COMMENTARY

Some enigmatic aspects of the marine cyanobacterial genus, *Trichodesmium*

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Nitrogen is essential for life on the earth, but despite being so abundant (78%) in the atmosphere, it is not readily usable by higher plants. Certain micro-organisms (prokaryotes) living in the soil (*Azobacter, Clostridium*), in plant root nodules as symbionts (*Rhizobium*) and a few cyanobacteria convert dinitrogen into a usable form such as ammonia, the process now popularly known as 'Biological fixation of nitrogen'. Cyanobacteria also referred to as blue-green algae fix molecular nitrogen with the help of enzyme nitrogenase. Since this enzyme is very sensitive to oxygen, the algae protect it from O_2 that evolves during photosynthesis. Fay *et al.*[1] were the first to suggest 'heterocytes' as well protected centres of nitrogenase activity. Heterocysts are vegetative cells specialized with a distinct thick refractive wall and hyaline content.

Over 50 species of the genera, viz. *Anabaena, Calothrix, Nostoc, Scytonema, Stigonema* are heterocystous nitrogen fixers. While the explanation on biological N_2 fixation in heterocystous members appeared convincing, the observation of Dugdale *et al.*[2], identifying *Trichodesmium*, a non-heterocystous genus, as a nitrogen fixer stirred the traditional belief that only heterocystous members fix N_2. *Trichodesmium*'s ability was a paradox, and several hypotheses have been proposed as to how *Trichodesmium* might fix N_2 aerobically, but none provided a universal explanation.

It is believed that protection of nitrogenase, as well as operation of both N_2 fixation and photosynthesis in *Trichodesmium* is achieved by temporal and spatial segregation; N_2 fixation taking place from morning to mid-day, but photosynthesis put on hold[3]. Fogg was the first to offer a provocative hypothesis that a spatial segregation of O_2 evolution and nitrogenase activity occurs within *Trichodesmium* colonies, and that Photosystem II (PS-II) responsible for O_2 production operates in trichomes near the periphery of the colony, whereas nitrogenase activity is confined to the inner portions of the colony that lacked PS-II[4]. It has been observed that a few central cells of the colony develop reduced pigmentation, thus reducing the potential of photosynthesis while enhancing that of N_2 fixation[5]. Immunofluorescence studies have corroborated this observation, as nitrogenase has been found synthesized in about 20–40% of the cells, mostly concentrated around the centre of the filament. The rate of N_2 fixation dropped when filaments in the colony separated due to wave turbulence, thereby exposing more to O_2. It was also observed that in a typical colony of *Trichodesmium* comprising 50–200 trichomes, and each trichome with about 100 cells, the N_2 fixation occurred only during the day and not at night[6].

Biological fixing of nitrogen that requires high energy by nitrogenase, assumes that temporal and spatial segregation of nitrogen fixation and photosynthesis, might be the energy efficient strategy adapted by *Trichodesmium*. But we find it hard to comprehend, why a mechanism so successful in heterocytous members is absent in *Trichodesmium,* and how the present system proposed to be operating, which we know is more constrained by environmental conditions, gives *Trichodesmium* an ecological edge over other plankton? We also know that *Trichodesmium* prefers a warmer (20–30°C temp.) and calm sea condition to bloom, which is yet another environmental constraint affecting the prospects of *Trichodesmium* proliferation. These features will continue to be enigmatic until more work unravels the exact mechanism[6].

The presence of gas vesicles (gas vacuoles) was the second enigma that ignited global debate[7]. Gas vesicles and the photosynthetic products are considered the key factors in providing buoyancy. This also enables *Trichodesmium* in vertical excursions in the water column and maintains the population at a favourable depth as well (depth-keeping/cell ballasting)[7–9]. The depth keeping is suggested to maximize availability of light and nutrients and help to avoid mid-day irradiance[10,11]. Though there are some observations on the vertical excursion of *Trichodesmium* and its population maxima at subsurface depths of 15–25 m, how exactly the mechanism works is yet to be clear. Experimental studies on *Trichodesmium thiebautii* suggest protein to carbohydrate ratio to be the best predictor of buoyancy and for estimation of potential for vertical excursions within water depths, but it did not hold good for depths[12] beyond 70 m. A complex relationship between light, nutrients and compositional changes was also observed during the buoyancy reversals.

Trichodesmium forms dense surficial blooms, at times of the order of 2×10^6 km^2 imparting red, yellow or brown colours to the surface waters[6]. However, a large population goes undetected, as satellites capture only the surficial events and not the sub-surface ones. Even *in situ* water samplings that are normally planned only for a few depths, are likely to miss a large sub-surface biomass. Capone *et al.* in a recent study estimated the bloom that occurred in the Arabian Sea during 22–27 May 1995 is of the order of 2×10^6 (20% of the Arabian Sea surface). However, satellite scanning is unable to detect blooms in the afternoon, as a large population observed at the surface in the morning may not be available for satellite detection due to their negative buoyancy[6,12], besides difficulties are faced in distinguishing spectral signatures of *Trichodesmium* bloom and that of sediment plumes, as they are identical[6,13]. Though occurrence of *Trichodesmium* bloom at depth is rare, its blooms have been reported as deep as 240 m in the North Pacific and in the Red Sea.

What environmental and ecological factors control production and N_2 fixation in *Trichodesmium*? The amount of N fixed annually is poorly known but expected at least 5.4×10^{12} g per year[6]. Capone *et al.*[6,14] have provided a revised estimate based on their 15 years of intensive research and available information on distribution and abundance of predominant diazotrophs (Table 1).

The diversity among *Trichodesmium* species has prompted debate concerning its taxonomic and phylogenic position within cyanobacteria. Geitler and Sournia have placed *Trichodesmium* in the genus *Oscillatoria*, and recently, Anagnostidis and Komarek placed it in the family

Table 1. Estimate of N_2 fixation by *Trichodesmium* spp in the world oceans on a seasonal basis[14] $(g \times 10^9)$

Oceanic Basin	Spring	Summer	Autumn	Winter	Total
Pacific	11	163	162	0.9	337
Atlantic	101	474	133	614	1322
Indian	1890	0.5	267	966	3124
S. China and Arafuru Seas	7	0.9	0.1	10.2	18
Total	2009	638	562	1591	4801

Phormidiaceae of the order Oscillatoriales respectively[15–18]. However, it is intriguing to see *Trichodesmium* frequently referred to as *Oscillatoria* even in the recent literature. *Oscillatoria* lacks a visible sheath around its trichome and exhibits oscillating or gliding movement. *Trichodesmium* also shows similar trichome structure, but is surrounded by mucilage and is known to form colony and red or yellow blooms. Within the genus of *Trichodesmium*, however, it is difficult to distinctly identify some species merely on morphological features based on colony shape, trichome diameter, and ratio of cell length to cell width and position of gas vacuoles[19] as is normally followed.

Based on a careful study with the aid of both light and electron microscopy, Janson *et al.*, identified six species, viz. *Trichodesmium erythraeum*, *T. thiebautii*, *T. tenue*, *T. hildebrandtii*, *T. contortum* and *T. aureum* and suggested clustering of four based on both morphological characteristics and DNA sequence[17]. The two most reported species: *Trichodesmium erythraeum* and *T. thiebautii* have been identified as toxic, and reported to contain a type of neurotoxin commonly associated with paralytic shellfish poisoning (PSP)[20–23]. Despite availability of such published information we still find these species grouped as non-toxic. Since *Trichodesmium* forms extensive bloom and at times lead to mass mortality of fish and other invertebrates, it becomes essential to examine constituent organisms of each bloom very closely at the sub-cellular and biochemical level, besides the conventional morphology. A perusal of over 30 research papers published on aspects of *Trichodesmium* bloom in the Indian Ocean region, where *Trichodesmium erythraeum* and *T. thiebautii* are most frequent, showed that the cause for fish mortality whenever it occurred, is mostly attributed to asphyxiation and gill damage by the filaments of the alga.

Finally, we are left with an intriguing question. It is difficult to work out the travel time in a dynamic water column of 15–25 m and beyond 70 m as stated earlier, and also to estimate the number of trips that a species is likely to undertake in a day or during the bloom as dictated by the water conditions. Why did *Trichodesmium* not evolve flagella for motility? Let us agree that 'Nothing in the sea falls haphazard; if we cannot predict, it is because we do not know the cause, or how the cause works...' Henry Bigelow, 1929.

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