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Indications from Photosynthetic Components that Iron is a Limiting Nutrient in Primary Producers on Coral Reefs

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Abstract

Because iron is not available generally in oxygenated sea water, it may be a limiting factor in marine primary production. This hypothesis was tested in the context of Davies Reef, Latitude 18°50′S (one of the coral reefs in the central region of the Great Barrier Reef system). Samples were collected for study in the period August, 1980 to March, 1981. Sea water around the reef contained $\leqq 2\times10^{-8}$ *M* Fe, surface sediments from the reef contained 66 ± 26 (1 SD) ppm total Fe, and interstitial water near the surface contained $\geqq 5\times10^{-7}$ *M* Fe. Thus, Fe constituted a trace component of the reef environment, but limited Fe should be available to algae associated with the sediments. Specific biochemical analyses to test the Fe status of benthic photosynthetic organisms were carried out with a common blue-green alga, *Phormidium* sp., and a ubiquitous symbiotic dinoflagellate, *Gymnodinium microadriaticum* (zooxanthellae). The blue-green alga contained the electron transport protein, flavodoxin, which is found only in Fe-deficient organisms. Supporting evidence for Fe stress in this organism included chlorosis in the presence of plentiful biliprotein, and very low extractable photosynthetic cytochrome, *c*-553. The latter observations were shown to be the result of Fe deficiency in laboratory cultures of a blue-green alga, *Synechococcus* sp. These cultures showed that production of flavodoxin is not a universal response of algae to Fe stress, but that lowered cellular concentrations of Fe-containing proteins involved in photosynthesis probably is universal. The zooxanthellae from a soft coral, *Sinularia* sp., had three-fold lower total Fe and ferredoxin (an electron transport protein), than the same alga from a clam, *Tridacna maxima*. Thus, some algae in symbiotic associations may also suffer Fe-deficiency. It was concluded that the degree and extent of Fe-stress in primary producers on a coral reef may influence growth rates, biomass, and distribution of species.

Introduction

In the open surface waters of tropical seas, the low concentrations of N and P available for photosynthetic organisms have commonly been considered the major constraint on the pelagic biomass (Goldman *et al.*, 1979). The primarily benthic community of coral reefs, though surrounded by such water, cannot be constrained similarly by N and P. The availability of inorganic N does appear to limit short-term growth of benthic algae in some reef habitats (Hatcher and Larkum, 1983), but there has been recognition of widespread fixation of nitrogen, which can generate usable forms of N for organisms which do not fix N (Lewis, 1981); the exportation of fixed N to the surrounding water has also been reported (Webb *et al.*, 1975; Andrews and Müller, 1983). Work in our laboratory has revealed that coral reef sediments contain a pool of P, much of which is potentially available to photosynthetic organisms associated with the sediment (Entsch *et al.*, 1983). Thus, it is clear there can be an enhanced local supply of N and P in coral reefs.

Nitrogen and P are not the only mineral nutrients which may influence biomass and productivity in marine environments. Iron and manganese are indispensible components of photosynthesis, with Fe required in considerable quantities in chloroplasts (Price, 1968; Spiller and Terry, 1980). In addition, iron and molybdenum are essential to nitrogen fixation, with 34 atoms of Fe in each molecule of nitrogenase (Eady and Smith, 1979) and more Fe required in associated electron-carrier proteins. Iron is present in the open sea in only trace amounts due to the insolubility of the ferric cation in oxygenated sea

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water (Huntsman and Sunda, 1980). Over many years, there have been occasional references to the importance of available Fe in the regulation of phytoplankton growth (Tranter and Newell, 1963; Provasoli, 1979; Huntsman and Sunda, 1980). Any input of Fe from land is quickly lost by precipitation into bottom sediments (Boyle *et al.*, 1977; Moore *et al.*, 1979). Thus, some shallow sediments can contain plentiful mineral nutrients for benthic organisms. However, most of the reefs of the Great Barrier Reef Complex are well away from the continental shoreline and are not likely to be enriched in Fe from the water column (even with flood input by rivers: Wolanski and Jones, 1981). Sediments of the reefs are generated *in situ* by calcifying organisms (Milliman, 1974), and are thus unlikely to contain much Fe from surrounding sediments.

Our initial investigation of the iron status of primary producers on coral reefs was concentrated on a designated test reef (Davies Reef), typical of the reefs in the central region of the Great Barrier Reef Complex, and the results obtained should be applicable to most coral reefs containing only carbonate sediments.

The biological availability of Fe depends on the organism involved as well as the chemical species in the environment. Many microorganisms are capable of specific uptake of ferric iron by excretion of siderophores (Neilands, 1981). Recent literature suggests that, like higher plants, some macroalgae may use a mechanism which involves reduction of ferric to ferrous iron before uptake (Manley, 1981). Generally, there is very little information on this aspect of marine algae, although siderophores have been detected from blue-green algae (McKnight and Morel, 1980). Thus, chemical measurements of various forms of Fe in the environment are not good indicators of the Fe status of a group or organisms. Specific physiological markers are the most effective way of indicating deficiency or sufficiency. Where physiological investigations of Fe stress have been made (in some microorganisms and higher plants), the responses detected were multiple (Light and Clegg, 1974; Terry, 1980). Most changes were negative in the sense of a decrease of some component, such as an Fe-containing protein. However, one distinctive and useful positive change has been observed – the displacement of soluble ferredoxin (2 or 4 Fe per molecule) by flavodoxin (no Fe present) when Fe supply is limiting (Mayhew and Ludwig, 1975; Hutber *et al.*, 1977). The physiology of this change and its environmental implications have not been investigated.

To apply the physiological information on Fe deficiency to photosynthetic organisms of a coral reef, we selected two groups of microalgae: (1) Blue-green algae, an important component of the epipelic and epilithic algal communities (Cribb, 1976), which are of primary trophic importance on coral reefs (Hatcher, 1983a, b). A widely distributed blue-green is the large, filamentous organism, *Phormidium* sp. If Fe stress is present in the blue-green group, then there is a reasonable chance that the same stress applies to other algae dependent upon mineral sources of Fe. (2) Algae in symbiotic associations with reef animals were also investigated. The most common organism of the latter group is the dinoflagellate *Gymnodinium microadriaticum*, most prominently found within the tissues of reef-building corals. This alga (like other algae in symbiotic associations) may depend on the predatory success of the host animal for sources of some nutrients such as N and Fe (Muscatine and Porter, 1977), so its nutritional status may correspondingly vary with host.

This paper reports the levels of iron found in the environment of the test reef, establishes that the limited availability of Fe must influence the physiology of photosynthesis, and hence that Fe could be a limiting nutrient of importance to at least a portion of the photosynthetic organisms of a reef. (A preliminary report of this research was presented at the Thirteenth International Botanical Congress, Sydney, August, 1981.)

Materials and Methods

Reagents

All inorganic reagents and solvents for analysis and manipulations were analytical reagent grade or better where required. The exceptions were calcium hypochlorite (called "dry chlorine", from Olin Chemicals, Stamford, Connecticut, USA) and carbon dioxide (medical grade from Commonwealth Industrial Gases, Australia).

The source and grade of specific biochemicals mentioned in the text are listed below. Flavodoxin (as a standard) from *Megasphaera elsdenii* was a gift from Professor V. Massey, Ann Arbor, Michigan, USA. The rest of the biochemicals came from the Sigma Chemical Company, and grading refers to Sigma products only. Flavin mononucleotide (FMN) was Grade 1; cytochrome *c*, horse-heart Type VI; NADPH, enzymatically reduced, Type III; ferredoxin-$NADP^+$ reductase (EC 1.18.1.2 and formally 1.6.7.1), from spinach; deoxyribonuclease I (EC 3.1.4.5), bovine Type III; phenylmethylsulphonyl fluoride (PMSF); Bicine, and Tris (Trizma grade).

Study Site

The Australian Institute of Marine Science is situated on Cape Ferguson, just south of Townsville, Australia. Davies Reef is one of a large number of individual coral reefs on the eastern (outer) half of the continental shelf off Townsville. The reef, with dimensions of approximately 5.9 × 2.6 km, is 70 km E.N.E. of Townsville at Latitude 18°50′S. There are no major reefs between Davies Reef and the continent, although there are larger and smaller reefs further east. The windward face of Davies Reef comprises a solid wall, with many large indentations and a high coral cover on the southeast corner. The leeward side is a broken wall with channels and actively growing areas. None of the surface is exposed, except at very low tides. There is a series of basins inside the front at the northern end, and a large

open central lagoon (from 10 to 25 m), with many patch reefs. All sections inside the reef are subject to tidal flushing. There is a small, but distinct seasonal variation in water temperature (from 23° to 29 °C).

Samples of water, sediments, corals, and algae were collected at Davies Reef in August and November, 1980 and March, 1981.

Iron in Water Samples

Water samples were collected in 4-litre polythene Niskin bottles attached to a nylon rope. Care was taken at all times to prevent exposure of samples to iron and steel. Water from the Niskin bottle was filtered through acid-washed, 20 μm plankton net attached to the outlet, and samples of 1 litre were stored in sealed, acid-washed polythene bottles. These bottles were stored at 4 °C and analysed as soon as possible after return to base. After samples had been filtered through a 0.45 μm HA Millipore filter, iron in the material collected on the filter was regarded as "particulate" iron, while that in the filtrate was regarded as "soluble". These classifications were arbitrary, since Fe can be colloidal in natural water samples. Particulate and soluble Fe were analysed by different procedures, as described by Strickland and Parsons (1972).

Interstitial water samples were collected from the surface sediments with minimum disturbance. Ceramic aquarium stones used (15×20×100 mm) did not contaminate sea water with Fe. The stones were connected via Tygon tubing to 50 ml polythene disposable syringes, and the whole assembly was washed with filtered sea water. SCUBA divers inserted the stones horizontally just below the surface of the sand and a sample was drawn into the syringe over a period of about 20 s, after allowing about 1 min for equilibration in the sand. Moderately slow sampling helped to minimize dilution from the water column and prevented fine particles being drawn into the syringe. The sample syringes were capped and later stored at 3 °C for analysis. Only total Fe was measured in these samples. Each sample of 50 ml was digested with a mixture of $HClO_4$ and HNO_3. The residue was dissolved in HCl, and Fe was measured by the method for soluble Fe (as above). The useful limit was approximately 10^{-7} *M*, because of higher blanks compared to water samples prepared by filtration through 0.45 μm filters, without digestion.

Iron in Sediments, Corals, and Algae

Surface-sediment samples from Davies Reef (to a maximum depth of about 10 cm) were collected in polythene bags (about 400 g sediment per bag) by SCUBA diving and stored at −20 °C. Samples of young, calcified tips of *Halimeda* spp. (free of epiphytes), and young, growing tips of several coral species were collected and added live to a saturated solution of calcium hypochlorite in sea water at Davies Reef. The samples were gently mixed periodically for several hours at room temperature. The bleached skeletons (free of tissue) were washed several times with fresh sea water, air-dried, and sealed in plastic bags for storage. In the laboratory, sediment samples were dried overnight at 100 °C, and subsamples of approximately 50 g were ground with a ceramic pestle and mortar for total iron analysis. Samples of 0.5 g of finely ground sediment were dissolved and then digested in a 2:1 (v/v) mixture of concentrated nitric and perchloric acids, which were then removed by evaporation after the addition of sulphuric acid. The digests were mixed with water, and calcium sulphate was allowed to settle before aliquots of the solution were taken for analysis using the procedure for particulate Fe in H_2O (Strickland and Parsons, 1972). After colour development, it was necessary to allow any fine, white particulate matter to settle before measurement.

Various epilithic algae were collected from the Reef by hand while SCUBA diving, roughly freed of excess water with chromatography paper, and stored in sealed, plastic bottles at −20 °C. Only a few of the samples collected had a small fraction of entrained sediment by weight, and these were analysed. About 10 g of frozen tissue was dried at 100 °C, and samples of about 0.25 g of dried tissue were digested and measured for Fe as described above for sediments.

General Analyses for Algal Extracts

Chlorophylls *a* and c_2 were determined by the method of Jeffrey and Humphrey (1975); soluble protein was assayed by the method of Bradford (1976).

Detection of flavodoxin in chromatographic fractions required several techniques. When flavodoxin occurred in concentrations similar to ferredoxin, it was detected by its characteristic absorption spectrum (Mayhew and Ludwig, 1975). This procedure was effective at $\geqq 10^{-5}$ *M* flavodoxin, but when a lower detection limit of 10^{-6} *M* protein was required, flavin fluorescence was used. Bound to flavodoxins, FMN has virtually no fluorescence, but is highly fluorescent in free solution (Mayhew and Ludwig, 1975). Thus, an unknown sample was acidified to pH 1 to 2 with solid trichloroacetic acid to release FMN from any flavodoxin and the protein precipitate formed was removed by centrifugation. Fluorescence due to FMN was detected by scanning for the emission peak at 530 nm in a fluorometer, upon excitation of the sample at 450 nm. This procedure does not detect biologically active flavodoxin. A test of biological activity at the required sensitivity (10^{-6} *M* protein) and specificity was achieved with the enzyme ferredoxin reductase (Foust *et al.*, 1969; Davis and San Pietro, 1977). The basic assay solution in 1 or 3 ml at 25 °C contained 10 m*M* potassium phosphate (pH 6.9) 35 μM mitochondrial cytochrome *c*, 150 μM NADPH, 0.05 μM enzyme, and ferredoxin (from *Synechococcus* sp. cells) or flavodoxin in the range of 0.1 to 0.5 μM. The reduction of cytochrome *c* was followed at 550 nm in a spectrophotom-

eter with a reference containing 35 μM cytochrome *c* to balance the absorbance. The low blank rate (without ferredoxin) was subtracted from the rate with ferredoxin or flavodoxin, which were added last to the assay. Molecular activity varied with each specific electron carrier protein.

Collection and Extraction of *Phormidium* sp.

Samples of the filamentous blue-green alga *Phormidium* sp. were collected by SCUBA diving on Davies Reef. Clumps of filaments were cleared of sediment, animals, and senescent tissue, then washed with sea water. Extracellular water was squeezed out, and sealed lumps of about 100 g of cells were rapidly frozen in solid CO_2, and stored at −76 °C until use.

Samples of 1 to 2 g of tissue were fractured from deep-frozen material, dried, and digested for total Fe, as described before for algae. A known weight of about 100 g of compacted filaments was thawed with 100 ml of distilled water. The mixture was homogenized to a uniform soup in a Sorvall Omnimix at 0 °C; then samples of about 2 g were taken for determination of dry weight and chlorophyll *a*. Extraction buffer (100 ml) was added to give a final concentration of 80 mM Tris-Cl, pH 8.3, at 0 °C, plus 1 mM PMSF. The mixture was homogenized with an Ultra-Turrax at full power to fragment the filaments. The total volume (300 to 350 ml) was then sonicated at 0° to 10 °C in aliquots of 50 ml to break the cells. Sonication was halted when at least 90% of cells were broken (determined by microscopic examination). About 3 mg of deoxyribonuclease was added and then a second addition of 1 mM PMSF. The homogenate was centrifuged for 15 min at 2 °C, and 30 000 × *g*. The pellet contained grey to white cell ghosts with green photosynthetic lamella on top. The supernatant was a clear, dark red solution with brown fluorescence. Samples were taken for determination of soluble protein and phycoerythrin (responsible for the red colour). The absorbance spectrum of the supernatant was characteristic of B-phycoerythrin and this protein was determined by using absorbance of 1% solution at 546 nm of 82.3 (Ó Carra and Ó hEocha, 1976). To remove an organic component from the extract which reacted with oxygen and darkened the solution over a period of hours, the supernatant was passed through a column (2.5 × 6.0 cm) of DEAE-cellulose (Whatman DE-52) equilibrated with 10 mM Bicine, pH 7.8 at 3 °C. the column was then washed with 0.25 M NaCl in Bicine and the desired fraction eluted in 60 ml with 0.45 M NaCl in Bicine. To obtain an estimate of the extraction of soluble photosynthetic cytochrome *c*, this column separation had to be replaced by ammonium sulphate fractionation, since the cytochrome *c* was lost from the column by the former procedure. The original supernatant solution from separate extractions was fractionated and chromatographed essentially as described for extracts from *Synechococcus* sp. (see "Growth and Analyses of *Synechococcus* sp.").

The eluate was made up to 0.1 M in potassium phosphate, pH 7.5, and solid ammonium sulphate was added to 55% saturation at room temperature. The precipitate formed was red (mostly phycoerythrin), and the supernatant was desalted with an Amicon Diaflo membrane (YM-10) to give a conductivity less than that of 0.15 M NaCl. This solution was loaded onto a 5 × 1.5 cm column of DEAE-cellulose equilibrated with 10 mM Tris-Cl, pH 7.8, at room temperature. The column was washed with 0.27 M NaCl in buffer until all traces of pink phycoerythrin had been eluted, leaving a yellow band in front of a red band on the column. The coloured proteins were eluted with 0.35 M NaCl followed by 0.5 M NaCl. The earliest fractions contained flavodoxin (as identified by flavin spectrum and ferredoxin activity in the assay of ferredoxin reductase) and the last, ferredoxin (identified as described for zooxanthellae). Flavodoxin was estimated by using an extinction value at 461 nm of 10 mM^{-1} cm^{-1}. Flavodoxins have extinction values for the absorbance maximum in the visible range between 9 and 11 mM^{-1} cm^{-1} (Mayhew and Ludwig, 1975). In the mixed protein fractions, each protein could be separately estimated, as only ferredoxin had an absorbance at 600 nm.

All analyses on the extracts of *Phormidium* sp. were corrected for sampling, losses, etc., and reduced to a basis of 100 g dry weight of cells. All results were underestimated by an unknown small factor, due to small residues of reef sediments in each sample of algae extracted.

Collection and Extraction of Zooxanthellae

Samples of the symbiotic dinoflagellate *Gymnodinium microadriaticum* (commonly called zooxanthellae) were obtained from clams (*Tridacna maxima*) and a soft coral [a *Sinularia* sp., similar in morphology to *S. capillosa* and *S. mollis* (Verseveldt, 1980)]; these were selected as they provided a high yield of cells free of animal tissue. Clams were collected from the reef by SCUBA diving and processed the same day on board the research vessel. Small slices of mantle tissue containing algae were homogenized at 0 °C for 1 min in sea water with a Sorvall Omnimix. The blended mixture was squeezed through 20 μm nylon plankton net, which retained clam tissue. The effluent was centrifuged in polycarbonate tubes at approximately 800 × *g* for 5 min (just long enough to pellet intact algae) at room temperature. The supernatant was discarded and the process was repeated until pellets of 5 ml of compacted algae had been collected. The pellets were washed twice with sea water in the centrifuge tubes, snap-frozen in solid CO_2, and stored at −76 °C for use later in the laboratory. Samples of a colony of *Sinularia* sp. were collected by SCUBA diving and processed on the research vessel. Pieces of the coral were taken directly from sea water and simply squeezed into a polythene basin by hand pressure. This ruptured the coral tissue and released freely suspended algae, as the skeleton changed from dark brown to yellow-white. The suspended algae were passed through plankton net, and then processed as described for the algae from clams.

About 15 ml of compacted cells of known weight were thawed in the presence of 45 ml of extraction buffer (0.15 *M* Bicine plus 1 m*M* PMSF, pH 7.8). Samples were taken from the slurry of cells for determination of dry weight and total Fe. Aliquots of the slurry were sonicated in an ice bath until more than 90% of cells had disintegrated (determined by microscopic examination). The homogenate (made up to 1 m*M* in PMSF again) was centrifuged at 2 °C in a preparative ultracentrifuge at 160 000 × *g* for 1 h. Samples of supernatant (dark orange-brown) were taken for determination of soluble protein and chlorophyll *a* (as bound in the soluble peridinin-chlorophyll-protein complex of dinoflagellates and referred to here as PCP), and one complete pellet (dark brown) was used for chlorophyll *a* plus c_2 analysis. Solid ammonium sulphate was added to 55% saturation at 2 °C, and the pH was kept at 7.2 with dilute NH_3 solution. After 30 min, the protein precipitate was removed by centrifugation, leaving a dark orange-brown supernatant. Ammonium sulphate was added to give 100% saturation at 2 °C, resulting in a protein precipitate which was orange-black, and represented about a third of the total soluble protein. The precipitate was dissolved in 10 m*M* Bicine, pH 8.0, and diluted with buffer until the conductivity was less than that of 0.15 *M* NaCl in the same buffer. The solution was run through a 4.0 × 1.5 cm column of DEAE-cellulose (Whatman DE-52) equilibrated with 10 m*M* Tris-Cl, pH 7.8, at 23 °C, and the column then washed with 0.15 *M* NaCl in the same buffer. The effluent contained PCP and soluble photosynthetic cytochrome *c*. At this stage, any soluble ferredoxin and flavodoxin from the cells should be adsorbed to the top of the column (Mayhew and Ludwig, 1975). The column was washed with 0.29 *M* NaCl in buffer, and an orange-yellow band was eluted, leaving a red-brown band at the top of the column. The eluted band contained an isomer of PCP (as distinguished by the characteristic carotene and chlorophyll *a* absorption bands present), and displayed no activity in the ferredoxin reductase assay. The column was finally washed with 0.5 *M* NaCl in buffer, which removed a single red-brown band with the characteristic absorption spectrum of a soluble, plant ferredoxin (Palmer, 1975). This solution was active at typical ferredoxin concentrations in the assay for ferredoxin reductase. Ferredoxin was estimated from the absorbance at 420 nm (extinction of 10 mM^{-1} cm^{-1}) and the Fe content of the solution (assuming 2 Fe atoms per molecule). All analytical measurements were reduced to amounts per 100 g dry cells.

Growth and Analyses of *Synechococcus* sp.

The organism used in laboratory cultures was a unicellular, marine, blue-green alga in axenic culture, isolated by L. J. Borowitzka (see Borowitzka *et al.*, 1980), and identified as a *Synechococcus* sp. using the classification system of Rippka *et al.*, 1979. Under this system, it is considered that *Agmenellum quadruplicatum* and *Coccochloris elabens* belong to the same group. Cells were maintained on agar slopes with a medium based on the *f*-mixture of Guillard and Ryther (1962; as described by McLachlan, 1973) without sodium silicate.

For physiological and biochemical studies, cells were grown in batch cultures of 17 litres each, from inoculums of 800 ml to 1 litre. The inoculum cultures were grown from slopes, using the modified *f*-mixture described above. Fe (as Fe^{III} EDTA) was made up to a final concentration in culture of 10^{-5} *M* Fe, without the presence of citrate. The medium, containing no precipitate, was sterilized by filtration through a 0.22 μm Millipore filter. Each inoculum was kept in flasks on a reciprocating shaker at 25 °C and illuminated from above and below by Sylvania cool-white fluorescent tubes. Growth rate of cultures was limited by the diffusion of CO_2 into the flasks. Before other nutrients became limiting, the flasks were used for inocculation of larger cultures.

Large cultures were grown in 20-litre Pyrex bottles with a sealed top containing an inocculation/sampling port, plus gas inlet and outlet ports. The growth medium used was again medium *f*, but with the following modifications: nitrate and phosphate were three-fold higher than in the original *f* medium, no silicate was added, and Fe was used in varying concentrations in the form of Fe^{III} EDTA (1:5 molecular ratio). The complete medium was added to 4 to 5 litres of distilled water and autoclaved – this method prevented precipitation. The solution was then made up to 17 litres with sea water filtered through 2 × 0.22 μm filters. For cultures with a regulated, low Fe supply, all glassware used was washed with detergent, 1 *M* HCl, and finally, filtered pure water. Iron deficiency was created by withholding Fe^{III} EDTA from large cultures.

After inoculation of large cultures, they were grown at 28° to 29 °C, stirred vigorously by a magnetic stirrer, and gassed with 1% CO_2 in air. Illumination was provided by a bank of fluorescent tubes (Sylvania cool-white) in a chamber with reflective walls and floor. At the surface of the bottles, the radiant intensity of visible light was approximately 200 μE m^{-2} s^{-1}. Cultures were monitored by several measurements. Cell density was followed by turbidity at 750 nm in a Varian, Superscan 3 spectrophotometer, with sample cells in the normal absorption position in the light beam. Readings were confined to the 0 to 0.5 absorbance range. Under these conditions, an absorbance of 1 was equivalent to 0.26 mg dry weight of cells per ml of culture in the linear growth phase. The occurrence of contamination (apart from low numbers of bacteria) was detected by phase-contrast microscopy. After cells had been removed by filtration, nitrate and phosphate in the medium were measured by use of an autoanalyser as described by Ryle *et al.* (1981). The relative photosynthetic pigment composition of whole cells was determined by recording the absorption spectrum of cell suspensions in the light-scattering position of an Aminco DW-2 spectrophotometer, where pigment absorption dominated over light-scattering in the visible light range. Differences in composition were detected by direct com-

parison of spectra when cell densities were matched at 750 nm. Chlorophyll *a* was recognized by a peak at 680 nm, phycocyanin at 630 nm, and carotenes at 485 nm. Each culture was harvested by passage through a Beckman JCF-2 continuous-flow centrifuge head at 15 °C. The pellet was collected, washed with 25% sea water (100% sea water = 35.5‰ S) twice, and stored at −80 °C.

Between 25 and 50 g of compacted cells were thawed with 4 to 5 vol of 70 m*M* Bicine (K salt), pH 7.8, and the slurry maintained near 0 °C and stirred thoroughly. Samples were taken to estimate dry weight, chlorophyll *a*, and total Fe. Total Fe was determined after acid digestion as already outlined for the interstitial water analysis (see "Iron in Water Samples"). The cell suspension was extracted at about −20 °C with acetone, as described previously by Smillie and Entsch (1971) for a similar *Synechococcus* sp. The resulting cell powder was extracted with 3 vol of the Bicine buffer per volume of compacted cells (thawed). Deoxyribonuclease (2 to 3 mg) was added and the slurry of cells was fractionated as described by Smillie and Entsch as far as the addition of ammonium sulphate to 100%, when the preparation was left overnight at 23 °C. The precipitate was collected by centrifugation, dissolved in 10 m*M* Tris-Cl, pH 7.8, and diluted with buffer to a conductivity equivalent to 0.15 *M* NaCl.

The protein solution was loaded on a column of DEAE-cellulose (DE-52 of Whatman), 3.5 × 1.5 cm, equilibrated with 10 m*M* Tris-Cl, pH 7.8. The effluent plus 10 ml of wash (0.15 *M* NaCl in buffer) was collected, and soluble photosynthetic cytochrome *c* was measured using an extinction at 553 nm of 29 mM^{-1} cm^{-1} – an average value for such cytochromes (see Mitsui, 1971). The column was washed with 0.25 *M* NaCl in buffer, followed by 0.35 *M* NaCl in buffer. The latter effluent was collected and tested for ferredoxin activity in the assay of ferredoxin reductase. Ferredoxin was eluted from the column by 0.5 *M* NaCl in buffer, and determined by using an extinction at 420 nm of 10 mM^{-1} cm^{-1}. All measurements were corrected for losses during fractionation, and recorded per 100 g dry cells.

Results

Iron in the Sea Around Davies Reef

Table 1 summarises measurements of Fe in the water column. Samples were collected below the surface from the top metre of water: (a) outside the windward face of Davies Reef; (b) from the back of the crest of the reef where water was flowing across the crest; (c) from the lagoon and three enclosed holes (large depressions 15 m or more deep in the reef flat behind the crest). These holes were further sampled within 0.5 m of the bottom sediment, at depths of 15 to 25 m. In all samples, Fe was at or below the limits of detection (Table 1). In some samples, the particulate fraction gave indications of Fe at the limit of detection. There were no differences in samples collected early in November and again in the following March after the annual wet season had added river run-off to coastal areas. Another group of samples was collected in March from the top metre of the water column over the continental shelf from the shore line to Davies Reef. Measurable quantities of iron were present close inshore, but these were variable, due to the mainly particulate form of the Fe here (Table 1). Fe concentration rapidly diminished with distance from the coast until it could no longer be measured with the techniques used.

Table 1. Iron measured in water column around Davies Reef, Latitude 18°50′S. Limits of detection for iron analyses, as described by Strickland and Parsons (1972), are: soluble, 1×10^{-8} *M*; particulate, 2×10^{-8} *M*

Sampling position and date	Average Fe values ($\times 10^{-8}$ *M*)	
	Soluble	Particulate
Davies Reef (24 samples, November, 1980, and March, 1981; combined data)	< 1	≦ 2
Transect to Davies Reef (March, 1981, calm weather: from Marine Institute shore line to reef)		
Off breakwater (inshore)	1	50
2 km offshore	7	10
22 km offshore	< 1	< 2
40 km offshore	< 1	< 2
58 km offshore	< 1	< 2
Behind Davies Reef (75 km)	< 1	< 2
Rapidly Processed Transect[a] (March, 1982, after moderate seas: off the coast towards Davies Reef)		
Off breakwater (inshore)	4	50
2 km offshore	4	23
6 km offshore	6	7
10 km offshore	6	4

[a] Samples analysed within 2 h of collection

There are two recurrent problems connected with the measurement of Fe in sea water. One is contamination, which can be overcome by careful sampling; the other is loss of Fe onto the surface of storage containers (Strickland and Parsons, 1972). This latter is impossible to overcome when samples have to be stored on an extended field trip. Thus, the validity of the reported results from stored samples was tested. A high-speed boat was used to collect samples up to 10 km off the coast towards Davies Reef, and analysis was commenced within 2 h of collection (Table 1). Although these samples were collected 12 mo after the samples of the longer transect to Davies Reef, the results obtained were very similar. In a further test of storage (not shown in Table 1), a separate 20-litre sample was collected close to shore at one site and quadruplicate samples were measured for particulate Fe immediately and again after standing for 4 d in sampling containers; no significant differences were found. The latter test was only possible at measurable Fe concentrations. However, losses due to storage could be significant when total Fe concentrations in the sample decrease to the detection limits (as recorded around Davies Reef).

Iron in Davies Reef Sediments and Algae

Table 2 shows the amount of Fe present in the surface sediment layer of Davies Reef. Sediment samples were collected to a depth of approximately 10 cm from various environments within the reef – sites of high coral and algal cover on the perimeter, lagoonal sediments, and from the bottom of enclosed large pools in the windward crest. Total Fe analyses showed that Fe was a trace component of the sediments, and was probably distributed uniformly over the reef. At least some of this Fe could be available to primary producers growing in association with the sediment if Fe were dispersed in soluble and colloidal species. Previous investigations (Entsch *et al.*, 1983) have shown that the surface layer of sediment is anaerobic, and that with increasing depth, electrochemical potentials develop which could result in reduction of Fe to the ferrous oxidation state. In the absence of specific chelating agents, ferrous Fe is much more soluble in sea water than ferric Fe, the oxidation state in the presence of oxygen (Stumm and Morgan, 1970).

Samples of interstitial water were collected at the same sites used for sediment collection. The method of collection was found previously to result in dilution of the interstitial water by water from above the surface (Entsch *et al.*, 1983); thus, oxidation and dilution of the Fe present was expected, and the concentrations of Fe measured (Table 2) must represent a lower limit of the *in situ* situation. Since the distribution of chemical species of Fe would be influenced by the method of sampling, only total Fe was measured. The concentration of Fe detected in interstitial water was of the order of one part in 10^4 of the Fe present in the sediments. At the interface with the water column, the presence of oxygen and soluble phosphate would result in precipitation of any soluble Fe and presumably its retention in the sediments (Entsch *et al.*, 1983). Apart from the soluble species of Fe, a further significant fraction of the pool of Fe could be available to organisms which have mechanisms for the solubilization of particulate Fe^{III} before uptake (e.g. some blue-green algae).

Tissue-free skeletons of growing tips of corals and algae contained very low concentrations of Fe, which suggests that iron is not incorporated into the carbonate matrix of a reef as a consequence of the process of calcification (Table 2). Thus, the Fe in the sediments was probably present in the following associations: with organic matter, adsorbed onto the surface of carbonates, and distributed as inorganic minerals among the sediment particles.

Apart from planktonic algae, the organisms most likely to have difficulty in obtaining a supply of essential Fe from the environment are the epipelic and epilithic (or "turf") algae, which are the most important primary

Table 2. Total iron contents of sediments and algae from Davies Reef. Each sediment and algal sample was measured in duplicate

Sample	*N*	Mean value or range
Mixed sediments – top 10 cm	20	66 ± 26 ppm
Interstitial water[a]	14	$(50 \pm 18) \times 10^{-8}$ *M*
Bleached, newly formed, coral and *Halimeda* spp. skeletons	10	0 to 2 ppm
Algae (Fe as % dry wt)		
Boodlea composita (Chlorophyta)[b]	1	0.0085 %
Ceramium gracillimum (Rhodophyta)[b]	1	0.017 %
Chlorodesmis comosa (Chlorophyta)	2	0.0105 %
Epilithic community (multispecies, filamentous turf)[b]	2	0.016 %
Filamentous blue-green mats (Cyanophyta)[b] *Lyngbia, Arthrospira, Phormidium* species	4	0.03 to 0.040 %

[a] Sampling technique resulted in some dilution with water from above surface of sediments
[b] Sampling technique resulted in minor contamination with other organic and inorganic material

producers on coral reefs (Hatcher, 1983a). These algae grow at the interface between the sediment (with some available Fe) and the water column (with much lower concentrations of Fe). The total Fe levels of a few important components of the algal population on Davies Reef are listed in Table 2. Caution must be exercised in interpretation of these measurements, since large systematic errors can occur. For example, entrained sediment in samples should mostly cause an underestimate of tissue Fe, but particulate Fe adsorbed onto tissues should result in an overestimate. The samples analysed (Table 2) showed a relatively low concentration of Fe in photosynthetic tissues (cf. algae in Healey, 1973; and cf. higher plants in Price, 1968), with the notable exception of the filamentous blue-green algae, which had a distinctly higher Fe content. Thus, any indication of Fe deficiency in epipelic blue-green algae should also be a good indication of deficiency in other algae.

Biochemical Indications of Iron Deficiency in Reef Algae

The most outstanding problem in the use of a protein marker for nutrient status is to select representative organisms suitable for study by biochemical techniques. The blue-green alga chosen has been tentatively identified as a *Phormidium* species, and is typical of the organisms in Section III of the classification of Rippka *et al.* (1979). It consists of a tangled mat of red-black, linear trichomes of uniform cells without heterocysts, with the trichomes enclosed in an outer cylindrical sheath. Cells are large – 15 to 30 μm wide and 5 to 10 μm along the sheath. This organism is widespread on the surface sediments in the lagoon of Davies Reef, and is also found growing from segments of the common coral *Acropora* sp. Compared to other blue-green algae observed on the reef, it could be collected as almost a monoculture in quantities sufficient for extraction and analysis.

The important symbiotic alga *Gymnodinium microadriaticum* (zooxanthellae) was isolated from the clam *Tridacna maxima* and a spiculated soft coral (a *Sinularia* species). There were two reasons for these choices: (1) Clean preparations of intact algae could be obtained readily at sea. (2) Algal cells from these animals might be subjected to different nutrient regimes. Cells from the siphonal tissues of the clam constitute only a tiny fraction of the mass of the organism (Trench *et al.*, 1981) and could be supplied with adequate micronutrients from the circulatory system of this organism, which filters particulate matter from the water. Cells from the coral comprise a substantial fraction of the living tissue of the total organism (Muscatine, 1980); a heavy demand by photosynthetic tissue for Fe and other scarce metals might not be satisfied by the predatory ability of this coral.

Table 3 records the occurrence of ferredoxin and flavodoxin in *Phormidium* sp. and *Gymnodinium microadriaticum* together with supporting measurements of other significant parameters. Several pieces of information were necessary in order to effectively assess the reliability of the results. Microscopic observation revealed that at least 90% of all cells were broken after sonication. Specific procedures were developed for fractionation of cell extracts before application to the key environmental samples collected. Since neither ferredoxin and flavodoxin display enzymatic activity, their identity and quantitation were achieved by isolation to a degree of purity where measurement of specific molecular properties was effective. The absorption spectra of the proteins from *Phormidium* sp.

Table 3. Biochemical indicators of Fe status in selected algae from Davies Reef. All results are mean values obtained from duplicate samples of each alga extracted separately. The *Phormidium* sp. was a widely distributed filamentous blue-green alga. The cells of *Gymnodinium microadriaticum* (zooxanthellae) were from *Tridacna maxima* (a clam) and a *Sinularia* sp. (soft coral). Dash = not detected; ND = not determined

Cellular component per 100 g dry cells	*Phormidium* sp.	*G. microadriaticum*	
		from clams	soft coral
Total chlorophyll (μmol)	220	2 700	1 700
Soluble protein (g)	4.4	19.5	17.6
Biliprotein (g)	1.05	0	0
Ferredoxin (μmol)	1.3[a]	7.5	2.6
Flavodoxin (μmol)	0.95	–[b]	–[b]
Soluble cytochrome (*c*-553 μmol)	0.20[c]	ND	ND
Total iron (g)	0.035	0.045	0.013
Chlorophyll: ferredoxin plus flavodoxin	98	360	650

[a] Other samples of this organism were extracted using a different procedure and no ferredoxin detected

[b] With the assays employed, any flavodoxin present must have been at concentrations < 0.1 μmol per 100 g dry cells

[c] Recovery of this cytochrome may not have been complete due to possibility of partial precipitation in dilute solution in the presence of saturated ammonium sulphate

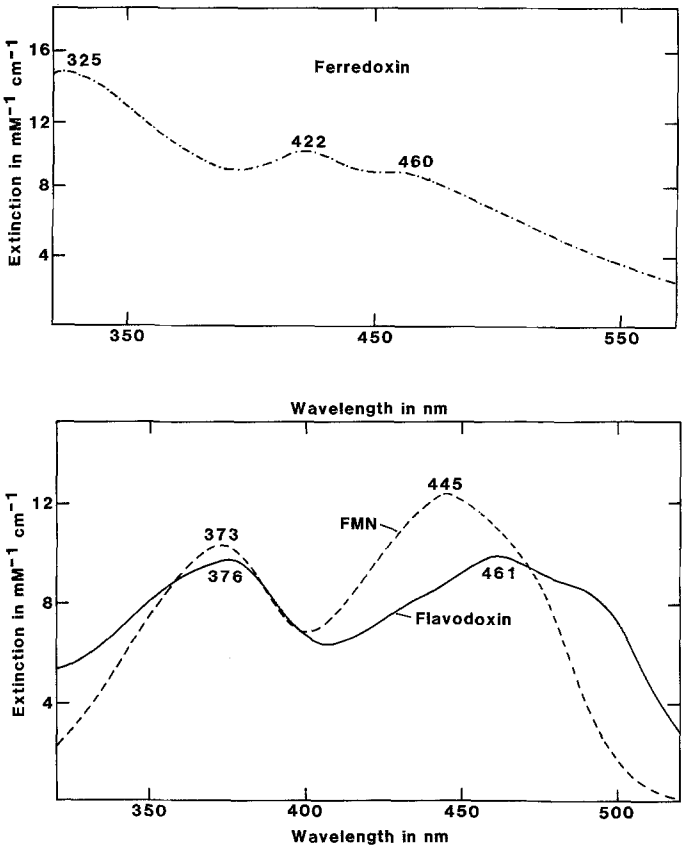

Fig. 1. *Phormidium* sp. Absorption spectra of ferredoxin and flavodoxin from blue-green algae collected from Davies Reef. The spectrum of ferredoxin is typical of the 2 Fe/2 S chromophore found in soluble plant ferredoxins (Palmer, 1975); the spectrum of flavodoxin is typical of a flavin nucleotide bound to a protein compared to the same nucleotide in free solution (FMN)

are illustrated in Fig. 1. The isolated ferredoxins and flavodoxin were tested for functionality as electron carriers in an assay of the enzyme, ferredoxin reductase (see "Materials and Methods – General Analyses for Algal Extracts"). Conditions were not optimized for a particular protein, but were chosen to detect the presence of both ferredoxins and flavodoxin. Purified samples of proteins from *Phormidium* sp. at 0.15 μM in the assay gave a ratio of activities for ferredoxin to flavodoxin of 5. With the procedures required for separation and analysis, there was some uncertainty in the quantitation of these proteins – the maximum relative error of the determinations with a specific extraction procedure was estimated at $\pm 25\%$ on a dry weight basis.

Two of 4 samples of *Phormidium* sp., extracted by the standard procedure, yielded very similar results for the parameters measured; the mean values are shown in Table 3. The other 2 samples differed in so far as no ferredoxin was detected. For these samples, a significant difference in the extraction procedure was introduced to recover photosynthetic cytochrome *c* (see "Materials and Methods – Collection and Extraction of *Phormidium* sp."), which may have resulted in the loss of ferredoxin. To test this hypothesis, a protein extraction from *Phormidium* sp. was split into equal parts at the stage of the cell-free supernatant. One half was fractionated by the standard procedure and yielded ferredoxin; the other half was fractionated by the procedure to recover cytochrome *c*, and no ferredoxin was detected. When the procedures were compared, it was clear that this ferredoxin was soluble when very dilute in saturated ammonium sulphate solution. The constancy of results for *Phormidium* sp. could mean that the samples were too large (> 100 g wet wt) to reveal any variation in environmental influence, and/or the reef sediments were spatially constant sources of nutrients. Compared to laboratory cultures of blue-green algae with excess Fe, chlorophyll and protein levels of wild *Phormidium* sp. were low (cf. for one example, *Phormidium* sp. results in Table 3 with high Fe cells, Table 4). About 25% of the extractable protein was comprised of biliprotein, dominated by phycoerythrin, which was responsible for the red colour of the cells. Approximately equal quantities of ferredoxin and flavodoxin in the extracts could be a consequence of partial replacement of ferredoxin under conditions of Fe deficiency, or of some cells being more deficient in Fe than others in a sample. A photosynthetic cytochrome *c* is commonly soluble in extracts of algae (Yakushiji, 1971). This cytochrome is a different protein from the membrane-bound cytochrome *f* of higher plants, and both cytochromes are found in algae (Wood, 1978). The *Phormidium* sp. cells liberated very low levels of soluble cytochrome *c*, even by comparison to ferredoxin and flavodoxin.

The results obtained from extracts of *Gymnodinium microadriaticum* (zooxanthellae) differed from those obtained from the blue-green alga (Table 3). Chlorophyll and protein concentrations were much higher, possibly indicating that the zooxanthellae came from a richer nutrient environment inside their host organism. Flavodoxin was not detected in cells from either host source, even at the very low level detectable by the ferredoxin reductase assay (see Table 3). There were very significant differences in the concentrations of ferredoxin and iron, with lower concentrations being found in the zooxanthellae cells from the soft coral. The data presented in the next section (see Table 4) indicate that these differences could result from Fe deficiency in the zooxanthellae cells from the coral.

Unfortunately, further support for iron deficiency by comparison of concentrations of photosynthetic cytochrome *c* was not possible. The cytochrome was certainly extracted, but was not chromatographically separable from the soluble PCP described by Prézelin and Haxo (1976): the PCP pigments were so much in excess of the heme of the cytochrome, that they swamped effective spectrophotometric quantitation. The higher chlorophyll to ferredoxin ratio in coral zooxanthellae compared to those from clams might result from pigment synthesis as an adaptation to light (Prézelin and Alberte, 1978): the coral was collected at a depth of 15 to 20 m, the clams within 5 m of the surface.

Effects of Fe Deficiency on Cultures of the Marine Blue-Green Alga *Synechococcus* sp.

This unicellular, marine blue-green alga was examined in the laboratory for the effects of Fe deficiency upon measurable gross changes in its photosynthetic apparatus. Deficiency in Fe was experimentally established in batch cultures. Cultures with very low concentrations of available Fe were compared to cultures grown simultaneously with excess Fe. Relative differences in the photosynthetic apparatus were followed with time.

Changes with time in directly comparable cultures are shown in Fig. 2. There is no ready explanation for the lags in growth at the start of these batch cultures (e.g. the time-lag of 14 h before commencement of measurable growth for the cultures shown in Fig. 2). The high-iron culture passed through a very brief exponential phase, followed by an extended linear phase up to the time of harvesting. Nitrogen and phosphorus were always in excess (Fig. 2), as was CO_2 (established by pH measurement of samples). The only nutritional uncertainty was the possibility of exhaustion of a micronutrient, such as Mn, Cu, or Mo towards the end of growth. The linear phase of growth resulted from light-limitation, which was to be expected in such large batch-cultures, and which was confirmed by an

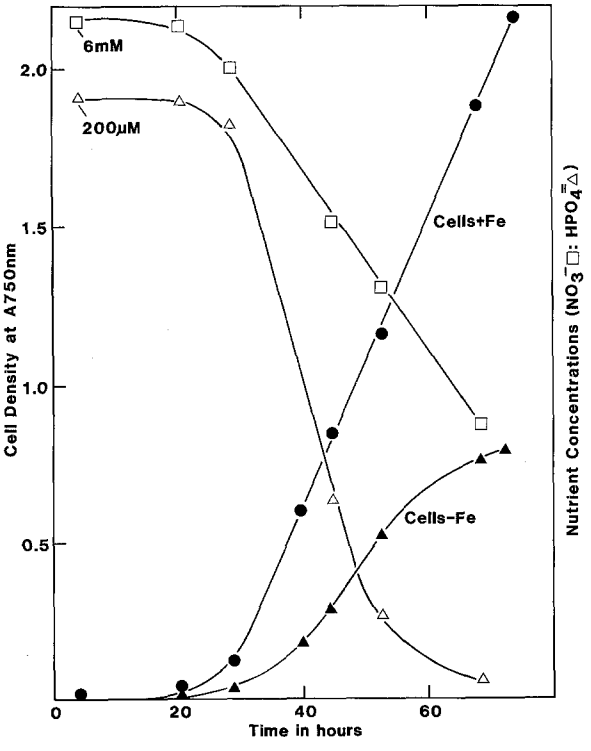

Fig. 2. *Synechococcus* sp. Changes in concentrations of culture components with time during growth in batch cultures of 17 litres. Cellular density changes illustrate differences in growth caused by absence of added Fe from cultures (▲), compared to presence of 2×10^{-5} *M* Fe (●). Time courses for nitrate and phosphate are for the culture with Fe. Phosphate was on the point of exhaustion in the medium at the time of harvest (after 70 h). The culture without Fe only partly utilized the N and P in solution

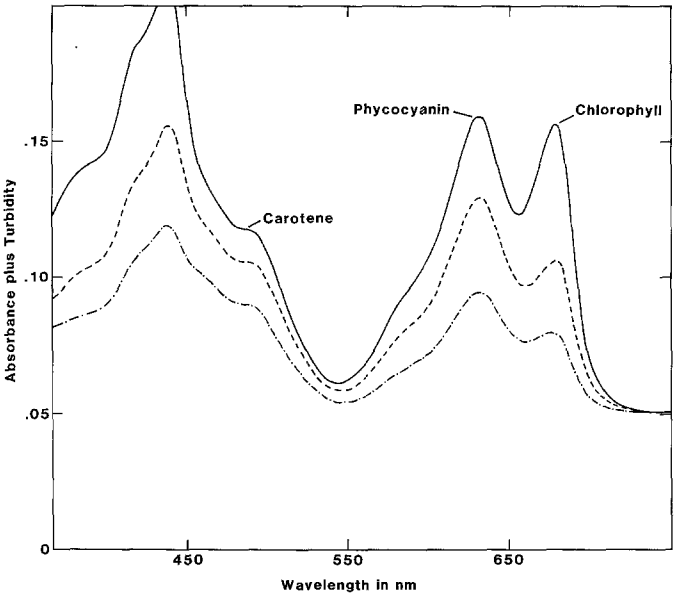

Fig. 3. *Synechococcus* sp. Comparison of whole-cell spectra from algal cultures, illustrating effect of Fe deficiency upon photosynthetic pigments. Spectra of cells plus Fe (——) and minus Fe (– –) were from cells at the same time after the start of the respective cultures. The additional spectrum of cells minus Fe (· – ·) was recorded from a sample taken from the same culture several hours later. [A sample taken from the culture with Fe at this same later time displayed the same spectrum as before (——).] All spectra were measured with cellular suspensions of equivalent turbidity at 750 nm

increased chlorophyll *a* and phycocyanin content per cell in the linear phase compared to the early exponential phase (cells adapt normally to lower light intensity by increasing the amounts of light-harvesting pigments: e.g. Senger and Fleischhacker, 1978). The low-iron culture grew more slowly, and was probably limited by Fe deficiency at all stages of growth. Net growth had ceased at the time of harvesting. Care was taken to maintain the same level of EDTA in all cultures, so that chelation of other metals would be basically similar in both high- and low-Fe cultures.

There were several qualitatively characteristic changes in Fe-deficient cells. The photosynthetic pigment composition changed continuously compared to control cells in the linear phase, with time (Fig. 3). Carotene, chlorophyll *a*, and phycocyanin all decreased per unit cell with time, with the added refinement that chlorophyll *a* decreased more rapidly than phycocyanin initially. Thus, deficient cells were a pale yellowish blue-green compared to the intense blue-green of controls. Microscopically, the Fe-deficient cells were larger than control cells, and formed longer chains of attached cells. Low-Fe cells had a 30 to 40% higher dry weight per unit cell density (as measured by light scattering). They were still viable, at least in the mid-phase of batch growths. A culture of Fe-deficient cells at a density equivalent to that attained after 50 h in Fig. 2 was supplemented with sterile Fe-EDTA. After a further 2.5 h, photosynthetic pigments were increasing per unit

Table 4. *Synechococcus* sp. Changes in selected biochemical indicators due to limiting Fe in laboratory cultures. All results are mean values obtained from duplicate samples of algae from separate cultures such as those illustrated in Fig. 2

Cellular component per 100 g dry cells	Excess Fe	Deficient in Fe
Chlorophyll (μmol)	1 650	300
Ferredoxin (μmol)	13.5	4.0
Flavodoxin (μmol)	–[a]	–[a]
Cytochrome *c*-553 (μmol)	13.3	2.6
Iron (g)	0.18	0.009
Chlorophyll:ferredoxin	120	75

[a] With the assays employed and material extracted, any flavodoxin present must have been at < 0.05 μmol per 100 g dry cells

cell density and, after a total of 18 h, had increased almost to the concentration in high-Fe cells, the largest increase being in chlorophyll *a*.

When cell extracts from high-Fe cells were compared to extracts from cells with very low Fe (i.e., the latter harvested when growth had almost ceased), no flavodoxin could be found in either extract (Table 4). This experiment was repeated, and appropriate chromatographic fractions were tested using the sensitive ferredoxin-reductase assay, but still no flavodoxin was detected. As already stated, differences in measured photosynthetic components in Fe-deficient cells compared to cells with excess Fe included decreased chlorophyll *a* in the former. Ferredoxin and soluble photosynthetic cytochrome *c* decreased several-fold, and were clearly sensitive to the supply of Fe to the cells. For this *Synechococcus* sp., approximately 0.01% total Fe represented extreme Fe-deficiency. The minimum level of Fe in cells which did not exhibit detectable symptoms of deficiency was approximately 0.06%. However, healthy cells could readily accumulate much higher concentrations of Fe (Table 4), indicating a possible mechanism for storage of Fe.

Discussion

The primary aim of this study was to evaluate the significance of Fe in relation to the nutritional limitations to primary production on coral reefs. The results show that Fe is a trace component of the reef environment examined, and that representative samples of important primary producers from the test reef exhibit characteristic biochemical indications of Fe deficiency. Thus, Fe is probably a limiting nutrient for many primary producers on coral reefs.

The low concentrations of Fe in the sea around Davies Reef (Table 1) are probably typical of the bulk of the water column around the Great Barrier Reef Complex, which is many kilometres from the continental shore. Betzer and Pilson (1970) reported equally low concentrations of total Fe in tropical surface water of the Sargasso Sea. Reef waters can experience few periods of nutrient enrichment apart from occasional intrusion of water from deeper layers beyond the continental shelf (Andrews and Gentien, 1982). Measurable concentrations of iron were present in the water column close to the shore (Table 1), originating presumably from terrestrial sources. Hence, fringing reefs should receive a higher input of Fe, only some of which may be available to algae (Lewin and Chen, 1971).

For a detailed study of the distribution of Fe in Barrier Reef waters, new, more sensitive measurements of Fe would have to be devised. The methods used in this paper show that the total concentration of Fe comprises 10% or less of the concentration of P in the water column, whereas Fe is required at about 20% of the level of P in photosynthetic tissues of algae under optimum conditions (Healey, 1973). Thus, there is an imbalance in favour of P availability relative to Fe availability, and hence, Fe may be as important as N in limiting planktonic biomass in the Barrier Reef region (particularly nitrogen-fixing organisms). Fixed N is normally present in insufficient concentrations relative to P in the waters outside coral reefs (Andrews and Gentien, 1982). The trace levels of Fe in the Davies Reef sediment represent a massive pool of the metal in comparison with its levels in the water column. Since offshore reefs such as Davies Reef are unlikely to receive a supply of terrestrial or sedimentary mineral Fe, presumably this pool of Fe is maintained by biological inputs. Concentration of Fe in the carbonate matrix during the process of calcification may occur in some species, but this is not a general phenomenon. Analysis of bleached coral and *Halimeda* spp. skeletons of several species revealed almost no Fe (Table 2), in contrast to reports of Fe in "organic-free" coral skeletons (Milliman, 1974). Organic matter remains as the probable source of inputs of Fe to the sedimentary pool. A small fraction of the pool of Fe is detectable in the interstitial water of surface sediments (Table 2), and should be available to some epipelic and epilithic algae in contact with micro-anaerobic environments. Exposure of interstitial water to oxygen should precipitate any soluble Fe (Stumm and Morgan, 1970), so that it is no longer available to many algae. Possibly the higher level of Fe in blue-green compared to other epipelic algae (Table 2) reflects the proposed superior ability of blue-greens to obtain Fe in a low-Fe aquatic environment (Murphy *et al.*, 1976). However, the demonstration of low levels of Fe in Davies Reef is not sufficient evidence that Fe deficiency is a primary limitation to the growth of photosynthetic organisms: scarcity of other nutrients and/or physical factors could prove more critical.

Concrete biological evidence for Fe limitation was obtained by the detection of a significant concentration of flavodoxin in the photosynthetic apparatus of samples of *Phormidium* sp. from Davies Reef (Table 3). By analogy, Fe limitation should occur in many other epilithic algae. This conclusion was supported by other evidence. The cells of *Phormidium* sp. were extremely chlorotic (on a dry weight basis), similar to the Fe-deficient laboratory culture of *Synechococcus* sp. studied (Table 4). Low concentrations of chlorophyll *a* in cells are indicative of N or Fe

deficiency. However, in the absence of a supply of N, blue-green algae lose all biliprotein (Allen and Smith, 1969; Stevens *et al.*, 1981), whereas the reef organism studied retained biliprotein, as did the Fe-deficient cultures of *Synechococcus* sp. studied (Fig. 3). Also, current experimental evidence (Stewart, 1980) indicates that blue-green algae similar to *Phormidium* sp. do not fix nitrogen to overcome a N-deficient environment. Approximately equal amounts of ferredoxin and flavodoxin in cells (Table 3) may still occur with severe Fe deficiency. A recent study of a *Nostoc* sp. when under conditions of extreme Fe deficiency, showed that one of two molecular species of soluble ferredoxin was replaced by flavodoxin, resulting in about equimolar amounts of ferredoxin and flavodoxin in the cells (Hutber *et al.*, 1977). Glover (1977) has shown that *c*-type cytochromes from the photosynthetic apparatus decrease in planktonic algae under Fe stress. The soluble algal cytochrome *c*-553 was measured in laboratory cultures of *Synechococcus* sp. and, as reported in Table 4, cellular concentrations decreased several fold under Fe stress. Hence, it was concluded that the very low amount of soluble cytochrome *c*-553 found in *Phormidium* sp. (Table 3) provided further evidence of Fe deficiency. However, this conclusion may not be correct if this cytochrome is suppressed in *Phormidium* sp. in the presence of an adequate supply of copper, as reported in some other algal species by Wood (1978). The concentration of total Fe associated with the reef alga (Table 3) was anomalous in the sense that it would support a hypothesis of only moderate deficiency compared to the laboratory alga, unless some of the Fe measured in the former were extracellular.

Although the presence of flavodoxin in a photosynthetic organism is a specific indication of Fe deficiency, there are at present considerable limitations to a wider application of this marker to many organisms from the environment. For example, the green, siphonaceous, calcifying algae of the genus *Halimeda* are major photosynthetic organisms and a major source of sediment components on coral reefs, and are readily collected. Attempts were made to analyse samples of *Halimeda* spp., but problems of reproducibility and recovery of protein in extracts have not yet been solved. Many other algae are also difficult to extract, and even to obtain in useful quantities. There is no information in the literature on the distribution of algal genes for flavodoxin. The protein has been isolated from a number of procaryotes, from one green alga (Zumft and Spiller, 1971), and from one red alga (Fitzgerald *et al.*, 1978). The present negative results in Table 4 for *Synechococcus* sp. show that flavodoxin is not universally distributed, even amongst procaryotes. It should be noted that a closely related species, *Anacystis nidulans*, produces flavodoxin (Smillie and Entsch, 1971). There have been claims that flavodoxin is constitutive in at least one procaryote, *Azotobacter vinelandii* (Mayhew and Ludwig, 1975). It is clear that a great deal more work needs to be done to clarify the full environmental influences upon the expression of genes for flavodoxin, and how this expression relates to other components of the photosynthetic apparatus. Investigation of marine photosynthetic organisms should have precedence, since the sea generally contains only trace concentrations of available Fe in the euphotic zone.

Since no flavodoxin was found in samples of *Gymnodinium microadriaticum* (Table 3), this organism (like the culture of *Synechococcus* studied; Table 4) may not be capable of flavodoxin production. Low levels of available Fe generally results in a relative loss of soluble ferredoxin per unit cell compared to cells with excess Fe (*Synechococcus* sp., Table 4; see also Cammack *et al.*, 1979), and this loss is apparent in zooxanthellae cells from the soft coral relative to zooxanthellae cells from clams (*G. microadriaticum* Table 3), and correlates well with the total Fe. The capture of prey by their symbiotic partners, may therefore shield zooxanthellae from nutrient deficiencies. On the other hand, photosynthetic tissues require much more Fe than do animal tissues which possess no elaborate system of oxygen distribution involving Fe proteins such as hemoglobin (Bezkorovainy, 1980). Thus, the available evidence suggests that Fe deficiency can occur even in some symbiotic arrangements on coral reefs. Confirmation will require more information on the laboratory response of zooxanthellae to Fe stress.

The investigations presented in this paper illustrate the importance of the surface layer of sediment and limestone rock as a source of one essential trace metal (Fe) for the predominantly benthic community of a coral reef. A similar conclusion was also reached in investigations of P and N (Entsch *et al.*, 1983). The demonstration of Fe deficiency in a common reef alga points to a need for information on the influence of Fe (and possibly other "trace" metals) upon primary production. The degree of Fe deficiency in a species can influence its rate of growth and biomass, as illustrated in Fig. 2 for *Synechococcus* sp. A recent detailed study of Fe deficiency (with the higher plant *Beta vulgaris* L.) showed an absolute dependence of light-saturated photosynthetic rates upon Fe supply (Terry, 1980). Competition for available Fe is important in bacterial interactions (Neilands, 1977). Similar competition could influence the distribution of benthic algal species on a coral reef. Limiting supplies of Fe could also regulate the degree of N_2 fixation, which is considered so important to the maintenance of a coral reef (Lewis, 1981).

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