4,5}, have made the assumption that diatoms do not utilize the abundant HCO_3^- in seawater but rather take up CO_2 by diffusion⁶. This has led to the hypothesis that large diatoms may be CO_2 -limited in the oceans⁷. We now demonstrate active uptake of HCO_3^- in the field and a carbon-concentrating mechanism in coastal Atlantic diatoms. By manipulating p_{CO_2} we show that growth of large diatoms in the California upwelling is not

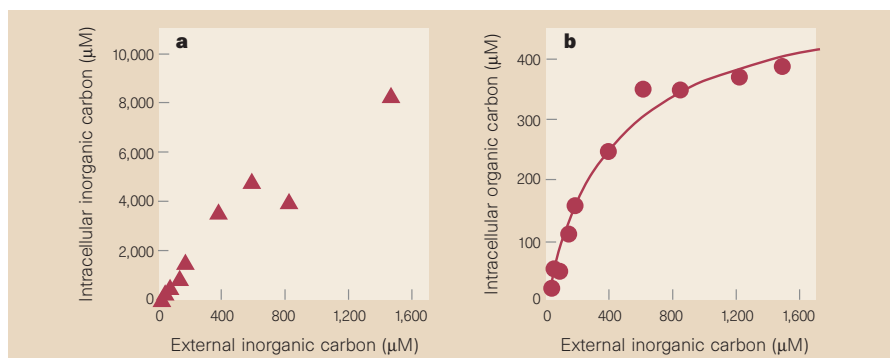


Figure 1 Short-term inorganic carbon uptake (a) and photosynthesis (b) in a marine diatom assemblage. Phytoplankton collected from Delaware Bay in April 1997 were filtered onto 5 µm polycarbonate filters and resuspended in low-carbon, buffered sea water, pH 8.2. Cell suspensions illuminated in an O_2 electrode chamber were allowed to deplete residual DIC until they reached the CO_2 compensation point (photosynthesis=respiration). Cell suspensions (190 µl) were incubated with varying concentrations of $\text{H}^{14}\text{CO}_3^-$ (50 mCi mmol⁻¹) for 10 s. Cells were centrifuged through a layer of silicone oil into a basic terminating solution (2.5 M NaOH, 10% methanol)⁸ and frozen cell pellets were assayed for organic (acid-stable) and inorganic (acid-labile) ¹⁴C activity by liquid scintillation counting. Total carbon counts were normalized to biovolume in cell pellets measured by incubating cell suspensions for 1 min with ³H₂O (ref. 8).

limited by CO_2 availability.

We ran short-term $\text{H}^{14}\text{CO}_3^-$ -uptake experiments⁸ using samples dominated by large (> 30 µm) diatoms (*Asterionella*, *Nitzschia* and *Rhizosolenia*) collected from Delaware Bay in 1997 during a spring phytoplankton bloom. The phytoplankton rapidly took up $\text{H}^{14}\text{CO}_3^-$ (within 10 seconds at pH 8.2) and accumulated intracellular dissolved inorganic carbon (DIC) at concentrations roughly 10 times higher than those outside (Fig. 1a). This resulted in high rates of photosynthesis at low ambient DIC levels.

The apparent cellular half-saturation constant (K_m) for photosynthesis was ~2.5 µM CO_2 (Fig. 1b), consistent with those measured in laboratory cultures of marine diatoms³. We have recently obtained similar results from 10-second uptake experiments in the California upwelling where the indigenous diatoms accumulated cellular inorganic carbon pools five times higher than external levels and showed photosynthetic half-saturation at ~2 µM CO_2 .

The rapid ¹⁴C uptake by the Delaware Bay diatom assemblage may have occurred through active transport of HCO_3^- or by extracellular catalysis of HCO_3^- dehydration to CO_2 coupled to active CO_2 transport⁹. The addition of 50 µM ethoxy-zolamide, an inhibitor of carbonic anhydrase, the zinc enzyme that catalyses HCO_3^- dehydration, had no effect on cellular DIC accumulation but did decrease carbon fixation to 30% of the control level. Carbonic anhydrase is therefore required to catalyse intracellular dehydration of actively imported HCO_3^- , thereby supplying CO_2 to the carboxylating enzyme Rubisco. Indeed, carbonic anhydrase activity seems to be essential for efficient photosynthesis in laboratory diatom cultures, particularly under low CO_2 conditions^{2,10}. In zinc-limited cultures, the activity of carbonic anhydrase is greatly reduced and

HCO_3^- utilization is impaired, resulting in CO_2 limitation¹⁰.

Diatoms that can use HCO_3^- as a source of inorganic carbon should not be dependent on free CO_2 for growth¹⁰. We tested this hypothesis using samples collected from the coastal California upwelling in June 1996 which we incubated with bubbled air containing CO_2 at various partial pressures. The growth of the phytoplankton, which was dominated by large diatoms (*Thalassiosira* spp., 30–40 µm diameter), was independent of p_{CO_2} (Fig. 2). Further, steady-state growth rates of the cells at 100 p.p.m. CO_2 (~1.4 cell divisions per day) were significantly larger than the maximum possible rates that could

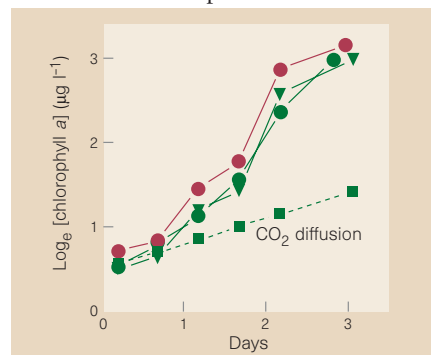


Figure 2 Growth of a Monterey Bay diatom assemblage at various p_{CO_2} levels. Surface water samples (20 litres), collected in June 1996, were incubated with trace-metal-clean techniques at ambient sea temperature and bubbled with air/ CO_2 mixtures containing 100 (green circles), 330 (purple circles), and 750 (green triangles) p.p.m. CO_2 . Growth was measured by daily chlorophyll *a* concentrations¹². Phytoplankton growth rates (~1 d⁻¹) were not significantly different between CO_2 treatments ($P>0.05$). Dotted line: theoretical maximum growth rate that could be supported by CO_2 diffusion to the cell surface (calculated for a cell radius of 15 µm, 300 pmol C per cell; 3 µM CO_2 in the bulk medium and no CO_2 at the cell surface⁷).

be supported by CO₂ diffusion to the cell surface⁷ (~0.3 divisions per day; Fig. 2). This provides compelling evidence that HCO₃⁻ is an important source of inorganic carbon for the natural diatom population.

Bloom-forming diatoms use HCO₃⁻ as a source of DIC, they possess a carbon concentrating mechanism, and as a result, are not limited by CO₂ availability. If this is the case in productive coastal waters it is probably also the case in the open ocean where higher ratios of DIC to nitrate and phosphate, lower maximum pH during the growth season, and smaller phytoplankton size should make the acquisition of inorganic carbon easier. It is doubtful that inorganic carbon is limiting in the open ocean, unless some other factor, such as low zinc availability¹¹, impairs its uptake and fixation by phytoplankton¹⁰. Our results have important implications for ocean carbon cycling, and suggest that a critical evaluation of carbon isotope fractionation models^{5,6} which assume passive CO₂ uptake by phytoplankton is needed.

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An atom-focusing mirror

The recent interest in atom-optics has mainly been directed at the manipulation of atomic beams by static fields or lasers^{1–3}. Using an alternative approach we have succeeded in focusing in two dimensions a neutral atomic helium beam at room temperature with a reflective optical element (an atom mirror). Such focusing relies on specular elastic scattering, which leaves the coherence of incoming wavepackets unchanged.

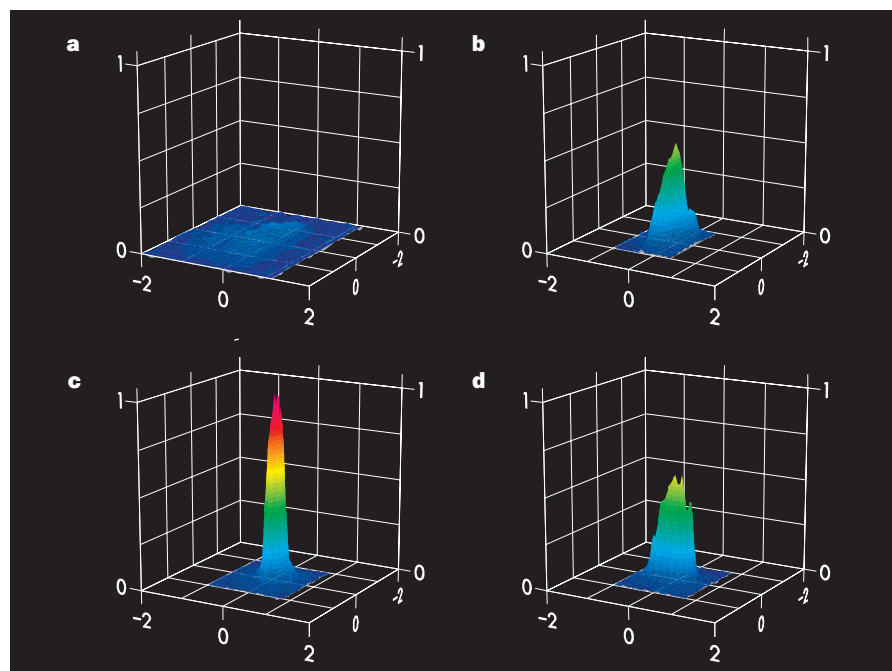


Figure 1 Beam cross-sections for various mirror curvatures (R). The horizontal axes span a plane perpendicular to the beam direction of travel (scale in mm). The vertical axis shows normalized intensity. **a**, Unfocused beam. **b**, First focus in scattering plane ($R=1.2$ m). **c**, Disk of least confusion ($R=0.8$ m). **d**, Second focus perpendicular to scattering plane ($R=0.5$ m).

We created the atom mirror from a single 50- μm -thick silicon crystal cut along the (111) crystal plane. The surface was hydrogen-passivated *ex-situ*⁴ making it inert (the reflectivity remained constant over several months at 10^{-6} mbar). The crystal was deformed electrostatically in an arrangement similar to a parallel plate capacitor to give a parabolic profile for focusing. The focal length can be varied *in situ* simply by adjusting the electric field.

We produced a helium beam in a supersonic expansion source⁵ at room temperature (corresponding to a wavelength of 0.52 Å). We placed the mirror 0.7 m from the source and 0.8 m from the detector with the beam incident at 45°. We measured beam cross-sections for different mirror curvatures (Fig. 1) by scanning the beam across a 100 μm pinhole in front of the detector. Focusing in the geometry used here is necessarily astigmatic. The intermediate disk of least confusion (Fig. 1c) has a spot diameter of 210 ± 50 μm . The solid angle of the unfocused beam is reduced by a factor of about 100. There is a corresponding increase in intensity. The spot size is not limited by aberrations of the mirror, but solely by the geometry of the system and the size of the object (confirmed by computer simulations to be the surface of last scattering in the supersonic expansion⁵).

The ultimate performance of the mirror is limited by the geometrical aberrations⁶ and diffraction⁷ due to the finite size of the mirror (elastic scattering gives no chromatic aberrations). The only other atom-focusing method that relies solely on the de Broglie

wavelength of the atoms is a Fresnel zone plate⁸. A zone plate is diffraction-limited because of the necessary constraints on the plate diameter (about 0.2 mm with current technology). A mirror, on the other hand, has no such inherent limit on its size. With normal incidence, a point source, and image planes 1 m and 0.1 m from the focusing element, the best spot size would be about 2.5 nm with the beam spanning about 2 mm on the mirror surface.

It follows that a helium microscope with nanometre resolution is possible. A helium atom microscope would be a unique non-destructive tool for reflection or transmission microscopy. It could be used to investigate fragile and insulating materials such as polymers and certain biological samples. Focusing mirrors also have the potential to increase spatial resolution and intensity in conventional helium-surface scattering instruments.

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