GROWTH AND PHOTOSYNTHESIS IN MARINE SYNECHOCOCCUS (CYANOPHYCEAE) UNDER IRON LIMITATION

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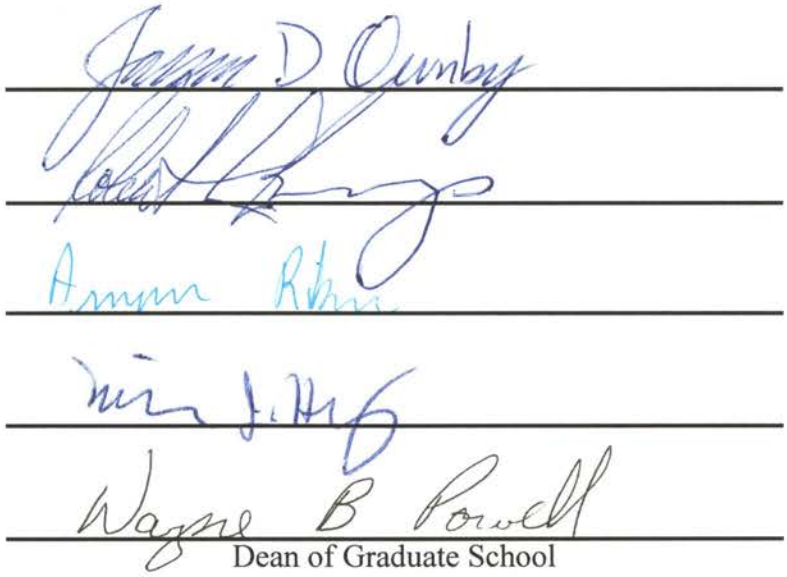

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This project was really a pioneer work to me. Before I began working on this program in 1993, I had not even realized that cyanobacteria is another name for blue-green algae. I still was not sure if I could successfully culture these mysterious tiny marine Synechococcus only three years ago. Over the years, I spent almost ½ of the time to figure out how to culture the cells and to measure P-I curves for the Fe-limited cells. I could not collect any useful, or publishable, data until about one and half a years ago. Without the help of a lot of people, this project could not have been done.

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INTRODUCTION

Problem statement

Since their discovery in 1979, marine Synechococcus have been found to be abundant in many regions of the oceans. One group of marine Synechococcus, containing phycoerythrin as the primary light harvesting pigment, can neither fix nitrogen nor produce siderophores. They occur commonly in both coastal regions and open oceans, and are frequently the dominant species in deep-mixing, oligotrophic waters because of their small size and efficient absorption of low light. Despite the advantage for nutrient uptake by the small size of coccoid Synechococcus, this group of prokaryotes are thought to have a higher cellular iron requirement (relative to macronutrients) than eucaryotic algae. Therefore, the dominance of Synechococcus in the high nutrient/low chlorophyll (HNLC) areas is paradoxical.

The effect of iron deficiency on Synechococcus growth rate and cell yield cannot be ambiguously determined in the field, although in situ data indicate that both growth rate and biomass of picophytoplankton (< 2 μm; including Synechococcus) could be iron-limited in HNLC regions. Variability and complications in the field also make it impossible to conclusively determine changes in photosynthesis and respiration of single phytoplankton species. These argue for accurately and conclusively determining specific growth rate, photosynthesis, and respiration of ecologically important Synechococcus in the lab. The respiration rate in darkness, which cannot be accurately measured by the ^{14}C technique commonly used in the field, can be easily determined from measuring oxygen evolution.

Cyanobacteria in surface waters are potentially exposed to high light for at least a short period of time. It is quite possible that iron deficit affects the susceptibility of marine cyanobacteria to photoinhibition.

Objectives

This project was to understand how marine Synechococcus responds physiologically to variations in iron availability and high light stress under strictly controlled conditions. The research program had three objectives:

1. Determine the relationship between iron concentration and Synechococcus growth rate and final cell yield.

2. Determine the effect of iron deficiency on photosynthesis and respiration in Synechococcus.

3. Test for interaction of iron deficiency with growth irradiance and acute high light stress.

The overall design of the experiment was to grow Synechococcus strains A2169 (from off the coast of Peru in Pacific Ocean) and WH7803 (from the Sargasso Sea in the North Atlantic) under various ecologically relevant combinations of iron concentrations and irradiances. Both batch cultures and semi-continuous cultures were investigated. Cultures acclimated to these conditions were analyzed for growth rate, pigment content, photosynthetic and respiratory O_2 exchange, and low temperature chlorophyll fluorescence signals. The changes in specific growth rates, O_2 evolution rates, and chlorophyll fluorescence were used as indications of the changes in the photosynthetic activity of Synechococcus in response to modifications in iron and growth irradiance

conditions. Since there is a strong basis to expect a large effect of growth irradiance on iron nutrition, I tested for interaction of light- and iron-limitation on the marine cyanobacterium Synechococcus in laboratory cultures.

This project will improve our understanding of iron limitation on growth and photosynthesis in marine Synechococcus and provide necessary knowledge for further assessing physiological mechanisms of photosynthesis using active fluorescence techniques. Results of this research will help to recognize and quantitatively describe physiological changes of photosynthesis in marine Synechococcus under iron-limitation and high light stress, which is fundamental to understanding their distribution and role in primary production in the open ocean.

LITERATURE REVIEW

General information of cyanobacteria

Cyanobacteria are the largest and most diverse group of oxygenic photosynthetic procaryotes. The sizes of cyanobacteria range from coccoid cells less than 1 μm in diameter to trichomes over 100 μm in diameter (Whitton 1992). Over the long evolution of approximately 3 billion years, they have become established in a wide range of ecosystems such as freshwater ponds, seas, hot springs, soils, and deserts. They are capable of surviving extreme changes in light, temperature, nutrients, currents and predation due to their physiological characters such as tolerance of high temperatures and desiccation, the abilities to utilize low light and CO_2 concentration, and the ability to fix N_2 (Stanier et al. 1981, Whitton 1992). Some members of cyanobacteria produce heterocysts, akinetes, terminal cells and hairs, and hormogonia in particular growth phase or adverse environmental conditions (Castenholz and Waterbury 1989).

Cyanobacteria, or blue-green algae, were traditionally treated as algae. However, based on the lack of membrane-bounded nuclei and organelles and the possession of water-soluble phycobiliproteins, they have been set aside as a separate class within the Gram-negative eubacteria (Stanier et al. 1981, Castenholz and Waterbury 1989).

Like eucaryotic algae and higher plants, cyanobacteria have two photosystems and carry out oxygenic photosynthesis using CO_2 as well as HCO_3^- as carbon sources. The photosynthetic apparatus in cyanobacteria is similar to that of eucaryotic algae and higher plants in structure, function, and molecular aspects (Bryant 1986), but is very amenable to genetic manipulation (Bryant and de Marsac 1988). In recent years, cyanobacteria (especially freshwater and coastal strains) have become an increasingly

useful system for investigations of structural, functional, and genetic characters of oxygenic photosynthesis. As an experimental system, cyanobacteria are conveniently maintained at selected culture conditions and have a relatively short generation time (commonly 12-24 h).

Distribution of marine cyanobacteria

In the open ocean, the most important photosynthetic organisms are the single-celled oxygenic phytoplankton (Falkowski 1994), which all live in the euphotic zone. Phytoplankton should be regarded as an important factor in the carbon cycle in the world. Climate change may affect the surface environment and the abundance and type of marine phytoplankton, which may give rise to direct and indirect feedback effects on the climate system (Holligan 1992). They collectively fix between 35 and 50×10^9 tons of carbon a year, which is about 40% of the global total (Falkowski & Woodhead 1992, Falkowski 1994). This makes them an inevitable element in predicting the carbon cycle that affects the magnitude of the greenhouse phenomenon.

Cyanobacteria are an important component of marine phytoplankton. They are found in all the world's oceans. Some activities of marine cyanobacteria that have profound economic effects are their role in the global carbon cycle by removing atmospheric CO_2 and their support of the world's fisheries by feeding the protists and small animals that feed larger creatures (Kudol et al. 1990, Gallager et al. 1994).

We know that there are over 100 species of cyanobacteria in freshwater habitats. In contrast, there are only a relatively small number of cyanobacterial genera in the open ocean. The two best-known genera are Trichodesmium and Synechococcus. In recent

years, Prochlorococcus also have been found to be an important component of phytoplankton in various regions of the open ocean (Olson et al. 1990, Falkowski et al. 1994, Vaulot et al. 1995).

Characteristics and distribution of marine Synechococcus

The genus Synechococcus are coccoid unicellular organisms dividing by binary fission in a single plane. There is no formal delineation of species in the genus due to lack of phenotypic and genetic analyses. The cells are < 3 μm in diameter, contain photosynthetic thylakoids located peripherally, and lack structured sheaths (Castenholz and Waterbury 1989).

Since their discovery in 1979, marine Synechococcus have been found to be abundant in many regions of the oceans. There are two distinct subgroups of marine Synechococcus. Coastal isolates lack phycoerythrin and have phycocyanin as the primary light-harvesting pigment. They do not require elevated salt for growth and have never been isolated from the open ocean. They are assumed to be terrestrial freshwater forms that have invaded the marine environment but are unable to compete successfully in the open ocean (Waterbury et al. 1986). They have been a preferred tool of genetic study in recent years. This project will not discuss this group of Synechococcus.

The second group of marine Synechococcus contain phycoerythrin as the primary light harvesting pigment and have elevated salt requirements for growth. They occur commonly in both coastal regions and open oceans, and are sometimes called oceanic Synechococcus. Nitrogen fixation has never been reported in marine Synechococcus collected from the open ocean (Whitton 1992). It is this group of marine Synechococcus

that this project will focus on. I will refer to them as marine Synechococcus according to Waterbury et al. (1986).

Marine Synechococcus are small coccoid to rod-shaped unicellular forms (0.6-0.8 × 0.6-1.6 μm) that occur abundantly in surface waters of the temperate and tropical oceans. They have been found in the euphotic zone in all oceans except the Antarctic (Waterbury et al. 1986, Fogg 1987).

Marine Synechococcus usually account for 5 to 50% of the total primary production in the oceans. By counting picophytoplankton cells (< 2 μm) under an epifluorescence microscope, Takahashi et al. (1985) reported that picocyanobacteria (Synechococcus) contribute up to about 80%, mostly over 50%, of the total biomass of coastal picophytoplankton. Even in oligotrophic tropical waters Synechococcus are present throughout the year at concentrations ranging from 10^3 to the low 10^4 cells·mL^{-1} (Waterbury et al. 1986). Some investigators reported values between 50-80% of the primary production for the <3 μm fraction of phytoplankton (predominantly Synechococcus and Prochlorococcus) (Takahashi and Hori 1984, Glover et al. 1985, Olson et al. 1990, Pena, et al. 1990, Vaulot et al. 1995). The percentage contribution by picophytoplankton to primary production tends to increase with depth in the euphotic zone and in more oligotrophic regions of the world's oceans (Stockner 1988). Murphy and Haugen (1985) sampled 50 stations in the North Atlantic and reported that the cyanobacteria picophytoplankton (Synechococcus) showed decreasing densities at higher latitudes. They suggested that cyanobacterial abundance is related in part to temperature.

However, nutrient availability and light quality in the euphotic zone also have great effect on the distribution of marine Synechococcus (Joint 1986).

The vertical distribution of marine Synechococcus has been examined in several studies. The maximum concentration of Synechococcus may occur at or near the surface (Waterbury et al. 1986), but in general, in well-mixed regions the concentrations are relatively constant with depth within the euphotic zone, whereas in stratified waters they are most abundant in the subsurface chlorophyll maximum (SCM) layer (Takahashi and Hori 1984, Murphy and Haugen 1985, Fogg 1987). Marine Synechococcus are frequently the dominant species in deep-mixing, oligotrophic waters because of their small size and efficient absorption of low light (Glover et al.1986, Fogg 1987, Ikeya et al. 1994). The absolute concentration of Synechococcus might not increase in these regions, but their relative abundance increases because other larger phytoplankton could not efficiently use low nutrient concentration and low light.

Photosynthetic apparatus of Synechococcus

The most observable cytoplasmic components of cyanobacteria are the thylakoids. The cells contain peripheral thylakoids and lack structured sheaths (Waterbury and Rippka 1989). The pigment-bearing thylakoid membranes are mainly independent of the cytoplasmic membrane but there are orderly attachment points or thylakoid centers associated with the periphery of the cytoplasm or the cytoplasmic membrane (Castenholz and Waterbury 1989). In the freshwater strain Synechococcus PCC7942, the major photosystem II (PS II) proteins are localized throughout the thylakoids, but the

photosystem I (PS I) proteins and ATP synthase proteins are mostly associated with the outermost thylakoid and with the cytoplasmic membrane (Sherman et al. 1994).

The photosynthetic apparatus comprises five multi-protein complexes. Four of these complexes are common to both cyanobacteria and higher plants: PS II, PS I, plastoquinol-plastocyanin oxidoreductase, and ATP synthase. The fifth complex serves as the light-harvesting antenna for PS II. In chlorophyll b-containing algae and higher plants, this complex is a chlorophyll-protein complex (CP II). However, in cyanobacteria this complex is the phycobilisome (see Bryant 1986 for a review).

The pigments in the cyanobacterial photosynthetic apparatus are chlorophyll a, phycobiliproteins, and carotenoids, but not chlorophyll b. The major light-harvesting complex of cyanobacteria is the phycobilisome. The phycobilisomes include the blue-gray pigment allophycocyanin (APC, absorption maxima = 650-670 nm), the blue pigment phycocyanin (PC, absorption maxima = 610-630 nm), and the red pigment phycoerythrin (PE, absorption maxima = 490-570 nm), among which the PEs have the greatest diversity in absorption spectral properties (Alberte et al. 1984). Synechococcus are the only marine cyanobacteria that contain PE as the major light-harvesting pigment. In strain WH7803, two absorbing forms of PE, phycourobilin and phycoerythrobilin, have absorption maxima at 500 and 550 nm, respectively (Alberte et al. 1984). Phycoerythrobilin predominates in oceanic strains of Synechococcus (Olson et al. 1990).

Evidence for iron limitation in the sea

Iron is the fourth most abundant element by weight in the earth's crust. However, it is an element with a low biological availability due to the poor solubility of Fe(III)

oxides and hydroxides in the presence of oxygen. Iron is needed for the synthesis of both chlorophyll and phycobiliproteins and is an essential cofactor in cytochromes, ferredoxin, iron-sulphur proteins and, in nitrogen-fixing species, nitrogenase (Carr and Wyman 1986). Under optimal nutrient conditions, 22-23 iron atoms are needed for a functional photosynthetic apparatus in cyanobacteria (Ferreira and Straus 1994). Thus, iron plays an important role in the photosynthetic electron transport chain and is an essential component of membrane-bound protein complexes of the photosynthetic apparatus in cyanobacteria.

Primary production in the open ocean is usually limited by the availability of nitrogen. However, in the western equatorial Pacific, the subarctic Pacific, and the Southern Ocean, over 20 % of the global oceans, major nutrients (nitrate, phosphate, and silicate) are high in the surface layer throughout the year but primary production or chlorophyll concentration is low (Martin and Fitzwater 1988, Martin et al. 1990, Cullen 1991, Martin et al. 1991, Geider and La Roche 1994). Synechococcus and Prochlorocuccus are frequently found to be the predominant species in these high nutrient/low chlorophyll (HNLC) regions. The reason for the low primary production has not been resolved, and there are many hypotheses to explain the low biomass of phytoplankton in these areas (Cullen 1991), among which the iron (Fe) limitation hypothesis has been intensively studied.

For a long time oceanographers have suggested a role for Fe in limiting phytoplankton growth in the sea (see references in Martin 1992), but all of the early bioassay experiments were discredited because of inadequate attention to sources of contamination (Martin and Fitzwater 1988, Geider and La Roche 1994). In the late

1980s, Martin and Fitzwater (1988) used trace metal clean techniques for the first time to study Fe limitation on the phytoplankton and resurrected the Fe limitation hypothesis.

The total dissolved Fe concentration in the ocean is usually 10^{-9} M or less (Brand 1991, Martin et al. 1990). However, the dissolved iron concentrations in the HNLC surface waters exist at an even lower level of 10^{-12} M (Martin et al. 1994, Gordon et al. 1997, Landry et al. 1997). Martin's Fe limitation hypothesis is based on observations of enhanced net phytoplankton growth in bottle incubations supplied with Fe. All observations in the subarctic Pacific (Martin and Fitzwater 1988), the Southern Ocean (Martin et al. 1990), and the equatorial Pacific (Martin et al. 1991) indicated that Fe limitation of phytoplankton may be a general phenomenon in HNLC regions. Fe deficit is most likely in the Pacific Ocean due to relatively low flux of eolian iron, the main source of iron input, to surface water (Brand 1991, Martin et al. 1990). Another possible source of nutrient variability other than eolian dust is the nutrient supplement from deep sea. Changes in climate and sea level in a particular ocean region might provide a mechanism that can drive large-scale nutrient variability regardless of whether or not local wind forcing is changed (Barber and Chavez 1991). Variations of Fe concentration from equatorial upwelling contribute to variations in primary and new production in this region (Gordon et al. 1997), although even upwelling Fe was suboptimal, thus open ocean phytoplankton have to obtain their Fe from eolian dust (Martin 1992). Recently Landry et al. (1997) suggested that rapid recycling of Fe by grazers also plays a key role in controlling Fe availability and thus phytoplankton abundance.

Recent research on Fe limitation on phytoplankton productivity has focused on the HNLC regions (Geider and La Roche 1994, Martin et al. 1994). There is strong

evidence that the dominant, small phytoplankton are better competitors for limiting Fe in the central equatorial Pacific because of their small size (Morel et al. 1991). By enriching an area of about 64 km^2 in the open equatorial Pacific Ocean with iron and tracking it for 10 days, Martin et al. (1994) recently provided strong but still debated evidence that all biological indicators showed an increased rate of phytoplankton production in response to the addition of iron. Other findings also confirm that iron-deficit may control phytoplankton biomass and productivity in the HNLC areas (Geider and La Roche 1994, Greene et al. 1994). However, most in situ data indicate that Fe limits only the specific growth rates of larger phytoplankton (mainly diatoms), whereas only the biomass but not the specific growth rate of smaller phytoplankton is Fe-limited (Wells et al. 1994). In situ experiments indicated physiological symptoms of Fe-stress in phytoplankton of all sizes, including the dominant components Synechococcus, Prochlorococcus, and eucaryotic picophytoplankton, while confirming the lack of growth limitation in picophytoplankton (Binder et al. 1996, Zettler et al. 1996). However, other recent studies also suggested that the both the growth rate and biomass of small dominant phytoplankton are limited by iron deficiency, although grazing also plays an important role in recycling iron (Coale et al. 1996, Landry et al. 1997).

Despite the advantage for nutrient uptake by the small size of coccoid Synechococcus, this group of prokaryotes has a higher cellular iron requirement (relative to macronutrients) than eucaryotic algae (Brand 1991, Geider and La Roche 1994). Therefore, the dominance of Synechococcus in the HNLC areas is paradoxical.

Synechococcus could be Fe-limited in the open oceans (Brand 1991), but the available data are quite limited. Rueter and Unsworth (1991) reported that cellular iron

of Synechococcus increases about 160-fold but the cell density and protein concentration increase only 7.8-fold comparing highest (10^{-6} M) to the lowest iron concentration (10^{-9} M). They suggested that under Fe limitation the cells could use iron more efficiently by regulating protein synthesis and cell ultrastructure. The photosynthesis capacity of Synechococcus PCC7002 decreased more than 4-fold in Fe-limited continuous cultures kept at the same growth rate as Fe-replete cultures, and growth rate was not a linearly related to iron availability (Wilhelm and Trick 1995). Kudo and Harrison (1997) recently reported that iron deficiency of Synechococcus is more severe, including suppression of growth rate and changes in cellular composition, when grown under light limitation. Batch culture study also has shown that Synechococcus growth rate as well as final cell yield can be reduced at low total Fe concentrations (Henley and Yin, in press).

Öquist (1971, 1974a, b) described chlorosis, the most obvious effect of iron deficiency, in Anacystis nidulans (= Synechococcus sp.). Iron deficiency has been shown to decrease the content of chlorophyll, phycobilisomes, carboxysomes, and membranes by controlling the ability of the cells to synthesize various components of the light-harvesting apparatus (Guikema and Sherman 1983, Sherman and Sherman 1983, Dubinsky 1992, Ferreira and Straus 1994). Many catalysts involved in electron transfer and reductive biosyntheses contain iron, and the abundances of most of these catalysts decline under iron-limited conditions (Geider and La Roche 1994). Although the ratio of phycocyanin to chlorophyll decreases in iron deficient freshwater cyanobacteria (Guikema and Sherman 1983), it is uncertain if marine Synechococcus, which contain phycoerythrin, respond in the same way. Cellular phycoerythrin concentration of marine strain WH7803, which is also used in this investigation, decreased more than 5-fold when

both iron and EDTA were omitted from the medium (Chadd et al. 1996). Unfortunately, the ratio of phycoerythrin to chlorophyll was not reported in the study.

One strategy phytoplankton have adopted to counter nutrient deficiency is the expression of specific proteins aimed at alleviating stress. Iron deficiency also leads to the synthesis of new proteins that are not present in normal cells (Guikema and Sherman 1983). Accumulation of a Chl binding protein with molecular weight of 36 kDa was reported in both freshwater Anacystis nidulans (Guikema and Sherman 1983) and marine Synechococcus WH7803 (Chadd et al. 1996). Freshwater cyanobacteria may synthesize siderophores to solubilize iron as siderophore-iron complexes under iron-stress condition (Ferreira and Straus 1994, Wilhelm and Trick 1994, Wilhelm and Trick 1995). Under moderate iron limitation, freshwater cyanobacteria also express flavodoxin, which serves as a functional substitute for iron-containing ferredoxin (La Roche et al. 1993, Pueyo and Gómez-Moreno 1993). However, neither siderophore (Rueter and Unsworth 1991, Chadd et al. 1996) nor flavodoxin (Chadd et al. 1996) has been reported in marine strains (including WH7803) under iron stress. In freshwater and coastal Synechococcus, iron limitation causes simultaneous de-repression of the flavodoxin gene and co-transcription of an adjacent gene (Laudenbach et al. 1988, Leonhardt and Straus 1992). The latter was found to encode a highly abundant chlorophyll-binding protein CP43', which binds up to half of the cell's chlorophyll under iron limitation (Riethman and Sherman 1988, Burnap et al. 1993).

Changes in the protein composition of thylakoid membranes are often accompanied by changes in light absorption, fluorescence excitation and fluorescence emission spectra (Guikema and Sherman 1983, Ferreira and Straus 1994, Geider and La

Roche 1994). In Fe-deficient cultures of the cyanobacterium Anacystis nidulans, the quantities of cytochromes and the P700:Chl a ratio were reduced (Öquist 1974b, Guikema and Sherman 1983) and the energy transfer was less efficient in PS I (Öquist 1974a).

In the ocean, phytoplankton are in darkness about half of each day, during which their respiration continuously consumes some fraction of diurnal production. Often, this consumption is assumed to be small based on comparison of rates of dark respiration (R_d) and light-saturated photosynthesis (P_m) for nutrient-replete cells. Geider and Osborne (1992) challenged this assumption because phytoplankton in situ are often neither light-saturated nor nutrient-replete, thus their gross photosynthetic rate (P) is less than P_m and the ratio R_d:P may be quite high. There is a disproportionate loss of photosynthetic capacity relative to respiratory capacity when cyanobacteria acclimate to Fe limitation (Guikema and Sherman 1983, Sandmann and Malkin 1983). This implies a differential regulation of photosynthesis and respiration, which could have important consequences for allocation patterns of limiting iron and sensitivity to photoinhibition (see next section).

The function of PS II is to act as water-plastoquinone oxido-reductase (WPOR): water is oxidized to O_2 and plastoquinone (PQ) is reduced to plastoquinol (PQH_2). The maximum change in the quantum yield of fluorescence ($\Delta\phi_m$) is a quantitative measure of photochemical energy conversion efficiency in PS II, the variability of which is determined by the functional organization of PS II as well as by electron transport between PS II and PS I. $\Delta\phi_m$ is calculated as the ratio of variable to maximum

fluorescence (F_v/F_m) and provides an estimate of nutrient stress or photoinhibition (Greene et al. 1994). Guikema and Sherman (1983) found that Fe-starvation decreased the efficiency of PS II indicated by a decline in $\Delta\phi_m$ in the cyanobacterium Synechococcus cedrorum, with no change in PS I or PS II activity per unit chlorophyll. Greene et al. (1994) suggested that Fe limitation and high light lead to loss of functional reaction centers and reduction of $\Delta\phi_m$. The effect of Fe limitation on the efficiency of photosynthesis is poorly characterized. Precise quantitative assessments of the effect of iron limitation or photoinhibition will require that the relationship between the effect on the ratio of F_v/F_m and on the photon yields of photosynthetic O_2 (ψ_{O_2}) be determined in laboratory experiments (Björkman and Demmig 1987).

Photosynthesis and respiration

There are two additional distinguishing characteristics of the photosynthetic apparatus in cyanobacteria: 1) The ratio of PS I reaction center to PS II reaction center is generally very high compared with eucaryotic algae (Raven 1990). In Synechococcus, The PS I:PS II ratio is 3:1 (Aoki et al. 1983). 2) More importantly, photosynthesis and respiration share part of the electron transport pathways (Peschek 1987, Scherer 1990, Shyam et al. 1993, Mi et al. 1994, Schmetterer 1994). Thus, they are especially interesting in that they must regulate aerobic respiration and oxygenic photosynthesis in the same compartment. Also, not all reactions or components of the respiratory electron transport chain are present in all strains or cell types of cyanobacteria. Schmetterer

(1994) suggested that there must be regulatory mechanisms that adjust certain reactions under different external conditions.

Oxygen consuming processes in cyanobacteria may include cytochrome-pathway respiration, alternative (cyanide-resistant) respiration, photorespiration, and the Mehler reaction (Lewitus and Kana 1995). Dark respiration refers collectively to cytochrome-pathway and cyanide-resistant respiration. The process produces energy (ATP) and consumes oxygen with an equimolar production of carbon dioxide (Grande et al. 1989). The respiratory components within the cyanobacterial cell are also located on the thylakoid membranes, consistent with shared respiratory and photosynthetic electron transport components (Carr and Wyman 1986, Scherer 1990). Respiration can feed electrons into the photosynthetic electronic chain, mediated by NAD(P)H dehydrogenase and possibly cytochrome b_6/f complex (cyt b_6/f) (Scherer 1990, Scherer et al. 1988, Schmetterer 1994, Lajkó et al. 1997). Cyt b_6/f of cyanobacteria, which is remarkably similar to the corresponding complex in higher plants (Hauska et al. 1983), is a component of both PET and RET (Scherer et al. 1988, Houchins and Hind 1983, Peschek and Schmetterer 1982, Sandmann and Malkin 1984). With KCN present in the light, all reducing equivalents originating from respiration are donated to the photosynthetic electron transport system (Scherer et al. 1982), providing additional evidence for shared respiratory and photosynthetic electronic transport chains.

The rate of dark respiration generally is found to depend on algal growth rate (Bannister 1979, Langdon 1987, Geider and Osborne 1986, Langdon 1993). However, how respiration changes under stress condition has not been studied intensely and the reports are contradictory. Grande et al. (1991) reported that photoinhibition of

Synechococcus WH5701 at high irradiances was due to inhibition of gross production rather than an increase in light respiration. However, Scherer and Böger (1982) found that Anabaena variabilis exhibits relatively high respiration under high light. By using the respiratory inhibitor NaCN, and the uncouplers FCCP and CCCP, Shyam et al. (1993) found that dark respiration of Anacystis nidulans (= Synechococcus sp.) protects against photoinhibition and aids recovery from photodamage. Also, respiration in Synechococcus was found to be capable of significantly regulating photosynthesis under heat stress (Lajkó et al. 1997).

Despite recent development of high resolution oxygen sensors and fluorometers for improved monitoring of phytoplankton primary production, variability and complications in the field make it impossible to conclusively determine changes in specific growth rate and dark respiration of single phytoplankton species. This argues for also accurately and conclusively determining growth rate and photosynthesis of ecologically important species such as cyanobacteria in the lab (Langdon, 1993). The respiration rate in darkness, which cannot be accurately measured by the ^{14}C technique commonly used in the field, can be easily determined from measuring oxygen evolution. Increase of respiration in nutrient-limited phytoplankton may be a useful tool for determining which particular nutrient limits production (Healey 1979, Elrifi and Turpin 1987).

Photoinhibition

Even though light is the driving force of photosynthesis, it can be harmful to the photosynthetic apparatus if its intensity is too high. Plants have evolved several mechanisms that protect the photosynthetic apparatus under potentially damaging light conditions. When the rate of photodamage exceeds the capacity of photoprotection, the plant is said to be under light stress, or photoinhibition. Photoinhibition is the light-dependent inhibition of the light-dependent reactions of photosynthesis (Osmond 1994).

Now it is generally accepted that PS II is the primary target of photoinhibitory damage. Once the capacity of the protective mechanisms and the repair cycle of PS II are exceeded, photoinhibition of PS II takes place. Exposure to inhibitory high light reduces the light utilization efficiency of photosynthesis due to the destruction of the functioning photosynthetic apparatus. Photoinhibition could be due to: 1) light-harvesting and primary photochemical reactions proceed while other metabolic reactions are limiting; 2) highly toxic singlet oxygen forms in the presence of excited pigments or when redox reactions are occurring; or 3) very high potential intermediates ($\geq$ 1.1 V) of electron transport damage surrounding proteins (Aro et al. 1993).

In order to avoid or minimize photoinhibition, plants have evolved several strategies. Damage to the 32kDa-QB binding protein (D1 protein) is thought to be the primary cause for photoinhibition. The D1 protein turns over at rapidly even under normal growth conditions. Thus repair of PS II via replacement of damaged D1 protein is the main photoprotective mechanism (Aro et al. 1993). Another protective mechanism in higher plants is the capability of plants to dissipate excess excitation energy via the

xanthophyll cycle (Demmig-Adams 1990). In higher plants, it has been found that protein phosphorylation can also afford partial protection to thylakoids when isolated thylakoids are exposed to photoinhibitory conditions (Horton and Lee 1985).

However, cyanobacteria do not have an active xanthophyll cycle (Demming-Adams 1990, Franklin et al. 1992, Falkowski et al. 1994). Protein phosphorylation is also not a protective mechanism in cyanobacterial cells because they lack grana stacking as in chloroplasts in higher plants (Öquist et al. 1995).. Thus cyanobacteria may rely mainly on D1 protein turnover, therefore PS II turnover, as an efficient means to resist photoinhibition (Öquist et al. 1995). Krupa et al. (1990) also suggested that the susceptibility to photoinhibition in freshwater Synechococcus is mainly regulated by changing the rate of turnover of the D1 protein. Kramer and Morris (1990) reported that Synechococcus WH7803 responds to an increase in irradiance with a rapid synthesis of RNA, in excess of that required for protein, followed by moderate increases in protein and DNA synthesis.

Photoinhibition is not restricted to conditions of high light, but may also occur under moderate light conditions in the presence of other environmental stress factors such as low temperature (Ögren 1994) or severe nutrient limitation. Because growth irradiance is expected to be the largest factor controlling iron use efficiency (Raven 1990), it is compelling to experimentally test for high light-iron interaction. The interaction of high light stress with nutrient limitation has been scarcely studied in phytoplankton (Kiefer 1973, Prézelin et al. 1986, Zevenboom et al. 1980). Especially, the interaction of photoinhibition with iron-limitation has not been studied in cyanobacteria. The sensitivity of marine Synechococcus to photoinhibition is uncertain, even though

Richardson et al. (1983) suggested that cyanobacteria might be photoinhibition sensitive. Marine Synechococcus is usually thought to obligately adapt to low light. Alberte et al. (1984) reported that 4 clones including WH7803 are light-saturated in the irradiance region of 40-80 μmol photons·m^{-2}·s^{-1} and are very sensitive to photoinhibition. However, another study reported that WH7803 reaches light-saturated photosynthetic rate (P_m) with no photoinhibition at 2000 μmol photons·m^{-2}·s^{-1} under nutrient replete conditions (Kana and Gilbert 1987a, b).

Photoacclimation to low light intensity may decrease the ratio of chlorophyll a to phycobilins (Castenholz and Waterbury 1989, Dubinsky 1992) and may result in an excess of photosynthetic light harvesting and electron transport proteins, such that they are idle much of the time (Raven 1990). The content of PE in marine Synechococcus WH7803 may depend on the light environment (Kana and Gilbert 1987a). Marine Synechococcus sp. growing under weak blue-green light at the bottom of the euphotic zone exhibits elevated PE, the main light-harvesting pigment for PS II, which permits active photosynthesis (Ikeya et al. 1994). However, the pigment changes under high light stress have not been intensively studied.

The extent of photoinhibition can be assessed by measuring photosynthetic electron transport as net oxygen exchange or chlorophyll fluorescence kinetics (Hall and Rao 1994). The optimal irradiance for photosynthesis is species-dependent, but it is also affected by physiological acclimation to irradiance level, nutrient supply, and temperature (Falkowski et al. 1994).

MATERIALS AND METHODS

Cyanobacterial strains and culture conditions

Two marine Synechococcus strains were used in this project. Synechococcus strain A2169, isolated in 1983 at 5°00.5'S, 84°29.4'W near Ecuador/Peru (Brand 1991) was kindly provided by Larry Brand. Synechococcus strain WH7803, isolated as in 1978 at 33°44.9'N, 67°29.8'W near Bermuda, was obtained from the Bigelow Center for the Culture of Marine Phytoplankton (CCMP1334). Both strains are of the phycoerythrobilin-dominant group.

Both clones were routinely maintained in glass flask batch cultures in autoclaved Gulf Stream seawater supplemented with f/10 nutrients (Guillard and Ryther, 1962) without Cu and Si supplements. The cultures were kept at 21-23°C and 50 μmol photons·m^{-2}·s^{-1} on a 14:10 L:D cycle. These lighting and temperature conditions were also maintained during the experiments. Illumination was provided by GE 20 W SP41 fluorescent lights, which have a spectrum similar to cool white. Continuous mixing was provided by an orbital shaker at 100 rpm. Stock cultures were maintained in glass flasks, and experimental cultures were kept in polycarbonate flasks, which do not significantly leach or adsorb trace metals (Price et al. 1989). The cultures were not axenic.

Media preparation

To avoid the uncertainty of trace-element nutrition in oceanic water, all experimental cultures were maintained in the artificial medium Aquil (Morel et al. 1979, Price et al. 1989). The medium and all stock solutions were prepared in Nanopure water

(~17 megohm-cm). Silicate was deleted as it is not needed for cyanobacterial growth. Nitrate was reduced to 150 μM to achieve a N:P ratio of 15:1, which approximates the Redfield ratio. Double-strength major nutrients stock (N, P) and double-strength synthetic ocean water (SOW) were passed separately through prewashed Chelex-100 columns to strip contaminating transition metals. After the Chelex treatment, SOW was autoclave-sterilized separately from major nutrients stock, and then equal volumes of SOW and major nutrients stock were mixed to give the final concentrations of solutes in Aquil. Filter-sterilized trace metals solution (including iron) and autoclaved vitamins were added to the autoclaved medium. After autoclaving, the pH of Aquil was adjusted to 8.0-8.1 by addition of filter-sterilized 1N HCl or 1N NaOH. All vessels used in the experiments were washed with detergent, soaked at least 24 hr in 1 N HCl, and rinsed with trace metal grade 1 N HCl followed by three rinses with Nanopure water.

For batch cultures, the final medium contained 5 μM EDTA and added Fe concentrations of 3.6, 18, 90 or 900 nM. For semi-continuous cultures, the final medium contained 5μM EDTA and added Fe concentration of 54 or 900 nM. The rationale behind using the EDTA complex was to present Fe in a restricted, but available form. The background iron contamination in Aquil has been estimated to be about 2 nM (Anderson and Morel 1982).

Incubation of batch cultures

Experimental cultures of 50 mL were grown in polycarbonate flasks under the same conditions for stock cultures described above. Stock cultures of A2169 and

WH7803 in f/10 (containing 5 μM EDTA and about 2.3 μM Fe) were transferred into 50 mL Aquil with 900 nM Fe in 125 mL polycarbonate flasks 5 days before experiment and then subcultured (40% v/v) three times in order to reduce iron reserves that may have accumulated in stock cultures.

The stock cultures used to initialize the experiment were in the middle log growth phase. The inocula were 4% and 6% for batch 1 experimental cultures of A2169 and WH7803, respectively. If we assume that stock cultures were Fe-replete and total cellular Fe is at least 50% of the total available (Rueter & Unsworth 1991), the Fe carryover from stock cultures was less than 18 nM for A2169 and 27 nM for WH7803.

Batch 1 cultures were subcultured into batch 2 cultures on day 5 using inocula of 3% for A2169 and 6% for WH7803, except for WH7803 at 3.6 nM added Fe, for which an inoculum of 8% was used. The larger inocula for WH7803 were intended to compensate for the lower cell densities compared to A2169.

In one experiment, batch 2 cultures were similarly subcultured into batch 3 to check the acclimation time of batch cultures. The subcultures were always initiated in late exponential phase.

Incubation of semi-continuous cultures

Stock cultures of A2169 and WH7803 in f/10 were transferred into Aquil with 900 nM Fe in polycarbonate flasks 5 days before experiment and then subcultured (40% v/v) three times in order to reduce iron reserves that may have accumulated in stock cultures.

Experimental cultures of 200 mL were grown in 500 mL polycarbonate flasks under the same conditions for batch cultures described above. A foil cap loosely covered the cotton stopper to prevent contamination. Cultures were grown at 50 μmol photons·m^{-2}·s^{-1} except for 0.43 d^{-1}–hl, which were grown at 100 μmol photons·m^{-2}·s^{-1}.

The stock cultures used to initialize the experiment were in the middle log growth phase. The inocula were 4 to 6% for semi-continuous cultures of A2169 and WH7803. Daily dilution of 25, 35, or 50% volume began on day 5 and continued 9, 7, and 5 days for 25, 35, and 50% dilution rate (corresponding to 0.29, 0.43, and 0.69 d^{-1}), respectively. Balanced cultures were confirmed by daily monitoring absorbance at 750 nm for several days before the physiological experiment.

Measurement of cell density and pigment

Cell densities were monitored every 1 or 2 days at the same time of day by measuring absorbance at 750 nm with a Shimadzu UV-160U spectrophotometer. Direct relationships (r^2 = 0.904, n = 32 for A2169 and r^2 = 0.908, n = 36 for WH7803) between cell densities (microscopic counts) in Aquil and absorbance at 750 nm were established from preliminary experiments. Specific growth rates (μ, d^{-1}) of individual batch cultures were calculated as the slope of the regression of ln(cell density) against time (r^2 >0.93, except for μ of WH7803 at 3.6 and 18 nM Fe) over the log growth period, which ranged from 3 to 5 days.

Chlorophyll a (Chl) was extracted by bringing 0.45 mL of the A_{750} or P-I samples to 4.5 mL total volume with acetone, and stored overnight in dark at -20°C. Cells and

precipitated materials were removed by centrifugation at 4000 × *g* for 15 min at 4°C and then Chl content in the extracts was measured with a Turner 111 fluorometer calibrated with spectrophotometrically-quantified Chl a (Shimadzu UV-160U, 2 nm bandpass).

Measurement and calculation of photosynthesis and respiration

Rates of photosynthetic and respiratory O_2 exchange were measured at 20°C in a Hansatech DW3 water-jacketed polarographic electrode chamber (15 mL), which was connected to a National Instruments 12-bit A/D board and custom-written software.

For batch cultures, cells were harvested in late exponential growth phase. For semi-continuous cultures, cells were harvested after equilibration to the tested growth rate for about three doubling times.

Cells were supplemented with 8 mM fresh $NaHCO_3$ just prior to examination to avoid CO_2-limitation. The samples in the O_2 chamber were exposed to a series of 13 incremented PFDs (4-7 min. each, depending on cell density) from darkness to more than 1000 μmol photons·m^{-2}·s^{-1}, with at least 5 irradiances in the linear initial slope region (Henley 1993). Light was provided from a slide projector and irradiance was adjusted with neutral density filters. The incident PFD was measured by a LI-COR Li-189 photometer with a Li-1000 quantum sensor located on the rear window of the O_2 chamber. Non-normalized rates of oxygen exchange (μmol O_2 ·hr^{-1}) were automatically calculated in real time by linear regression after each PFD.

To test short-term instantaneous photoinhibition, samples were subjected to high light treatment before measurement of P-I curves. For batch cultures, cells used to assess

photoinhibition were not the same ones used to measure P-I curve without high light treatment. However, subsamples from the same cultures were used to measure P-I curve with and without high light treatment (30 min at 1000 μmol photons·m^{-2}·s^{-1}) for semi-continuous cultures.

For batch cultures, cells were grown in Aquil with various Fe concentrations described above. Then batch 1 samples in late exponential growth phase were first exposed to 500 and 1000 μmol photons·m^{-2}·s^{-1} for WH7803 and A2169, respectively, for 30 min in the O_2 chamber. For semi-continuous cultures, samples in balanced cultures were exposed to 1000 μmol photons·m^{-2}·s^{-1} for 30 min for both strains. 8 mM fresh $NaHCO_3$ was added to the sample just prior to examination. To prevent oxygen supersaturation before the end of measurement, it was necessary to sparge the O_2 chamber with N_2 to reduce initial oxygen concentration for both strains at 900 nM Fe. The rates of O_2 exchange were calculated every 2.5 min during the 30-min high light treatment. After the 30-min high light treatment, the samples were adapted to dark for about 5 min and then the P-I curves were measured at a series of 13 PFDs as above.

The rates of net O_2 exchange were normalized to Chl content or cell density. Light-saturated gross photosynthetic rate (P_m), dark respiration rate (R_d), and initial slope at limiting PFDs (α) were determined by fitting individual curves to Bannister's mathematical formulation (Bannister, 1979). Fitted parameters for 3 – 4 replicate curves were then averaged.

Measurement of Chlorophyll fluorescence

Chlorophyll fluorescence ratio, F_v/F_m, was measured at liquid N_2 temperature (77K) using a custom-made fluorometer (Henley et al. 1991). Semi-continuous culture samples of 15 to 25 mL were filtered on GF/F filters and dark-adapted for 2 min with far-red light to induce high fluorescence state 1. Then chlorophyll fluorescence was measured at liquid N_2 temperature. F_v/F_m was automatically calculated. For high light treatment, the culture samples were incubated in the polarographic electrode chamber exposed to 1000 μmol photons·m^{-2}·s^{-1} for 30 min as in measurement of O_2 exchange, with or without 40 μg·mL^{-1} chloramphenicol (CAP). After the 30-min high light treatment, samples of 15 to 25 mL were filtered on GF/F filters and dark-adapted for 2 min with far-red light and then F_v/F_m was measured at liquid N_2 temperature. CAP was dissolved in ethanol and added to cultures so that the final concentration of ethanol was about 0.1%.

RESULTS

Preliminary experiments

Culturing conditions for marine Synechococcus

In order to avoid the dissolution of the silica and its associated trace metal contaminants during the autoclaving process, teflon bottles are the best choice for containing and autoclaving seawater (Brand et al. 1981, Brand 1991). Medium for semi-continuous cultures was autoclaved in teflon bottles, but medium for some batch cultures was autoclaved in borosilicate glass flasks. A parallel experiment was conducted to compare the effect of autoclaving in different type of vessels (Fig. 1). When grown

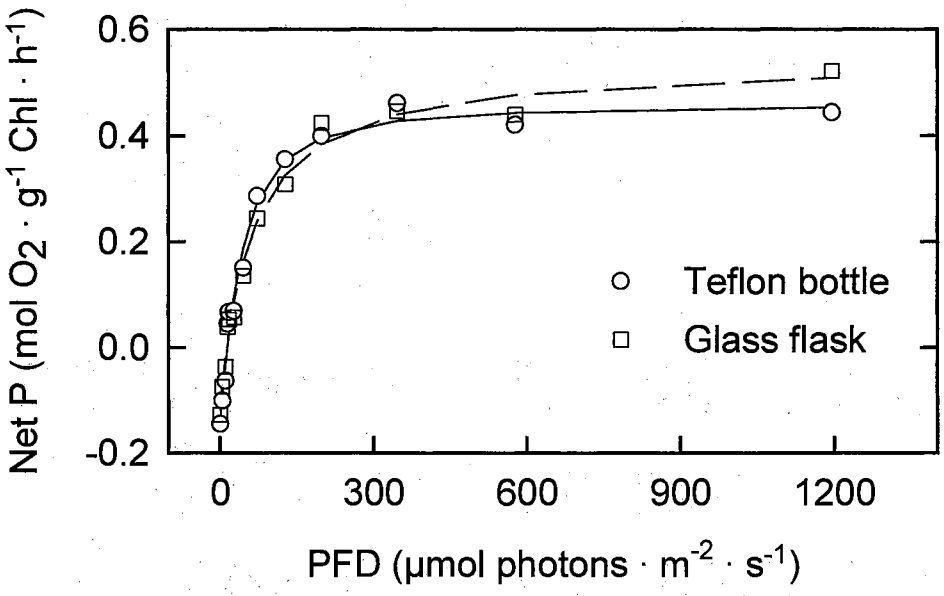

Fig. 1. Photosynthesis-irradiance curves of Synechococcus WH7803 batch 2 cultures at 54 nM Fe in Aquil . Cultures were grown at 50 µmol photons·m^{-2}·s^{-1}. The medium was prepared by autoclaving in a glass flask or teflon bottle. Error bars are omitted for clarity; n = 3.

in medium autoclaved in glass flasks, gross photosynthetic capacity of the cells (gross P_m^{Chl}, 0.68±0.08 mol $O_2 \cdot g^{-1}$ Chl $\cdot h^{-1}$) was not significantly different ($p > 0.05$, one way ANOVA) than that (0.60±0.06) of cells grown in medium autoclaved in teflon bottles. Initial slope at limiting light (α^{Chl}; glass flask: 0.01±0.01, teflon bottle: 0.01±0.01) and respiration rate in darkness (R_d^{Chl} ; glass flask: -0.14±0.02, teflon bottle: -0.12±0.02 mol $O_2 \cdot g^{-1}$ Chl $\cdot h^{-1}$) were also not significantly different. For both Synechococcus strains, cell yield (glass flask: 2.68±0.37, teflon bottle: 2.71±0.11 $\times 10^7$ cells $\cdot$ mL^{-1}) and specific growth rate (μ; glass flask: 0.54±0.07, teflon bottle: 0.50±0.09 d^{-1}) were not significantly different between cultures grown in media autoclaved in glass flask or teflon bottle. The absolute Fe concentration might be slightly higher when the medium was autoclaved in glass flasks, but it did not significantly affect the results in this investigation.

Standard curves for calculating cell density

Light-scattering is commonly used to monitor the growth of pure Synechococcus cultures (Shyam et al. 1993, Wilhelm and Trick 1995). In this investigation, cell density was calculated from absorbance at 750 nm (A_{750}) using different standard curves for A2169 and WH7803 (Fig. 2). Cells in various growth phase (early-, middle-, and late-exponential, and stationary phase) and in various Fe conditions (Fe-replete and Fe-limited) were counted microscopically using a Levy chamber at 400×. In all cases, at least 500 cells were counted. The results showed that there is a good linear relationship between counted cell density and A_{750}. I did not find an obvious change in cell size of

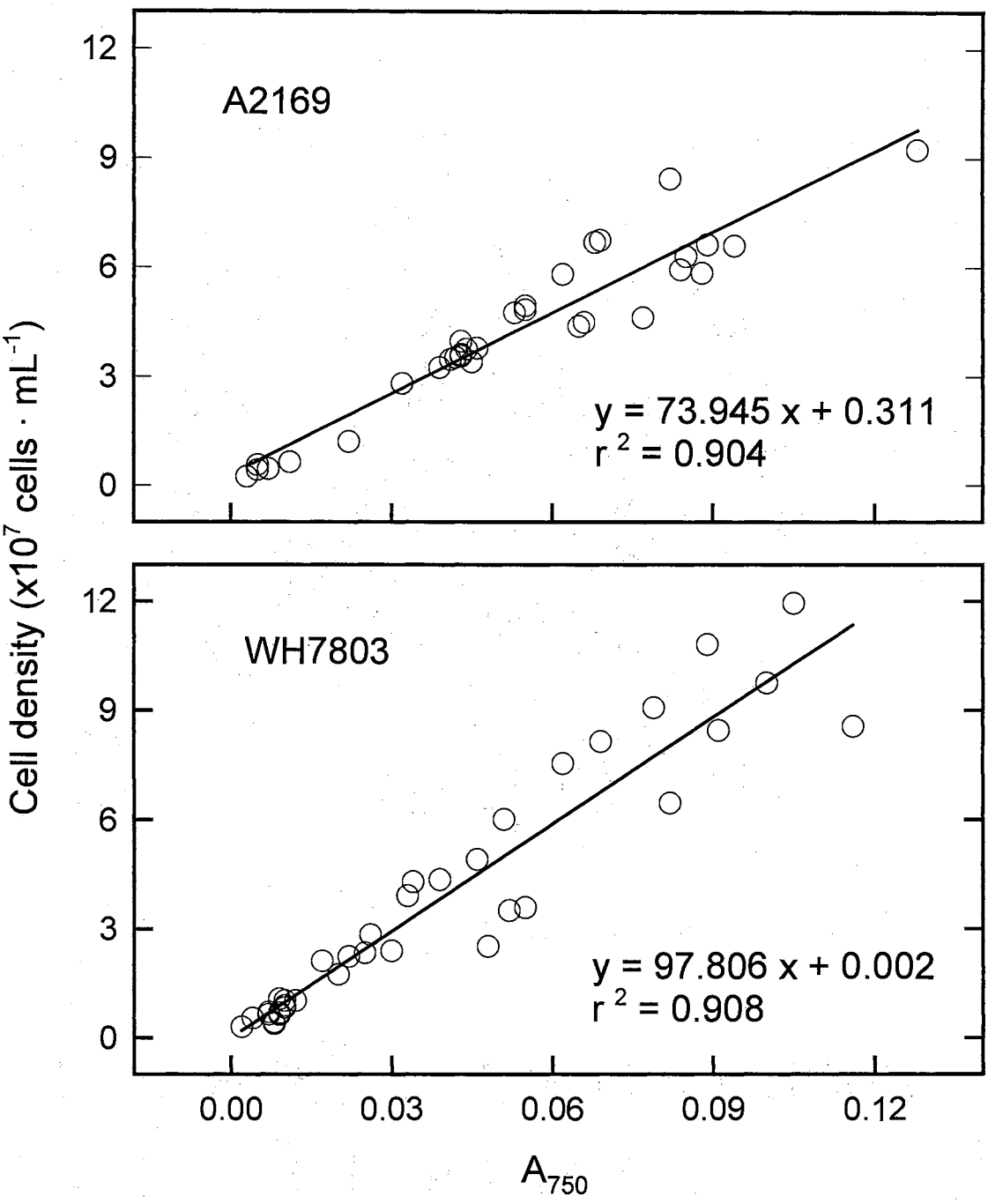

Fig. 2. Standard curves of A_{750} to cell density for Synechococcus A2169 and WH7803 in Aquil. Cell number was counted in 80 random fields (1/16 mm^2 each) using a Levy Chamber at 400×. Each sample was counted twice and the average was taken as the cell density.

the two marine Synechococcus strains under different growth phases or Fe conditions. If A_{750} of a sample was higher than 0.12, the sample would be diluted four- to five-fold before measuring A_{750}; and then calculated cell density based on the diluted A_{750} would be multiplied by the dilution factor to give actual cell density of the sample.

Measuring conditions for oxygen evolution

A batch culture may go through a lag phase, accelerating phase, exponential phase, and stationary phase. Each growth phase is a reflection of a particular metabolic state of the culture at any given time. Only during exponential phase does the growth rate and metabolic rates remain relatively constant. In this investigation, inoculated cells appeared to grow exponentially with negligible lag phase. Batch cultures on day 3, day 5, and day 7 represented early exponential phase, late exponential phase, and stationary phase, respectively. Photosynthetic capacity of cultures at these growth phases were significantly different (Fig. 3a). For Fe-replete culture, the maximum photosynthetic capacity occurs in early exponential phase, so that it would be desirable to measure P-I curve for cultures in early exponential phase. However, cell density in Fe-limited cultures in early exponential phase (data not shown) was too low to measure oxygen exchange precisely. Thus, I decided to measure oxygen evolution on day 5 (late exponential phase) for both Fe-replete and Fe-limited batch cultures in order to be consistent.

The time needed to measure a P-I curve was 50-70 min, depending on the cell density. Cells, especially Fe-replete cells, are actively photosynthesizing during this period of time thus may become CO_2-limited. To test this I added 4 or 10 mM $NaHCO_3$

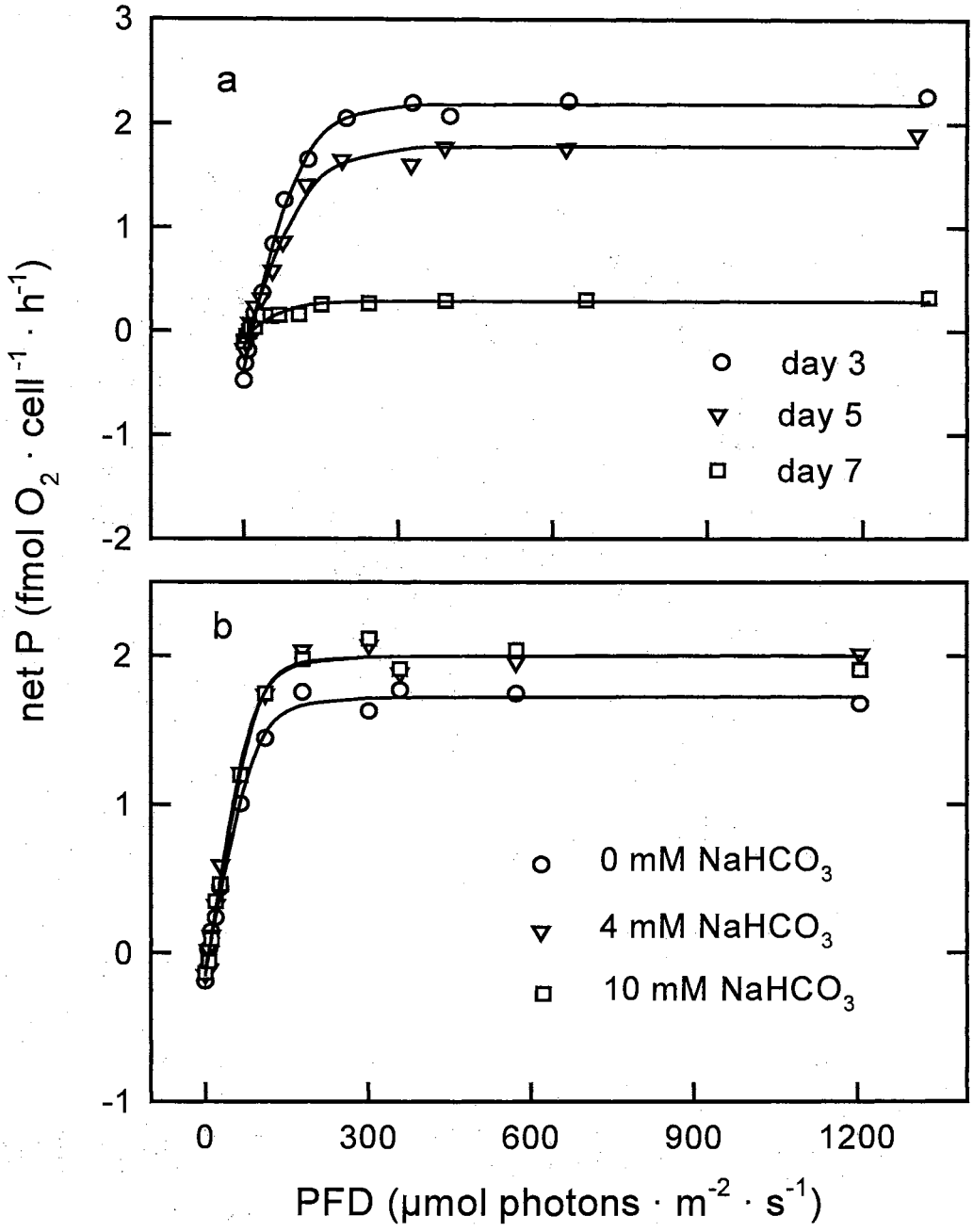

Fig. 3. Photosynthesis-irradiance curves for cultures in different growth phases (panel a) and cultures with $NaHCO_3$ added prior to measurement of oxygen evolution (panel b). Synechococcus WH7803 cultures in f/10 medium were used in this experiment; n = 3. Samples were aliquots of the same cultures on different day (panel a) or different added concentration of $NaHCO_3$ (panel b).

into the culture immediately prior to measurement of oxygen evolution. Photosynthetic capacity was saturated by an addition of 4 mM $NaHCO_3$ (Fig. 3b). For consistency and to ensure CO_2-saturation in all cases, 8 mM $NaHCO_3$ was added to every sample prior to the measurement of oxygen evolution in this investigation.

Batch culture experiments

Iron-limitation of cell yield and growth rate

Batch 1 Synechococcus cultures represent the acclimation period to the respective Fe concentrations, during which excess Fe from stock cultures was depleted. Consequently only moderate (but statistically significant) reduction of yield and growth rate (μ) was evident at low Fe in both strains (Fig. 4). A2169 grew exponentially for about two doublings at 3.6 nM Fe, three doublings at 18 and 90 nM Fe, and four doublings at 900 nM Fe. WH7803 grew exponentially for about two doublings at 3.6 nM Fe, three doublings at 18 nM Fe, and four doublings at 90 and 900 nM Fe. Batch 1 cultures in late exponential phase were subcultured into batch 2 cultures on day 5. Following subculture, yield and μ in batch 2 cultures were reduced to a greater extent in both strains, particularly at the two lowest Fe concentrations (Figs. 4, 5). Both strains grew exponentially for only about one doubling at 3.6 nM Fe, two doublings at 18 nM Fe, and four doublings at 90 and 900 nM Fe in batch 2 cultures. The differences between the highest and lowest added Fe were 12- and 17-fold in yield, 2.4-and 1.9-fold in μ for A2169 and WH7803, respectively. Final cell yields for A2169 (Fig. 5, open circles) averaged 123% (range 27-337% for individual Fe levels) higher than those for WH7803

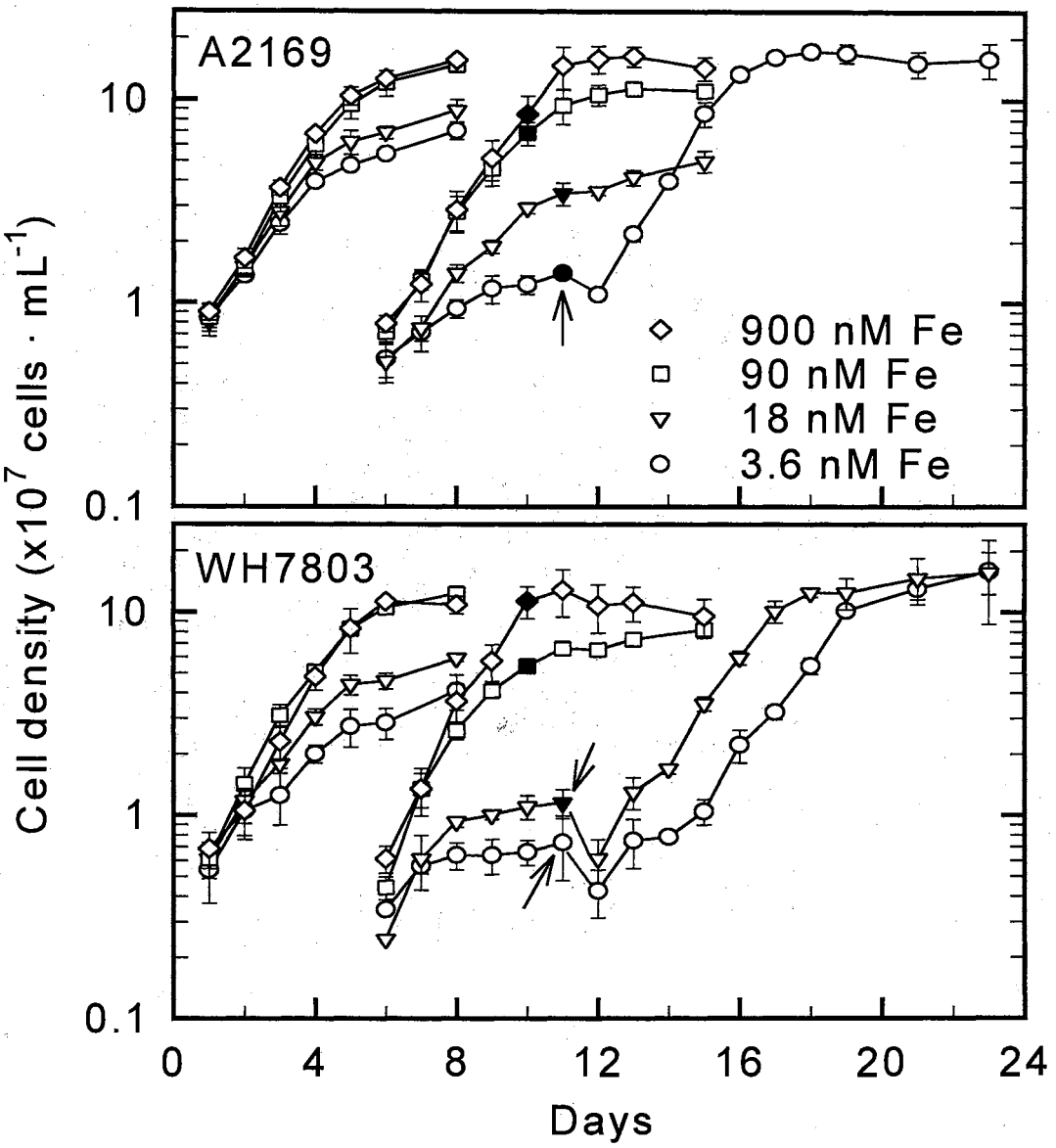

Fig. 4. Batch growth curves of Synechococcus A2169 (upper) and WH7803 (lower) in Aquil (mean ± SD, n = 4, except n = 3 for WH7803 at 3.6 nM Fe after day 11). Within each panel, the left series of curves are batch 1, and the right series are batch 2, subcultured from batch 1 cultures on day 5 using a 3% v/v inoculum for A2169 and 6% v/v for WH7803 (8% at 3.6 nM Fe). Arrows indicate addition of 900 nM Fe to 3.6 nM Fe A2169 and 3.6 and 18 nM Fe WH7803 batch 2 cultures on day 11. Filled symbols correspond to P-I data in Table 1.

(Fig. 5, open triangles). One-way ANOVA ($p < 0.001$) indicated significant effects of Fe concentration on yield and μ for both strains. Although I presented the μ for WH7803 at 3.6 nM added Fe, the growth rate was too low to be measured with confidence, because the A_{750} values were near the limits of detection (~0.005). Consequently the statistical differences among these treatments were unreliable.

Yield and μ of all Fe treatments within a strain differed significantly ($p < 0.05$; Tukey multiple comparison) except for μ at 90 and 900 nM Fe for both strains and μ and yield at 3.6 and 18 nM Fe in WH7803. Yields were significantly reduced at 90 nM Fe whereas μ decreased only at 18 nM Fe (Fig. 5). Although yield was more strongly affected than μ, the latter was reduced by about half at 3.6 compared to 900 nM Fe. The actual μ might be lower than calculated for WH7803 at 3.6 and possibly 18 nM Fe because the apparent log growth phase was brief and cell densities were very low. Relative μ/μ_{max} at 18 nM Fe was 0.72 for A2169 and 0.53 for WH7803, indicating greater Fe-dependency of μ in WH7803. Yield, both absolute and relative (normalized to yield at 900 nM Fe), also was inhibited more strongly by low Fe in the Atlantic strain WH7803 (normalized mean yields at 3.6, 18 and 90 nM Fe were 0.056, 0.088 and 0.59) than the Pacific strain A2169 (normalized mean yields 0.084, 0.30, 0.71).

To verify that Fe starvation was the sole cause of negligible batch 2 growth of 3.6 nM A2169, and 3.6 and 18 nM WH7803, 900 nM Fe (no additional EDTA) was added to these cultures on day 11 (arrows in Fig. 4). In response to the addition of Fe, 3.6 nM A2169, and 3.6 and 18 nM WH7803 all resumed exponential growth at about the same

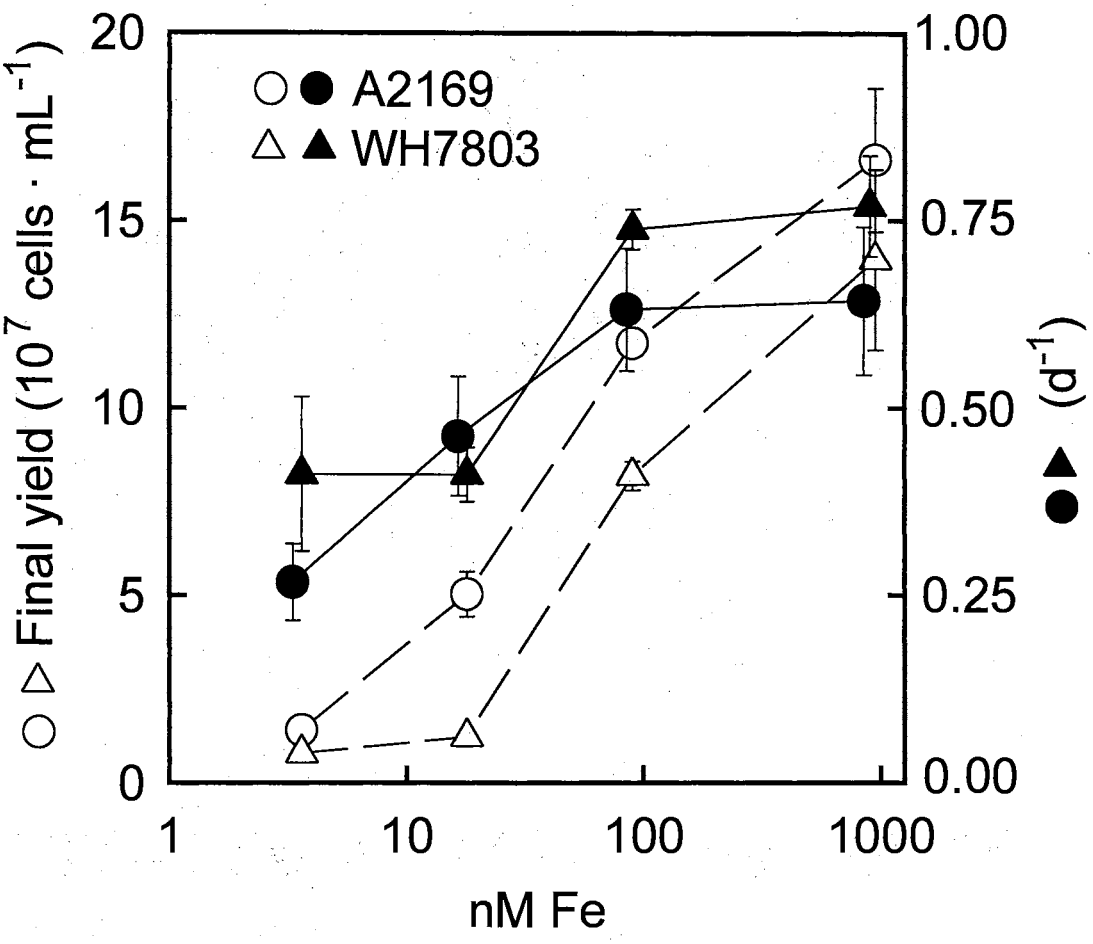

Fig. 5. Final cell yields (open symbols) and μ (filled symbols) of Synechococcus A2169 and WH7803 in Aquil as a function of total Fe concentration (mean ± SD, n = 4). Note the log scale of Fe concentration. Some points have been offset slightly on Fe axis for clarity.

rate and reached the same yield as in the cultures originally supplied with 900 nM Fe (Fig. 6). In response to the Fe addition, there were about 3-4 days of exponential growth following a lag phase of one day for 3.6 nM A2169, and three days for 3.6 nM and 18 nM WH7803. These results indicated that Fe concentration was the only limiting nutrient in these cultures.

Chlorophyll a (Chl) content per cell was generally high in early log growth phase and decreased progressively until stationary phase in batch 1 for both strains at all Fe concentrations, and at 900 nM Fe in batch 2 (Fig. 6). Chl content extended over about a 3- and 4-fold range for A2169 and WH7803, respectively. The cell Chl content attained highest levels during early log growth phase and rapidly declined through transition phase, most significantly in WH7803 batch 1 (Fig. 6, lower panel). The effect of Fe concentration on cell Chl content was more obvious in A2169 than in WH7803. In A2169 batch 2, the cell Chl content at 900 nM Fe was about double that at 3.6 nM Fe.

The temporal trend was absent or less obvious at the lowest Fe concentrations in batch 2, in which Chl remained fairly low even in early exponential growth. However, Chl concentration increased to a high level within 2 – 4 days of adding 900 nM Fe to these cultures on day 11, but eventually decreased again as stationary phase was reached (Fig. 6, filled symbols). The cell Chl contents in WH7803 batch 2 cultures at 3.6 nM Fe might not be reliable because the Chl concentrations in the cultures were near the limits of detection.

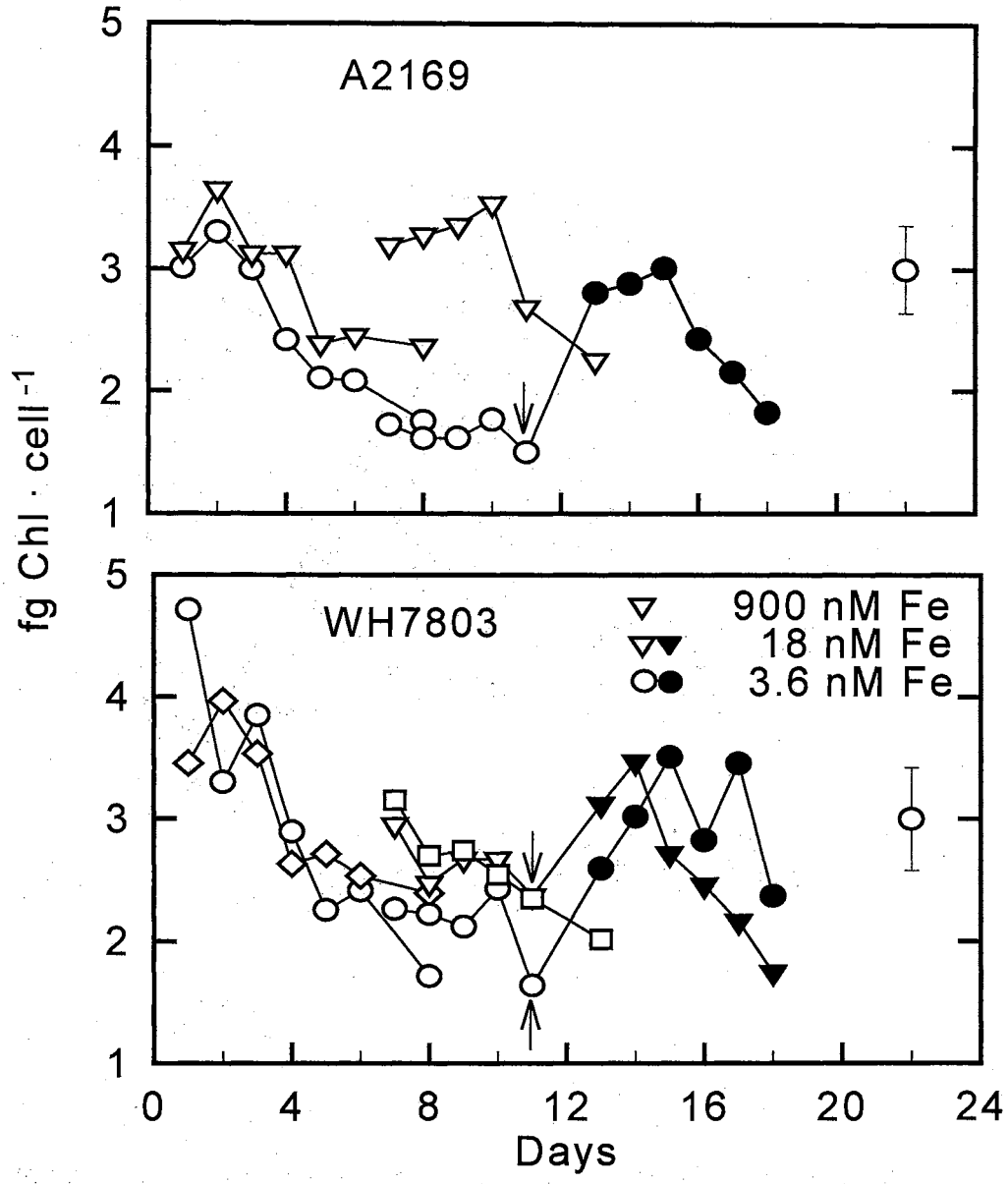

Fig. 6. Cell chlorophyll content during batch growth of Synechococcus A2169 and WH7803 in Aquil at selected total Fe concentrations; n = 3 (WH7803 filled circles only) or 4. Error bars are omitted for clarity; the average SD for each graph is plotted on day 22. Arrows indicate addition of 900 nM Fe to 3.6 nM Fe A2169 and 3.6 and 18 nM Fe WH7803 batch 2 cultures on day 11 and filled symbols denote the subsequent response of these cultures.

Effects of Fe on photosynthesis and respiration

To assess the effects of Fe concentration on photosynthesis properties of the cultures, I measured the Chl- and cell-specific rates of photosynthetic and respiratory O_2 evolution at a series of incremented PFDs for batch 2 cultures for both marine Synechococcus strains (Tables 1, 2). P-I curves were measured on days 10 or 11 (Fig. 4, filled symbols). For both strains, Fe concentration did not significantly ($p > 0.05$, one way ANOVA) affect the Chl-specific light-saturated gross photosynthetic rate (P_m^{Chl}) and apparent photosynthetic efficiency at limiting light (α^{Chl}) (Table 1). However, respiration rate in darkness (R_d^{Chl}) generally increased with decreasing Fe for both strains. The difference of R_d^{Chl} among Fe treatment was 6.4- and 6.5-fold for A2169 and WH7803, respectively. When O_2 evolution data were normalized to cell density, the results of the two strains were different (Table 2). For A2169, gross P_m^{cell} decreased significantly with decreasing Fe, but R_d^{cell} increased at 3.6 nM Fe. For WH7803, gross P_m^{cell} also decreased but not as much as A2169, and R_d^{cell} increased at low Fe. Gross P_m^{cell} changed much more than gross P_m^{Chl}, but R_d^{cell} changed less than R_d^{Chl} with different Fe for both strains.

The effects of Fe concentrations on R_d : gross P_m ratios are shown in Fig. 7. There was a large increase in R_d^{Chl} : gross P_m^{Chl} ratios in 3.6 nM Fe A2169 and 18 nM Fe WH7803, while R_d^{Chl} : gross P_m^{Chl} was unchanged at higher Fe concentrations. Compared to the next higher Fe concentration, R_d^{Chl} : gross P_m^{Chl} was about four- and eight-fold higher at 3.6 nM Fe in A2169 and 18 nM Fe in WH7803, respectively. The results indicated that WH7803 was Fe-limited at higher Fe concentration than A2169. The trend of R_d^{cell} : gross P_m^{cell} ratio, which were generally not reported in other

Table 1. Chl normalized photosynthetic capacity and dark respiration of Synechococcus A2169 and WH7803 in late exponential batch 2 cultures (day 10 or 11; filled symbols in Fig. 4). Values are means ± SD of 3 cultures. For each strain and parameter, values with different superscript letters are significantly different ($p < 0.05$, Tukey multiple comparison). α^{Chl} units are mol $O_2 \cdot g^{-1}$ Chl $\cdot h^{-1} \cdot \mu mol^{-1}$ photons $\cdot m^2 \cdot s^{-1}$; other variables are mol $O_2 \cdot g^{-1}$ Chl $\cdot h^{-1}$.

nM Fe	gross P_m^{Chl}	α^{Chl}	R_d^{Chl}	net P_m^{Chl}
		A2169		
3.6	0.33 ± 0.05^a	0.012 ± 0.007^a	-0.28 ± 0.03^a	0.04 ± 0.03^c
18	0.30 ± 0.05^a	0.005 ± 0.001^a	-0.07 ± 0.02^b	0.23 ± 0.06^b
90	0.27 ± 0.06^a	0.008 ± 0.005^a	-0.06 ± 0.01^{ab}	0.21 ± 0.11^b
900	0.41 ± 0.04^a	0.005 ± 0.0004^a	-0.04 ± 0.003^c	0.36 ± 0.04^a
		WH7803		
18	0.38 ± 0.13^b	0.011 ± 0.017^a	-0.31 ± 0.07^a	0.07 ± 0.09^c
90	0.69 ± 0.05^a	0.005 ± 0.001^a	-0.07 ± 0.01^b	0.62 ± 0.05^a
900	0.43 ± 0.10^b	0.006 ± 0.003^a	-0.15 ± 0.02^b	0.38 ± 0.08^b

Table 2. Cell normalized photosynthetic parameters of Synechococcus A2169 and WH7803 in late exponential batch 2 cultures (day 10 or 11; filled symbols in Fig. 4). Values are means ± SD of 3 cultures. For each strain and parameter, values with different superscript letters are significantly different ($p < 0.05$, Tukey multiple comparison). α^{cell} units are fmol $O_2 \cdot cell^{-1} \cdot h^{-1} \cdot \mu mol^{-1}$ photons $\cdot m^2 \cdot s$, other variables are fmol $O_2 \cdot cell^{-1} \cdot h^{-1}$.

nM Fe	gross P_m^{cell}	α^{cell}	R_d^{cell}	net P_m^{cell}
		A2169		
3.6	0.40 ± 0.13^{b}	0.009 ± 0.006^{b}	-0.26 ± 0.06^{a}	0.13 ± 0.18^{c}
18	0.56 ± 0.08^{b}	0.009 ± 0.002^{b}	-0.13 ± 0.04^{b}	0.43 ± 0.09^{bc}
90	0.73 ± 0.15^{b}	0.020 ± 0.010^{a}	-0.17 ± 0.03^{ab}	0.56 ± 0.12^{b}
900	1.41 ± 0.11^{a}	0.018 ± 0.001^{a}	-0.15 ± 0.01^{b}	1.25 ± 0.11^{a}
		WH7803		
18	0.60 ± 0.18^{b}	0.028 ± 0.025^{a}	-0.39 ± 0.07^{a}	0.21 ± 0.21^{b}
90	1.51 ± 0.13^{a}	0.011 ± 0.002^{a}	-0.16 ± 0.02^{b}	1.36 ± 0.13^{a}
900	1.12 ± 0.32^{ab}	0.015 ± 0.008^{a}	-0.12 ± 0.07^{b}	1.00 ± 0.26^{a}

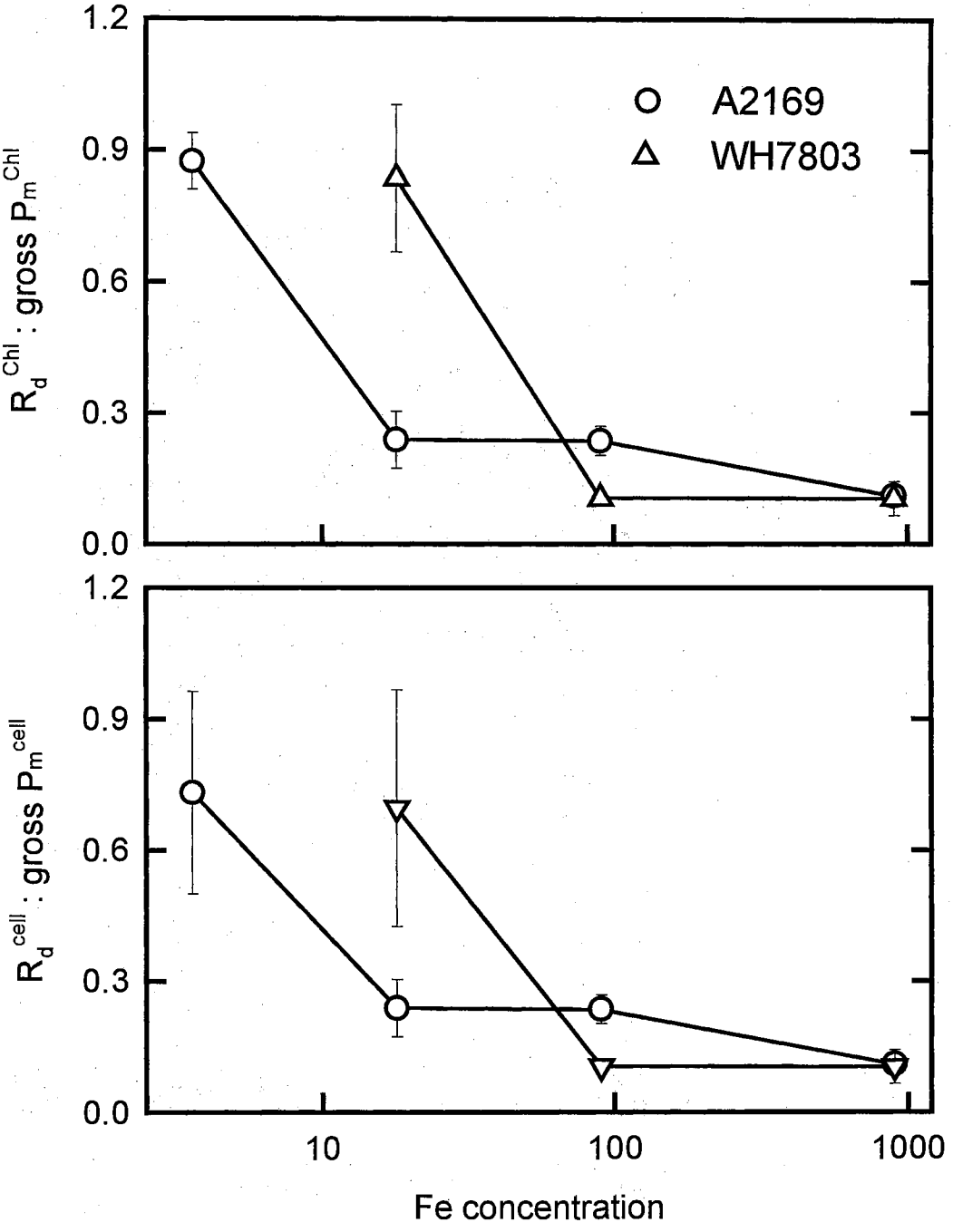

Fig. 7. R_d^{Chl} : gross P_m^{Chl} ratios (upper) and R_d^{cell} : gross P_m^{cell} (lower) of Synechococcus A2169 and WH7803 batch 2 cultures in Aquil as a function of total Fe concentration (mean ± SD, n = 3). Note the log scale of Fe concentration.

phytoplankton studies, was similar to R_d^{Chl} : gross P_m^{Chl} ratio in both strains.

In summary, gross P_m^{Chl} was unaffected by Fe concentration, whereas gross P_m^{cell} was significantly related to Fe concentration, with a two- to three-fold difference between Fe-replete and Fe-starved cells in both strains. R_d^{cell} was about two-fold higher at 3.6 nM Fe in A2169 and 18 nM Fe in WH7803 compared to the next higher Fe concentration. Because R_d^{cell} increased while gross P_m^{cell} decreased at low Fe concentrations, Fe-replete cultures had 7- to 9-fold higher net P_m^{cell} than low Fe cultures, and in the latter R_d^{cell} exceeded net P_m^{cell}. R_d : gross P_m ratio also indicated that WH7803 was Fe-limited at higher Fe concentration than A2169. Initial slope α^{Chl} was in some cases highly variable among replicate cultures and no obvious Fe-dependent trend was evident. Cell densities were too low to reliably measure O_2 exchange for 3.6 nM Fe WH7803 cultures.

Equilibration to Fe regimes and temporal trends in photosynthesis and respiration

The previous experiment at 18 and 900 nM Fe was repeated for both strains, except batch 2 cultures were subcultured into batch 3 (Fig. 8) in order to confirm that batch 2 cultures were already equilibrated to the respective Fe concentrations. Final cell yields and μ differed significantly between Fe treatments, but were identical in batch 2 and batch 3 (2 way ANOVA), confirming that cells were equilibrated to the Fe regimes in batch 2 (Table 3). The only exception was the significantly lower batch 2 μ of A2169 at 900 nM Fe, which was probably unrelated to Fe equilibration because these cultures were

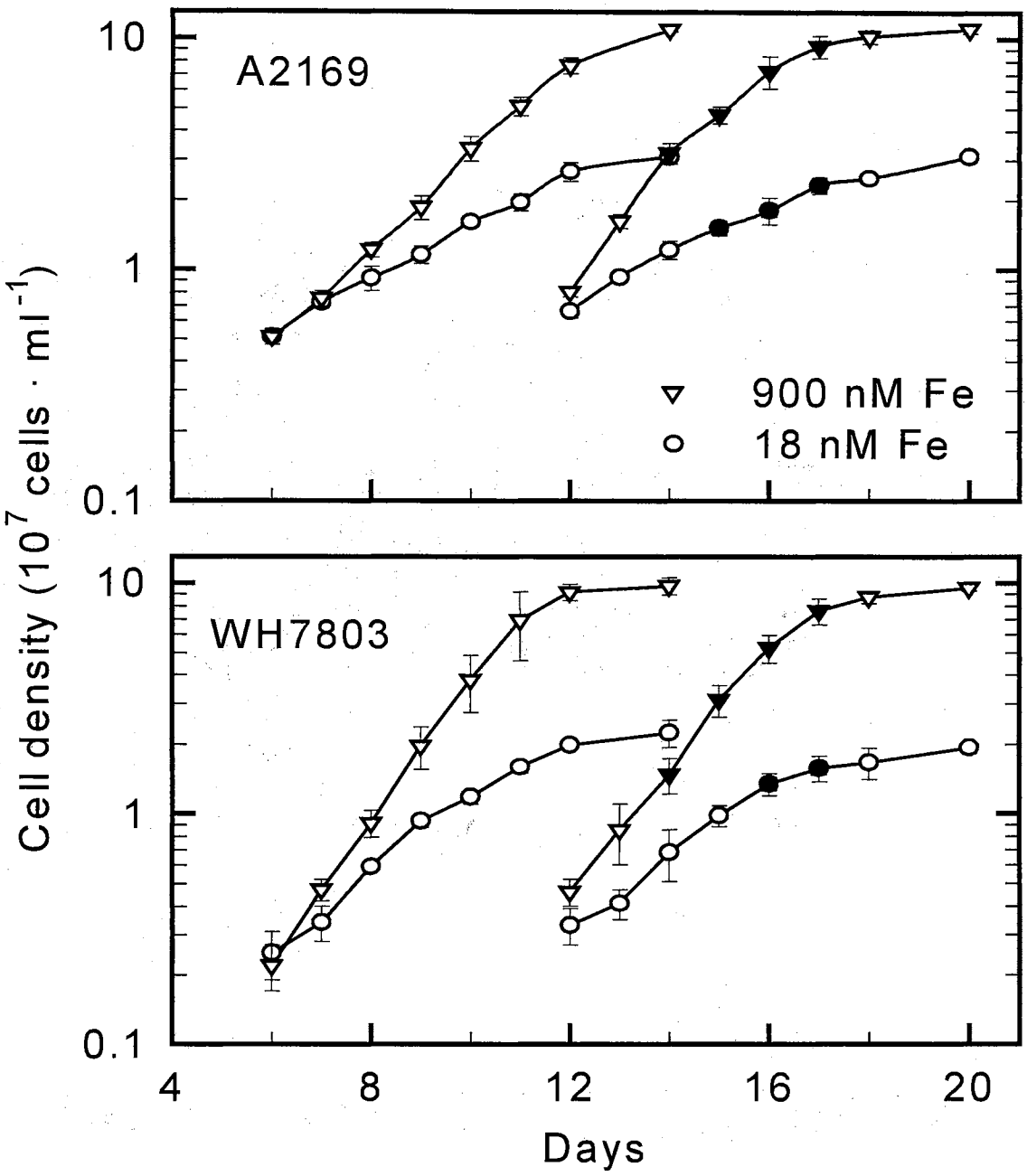

Fig. 8. Batch growth curves of Synechococcus A2169 (upper) and WH7803 (lower) in Aquil (mean ± SD, n = 4 for batch 2 and n = 3 for batch 3). Within each panel, the left series of curves are batch 2, and the right series are batch 3, subcultured from batch 2 cultures on day 11 using a 10% v/v inoculum for 18 nM Fe and 6% v/v for 900 nM Fe. Filled symbols correspond to photosynthesis and respiration data in Table 4.

Table 3. Final cell yields ($\times 10^7$ cells·mL^{-1}) and μ (d^{-1}) of Synechococcus A2169 and WH7803 batch 2 and batch 3 cultures in Aquil containing 18 and 900 nM Fe (means ± SD). For each strain and parameter, values with different superscript letters are significantly different ($p < 0.05$, Tukey multiple comparison).

Strain	Batch	n	nM Fe	yield	μ
A2169	2	4	18	3.1 ± 0.2^{b}	0.27 ± 0.04^{c}
	2	4	900	10.9 ± 0.2^{a}	0.47 ± 0.04^{b}
	3	3	18	3.1 ± 0.1^{b}	0.28 ± 0.02^{c}
	3	3	900	11.0 ± 0.4^{a}	0.60 ± 0.01^{a}
WH7803	2	4	18	2.2 ± 0.3^{b}	0.45 ± 0.09^{b}
	2	4	900	9.9 ± 0.8^{a}	0.71 ± 0.03^{a}
	3	3	18	1.9 ± 0.1^{b}	0.46 ± 0.10^{b}
	3	3	900	9.6 ± 0.2^{a}	0.63 ± 0.04^{a}

Table 4. Gross photosynthetic capacity and dark respiration (fmol $O_2 \cdot cell^{-1} \cdot h^{-1}$ or mol $O_2 \cdot g^{-1}$ Chl $\cdot h^{-1}$) of Synechococcus A2169 and WH7803 in mid- to late exponential batch 3 cultures (filled symbols in Fig. 8). Values are means ± SD of 3 cultures.

nM Fe		day 3	day 4	day 5	day 6
			A2169		
18 nM	gross P_m^{Chl}	- -	0.34 ± 0.08	0.33 ± 0.03	0.30 ± 0.04
	R_d^{cell}	- -	-0.33 ± 0.10	-0.28 ± 0.09	-0.30 ± 0.07
	gross P_m^{cell}	- -	0.75 ± 0.10	0.66 ± 0.12	0.66 ± 0.09
900 nM	gross P_m^{Chl}	0.51 ± 0.08	0.57 ± 0.10	0.33 ± 0.01	0.28 ± 0.02
	R_d^{cell}	-0.24 ± 0.05	-0.15 ± 0.03	-0.13 ± 0.05	-0.14 ± 0.03
	gross P_m^{cell}	1.56 ± 0.22	1.60 ± 0.11	0.89 ± 0.15	0.58 ± 0.09
			WH7803		
18 nM	gross P_m^{Chl}	--	--	0.32 ± 0.08	0.29 ± 0.08
	R_d^{cell}	--	--	-0.45 ± 0.12	-0.40 ± 0.08
	gross P_m^{cell}	--	--	0.55 ± 0.15	0.62 ± 0.14
900 nM	gross P_m^{Chl}	0.62 ± 0.08	0.63 ± 0.12	0.58 ± 0.08	0.53 ± 0.07
	R_d^{cell}	-0.21 ± 0.06	-0.15 ± 0.02	-0.15 ± 0.05	-0.15 ± 0.01
	gross P_m^{cell}	1.33 ± 0.18	1.24 ± 0.24	0.91 ± 0.23	0.70 ± 0.10

Fe-replete. The proportional decrease in yield and μ at 18 compared to 900 nM Fe was about the same for A2169, but less for WH7803, as in the previous experiment (Fig. 5).

Dark respiration and photosynthetic capacity were measured on four consecutive days for 900 nM Fe cultures and two or three days for 18 nM Fe cultures during batch 3. At 900 nM Fe, both species exhibited a large decrease in gross P_m^{cell} beginning on day 5 of batch 3, whereas P_m^{Chl} and R_d^{cell} decreased less markedly (Table 4), and R_d^{Chl} did not change (data not shown). Cell densities were too low at 18 nM Fe to measure O_2 exchange earlier in exponential growth. With a shorter time series, no trend was apparent in respiration and photosynthesis at 18 nM Fe (Table 4). Differences between P_m^{cell} at 18 and 900 nM Fe were greater early in exponential growth, at least for A2169 where three days can be compared. This supports the significant difference observed between P_m^{cell} in 18 and 900 nM Fe late exponential batch 2 cultures in the previous experiment (Table 1). The most obvious difference between the results of this experiment and the previous one is that A2169 R_d^{cell} in the batch 3 experiment was higher at 18 nM than at 900 nM Fe, as was the case in both experiments for WH7803.

Effects of iron on the P-I response to acute high light stress

To assess the sensitivity to photoinhibition among Fe treatments for both marine Synechococcus strains, batch 1 cultures were subjected for 30 min to 500 and 1000 μmol photons·m^{-2}·s^{-1} for WH7803 and A2169, respectively, before measuring the P-I curves. The low cell densities in the low Fe batch 2 cultures resulted in lower resolution of O_2 exchange measurements, so the effects of acute high light stress were tested using the less

severely Fe-stressed batch 1 cultures on days 5 or 6 (Fig. 4, filled symbols). The trend of O_2 exchange following transfer from darkness to high light (HL) is exemplified by A2169 grown in 90 nM Fe (Fig. 9). In the 30-min high light treatment, both Chl- and cell-specific net photosynthesis increased during the first 5 – 10 min, then declined.

Immediately after the HL exposure, P-I curves were measured. P-I curves with and without the HL exposure was exemplified by WH7803 grown in 18 nM Fe (Fig. 10). The R_d^{Chl} increased after the HL exposure. The photosynthetic parameters for all treatments are summarized in Table 5.

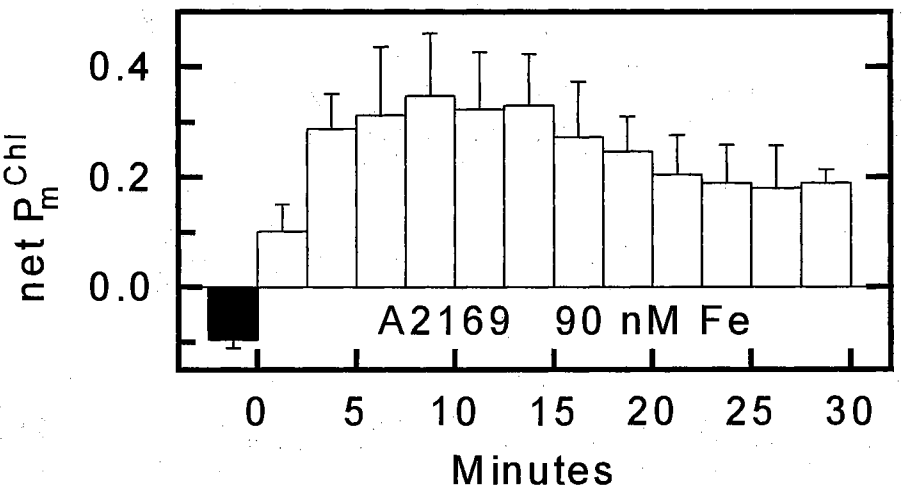

Fig. 9. Batch 1 Synechococcus A2169 grown in 90 nM Fe in Aquil: net photosynthetic performance following a dark to light (1000 μmol photons·m^{-2}·s^{-1}) transition at time 0 (mean ± SD, n = 4). Rates were calculated by linear regression at 2.5 min intervals through continuous digital O_2 data.

The difference of P_m^{Chl} among Fe concentration was not significant for both strains, with or without high light treatment. However, P_m^{Chl} of 90 and 900 nM Fe generally increased while P_m^{Chl} of 3.6 and 18 nM Fe did not change after high light treatment for both strains (Table 5). Compared to A2169, WH7803 had higher P_m^{Chl} at each Fe level with or without high light treatment. There was a trend of slightly increasing initial slope α (data not shown) with decreasing Fe but the change was not statistically significant, and the results were very variable.

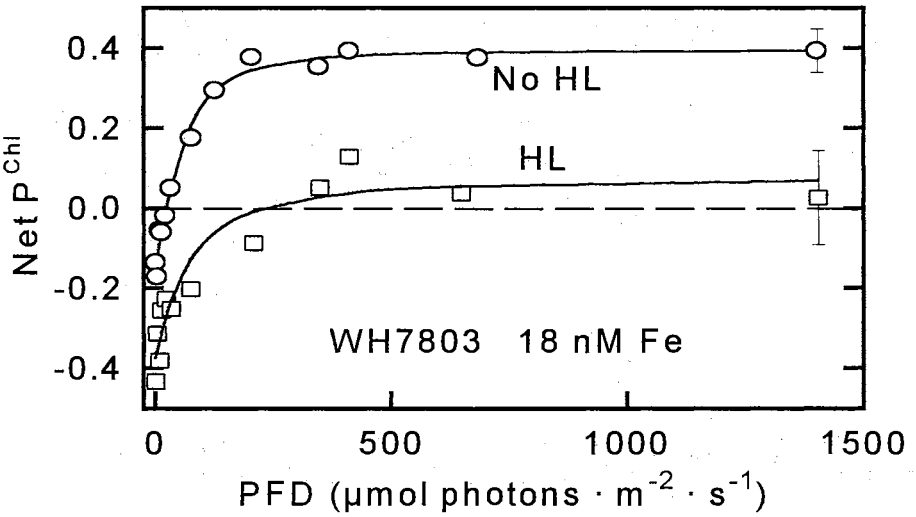

Fig. 10. Batch 1 Synechococcus WH7803 grown in 18 nM Fe in Aquil: photosynthesis-irradiance curves with (HL) and without (No HL) prior exposure to 30 min at 500 µmol photons·m^{-2}·s^{-1}. Means of 3 cultures; individual error bars are omitted for clarity, but the average SD is shown at the highest PFD for each curve. Results of such experiments for all treatments are summarized in Table 5.

Table 5. Gross photosynthetic capacity and dark respiration (mol $O_2 \cdot g^{-1}Chl \cdot h^{-1}$) of Synechococcus in late exponential batch 1 cultures (day 5 or 6; filled symbols in Fig. 4) with or without exposure to 500 (WH7803) or 1000 (A2169) μmol photons·m^{-2}·s^{-1} for 30 min prior to measurement. Values are means ± SD of 3 – 4 cultures.

	Control		High light	
nM Fe	gross P_m^{Chl}	R_d^{Chl}	Gross P_m^{Chl}	R_d^{Chl}
		A2169		
3.6	0.37 ± 0.12	-0.13 ± 0.07	0.36 ± 0.12	-0.19 ± 0.04
18	0.23 ± 0.02	-0.08 ± 0.01	0.43 ± 0.15	-0.18 ± 0.04
90	0.35 ± 0.02	-0.06 ± 0.01	0.48 ± 0.19	-0.07 ± 0.01
900	0.29 ± 0.05	-0.04 ± 0.01	0.47 ± 0.05	-0.04 ± 0.01
		WH7803		
3.6	0.47 ± 0.02	-0.09 ± 0.02	0.46 ± 0.12	-0.34 ± 0.04
18	0.52 ± 0.11	-0.14 ± 0.04	0.45 ± 0.14	-0.37 ± 0.05
90	0.51 ± 0.05	-0.06 ± 0.02	0.64 ± 0.05	-0.13 ± 0.04
900	0.42 ± 0.11	-0.04 ± 0.02	0.71 ± 0.02	-0.06 ± 0.02

Without the high light treatment, R_d^{Chl} at 3.6 nM was 3.8- and 3.4-fold higher than that of 900 nM cells, for A2169 and WH7803, respectively. R_d^{Chl} at 3.6 and 18 nM Fe increased significantly with high light treatment, enlarging the difference of R_d^{Chl} among Fe concentrations to 4.5- and 6.5-fold for A2169 and WH7803, respectively, still with the lowest values at 900 nM and highest values at 3.6 nM (A2169) or 18 nM Fe (WH7803). The interesting point is that, while R_d^{Chl} of WH7803 at each Fe concentration was similar to that of A2169 before high light treatment, R_d^{Chl} of WH7803 were much higher than those of A2169 after high light treatment, especially at 3.6 and 18 nM Fe.

The effects of high light treatment on Fe concentrations are more apparent in the R_d^{cell} : gross P_m^{cell} ratios (Fig. 11). The R_d^{Chl} : gross P_m^{Chl} ratios should change by the same proportion because Chl content per cell did not change (data not shown). Before the high light treatment, the R_d^{cell} : gross P_m^{cell} ratios in A2169 spanned 3.1- and 2.8-fold ranges for A2169 and WH7803, respectively, with highest values at 3.6 or 18 nM Fe, and lowest values at 900 nM Fe. After 30 min high light treatment, the difference of R_d^{cell} : gross P_m^{cell} ratios among Fe concentrations increased to 5.9- and 9.1-fold for A2169 and WH7803, respectively. Compared to the data without high light treatment, the R_d^{cell} : gross P_m^{cell} ratios for both strains at 3.6 and 18 nM Fe exhibited a significant increase after high light treatment, whereas the R_d^{cell} : gross P_m^{cell} ratios for 90 and 900 nM Fe generally did not change for both strains.

In summary, gross P_m^{Chl} in the low Fe cultures was unaffected by a 30 min high light exposure: 500 or 1000 μmol photons·m^{-2}·s^{-1} for WH7803 and A2169, respectively.

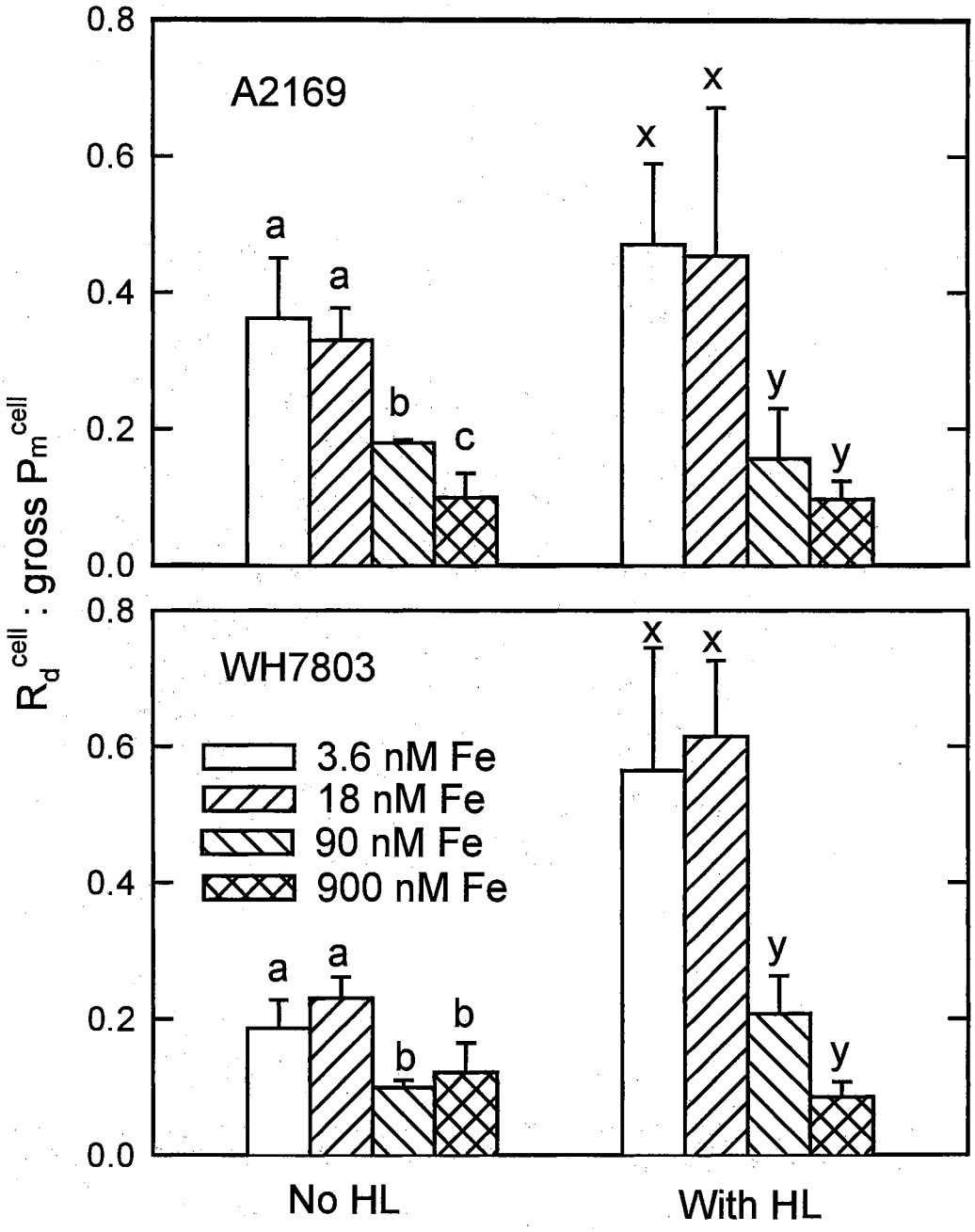

Fig. 11. R_d^{cell} : gross P_m^{cell} ratios of Synechococcus in late exponential batch 1 cultures (day 5 or 6; filled symbols in Fig. 4) with or without 30 min exposure to 500 (WH7803) or 1000 (A2169) μmol photons·m^{-2}·s^{-1} prior to measurement. Values are means ± SD. For each species and light treatment, Fe concentration having different letters differ significantly ($p < 0.05$, $n = 3$-4).

Gross P_m^{Chl} was slightly higher after high light exposure in high Fe cultures, especially strain WH7803, presumably indicating photosynthetic induction, i.e. Calvin cycle enzymes were not fully activated at the growth PFD, or partial deactivation occurred during the 5 – 10 min in darkness at the beginning of the P-I curve. In contrast, R_d^{Chl} was unaffected by HL exposure in high Fe cultures, but increased two- to four-fold in low Fe cultures, for example WH7803 at 18 nM Fe (Fig. 10). Thus, the difference in respiration, as well as R_d : gross P_m ratio, between low and high Fe cultures increased even further following high light stress. As in batch 2 cultures, α^{cell} was in some cases highly variable and did not appear to be affected by high light.

Semi-continuous culture experiments

Iron-limitation of cell density and Chl content

Semi-continuous Synechococcus cultures were equilibrated to dilution (growth) rates of 0.29, 0.43, and 0.69 d^{-1} at 50 μmol photons·m^{-2}·s^{-1} and at 100 μmol photons·m^{-2}·s^{-1} (0.43 d^{-1}-hl). Daily dilution of 25, 35, or 50% volume began on day 5 and continued 9, 7, and 5 days for growth rates of 0.29, 0.43, and 0.69 d^{-1}, respectively. Balanced cultures were confirmed by monitoring absorbance daily at 750 nm for several days before physiological experiment. Day-to-day variations in cell density was exemplified by WH7803 at 0.29 d^{-1} (Fig. 12). For low Fe WH7803 at 0.69 d^{-1}, cells

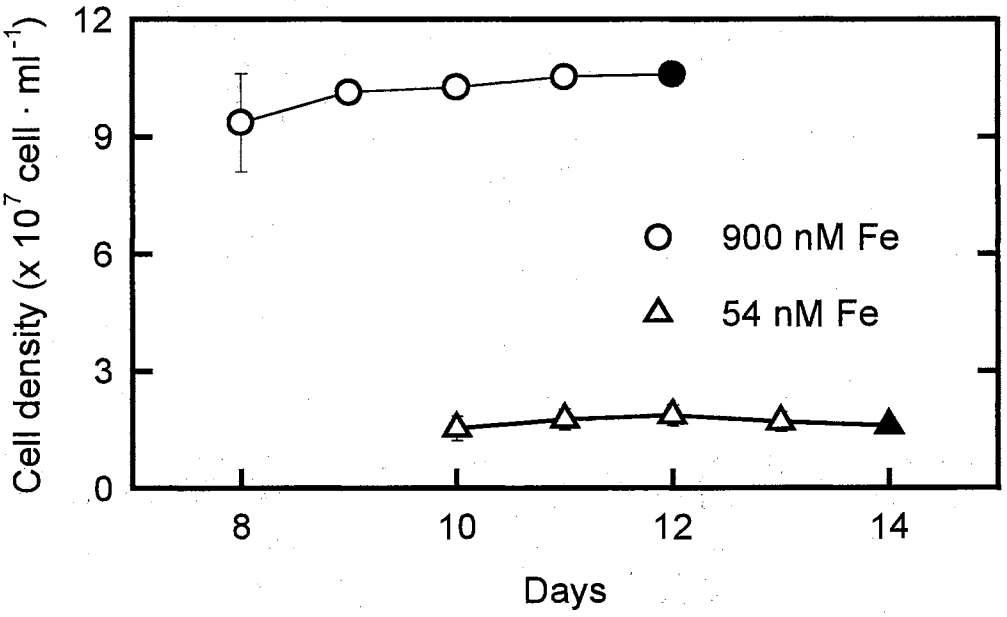

Fig. 12. Change of cell density of semi-continuous Synechococcus WH7803 grown at growth rate of 0.29 d^{-1} at 50 μmol photons·m^{-2}·s^{-1}. Daily dilution of 25% volume began on day 5 and continued 9 days for 54 nM Fe cultures. Filled symbols correspond to samples for physiological data in Figs. 13-21. In most cases, the SD was smaller than the symbol size.

could only be kept at the growth rate for a few days before being washed out. In all cases, the day-to-day variations in cell density were less than 15 % in the last three days prior to physiological experiment.

The cell density and Chl concentration of equilibrium semi-continuous cultures are shown in Fig. 13. Iron concentration significantly affected cell density and Chl content of both strains at all tested growth rates ($p<0.05$, $n = 3$-4), except for Chl content of WH7803 at 0.29 and 0.69 d^{-1}. There was no obvious relationship among cell density and Chl of different growth rates. For Fe-limited cells of A2169, Chl concentration decreased more than the cell density, so that Chl content per cell was lower than corresponding Fe-replete cells at the same growth rate (Fig. 13a, b). Chl content of A2169 was significantly different between Fe treatment at each growth rate. Low Fe availability in the medium limited the production of Chl. Chl content of WH7803 was more variable than that of A2169: it was lower in 0.29 and 0.69 d^{-1} but higher in 0.43 d^{-1} Fe-limited cells, comparing to corresponding Fe-replete cells. Growth under higher light (100 μmol photons$\cdot m^{-2}\cdot s^{-1}$; 0.43 d^{-1}, hl) resulted in lower Chl content in Fe-replete cells but not in Fe-limited cells for both strains.

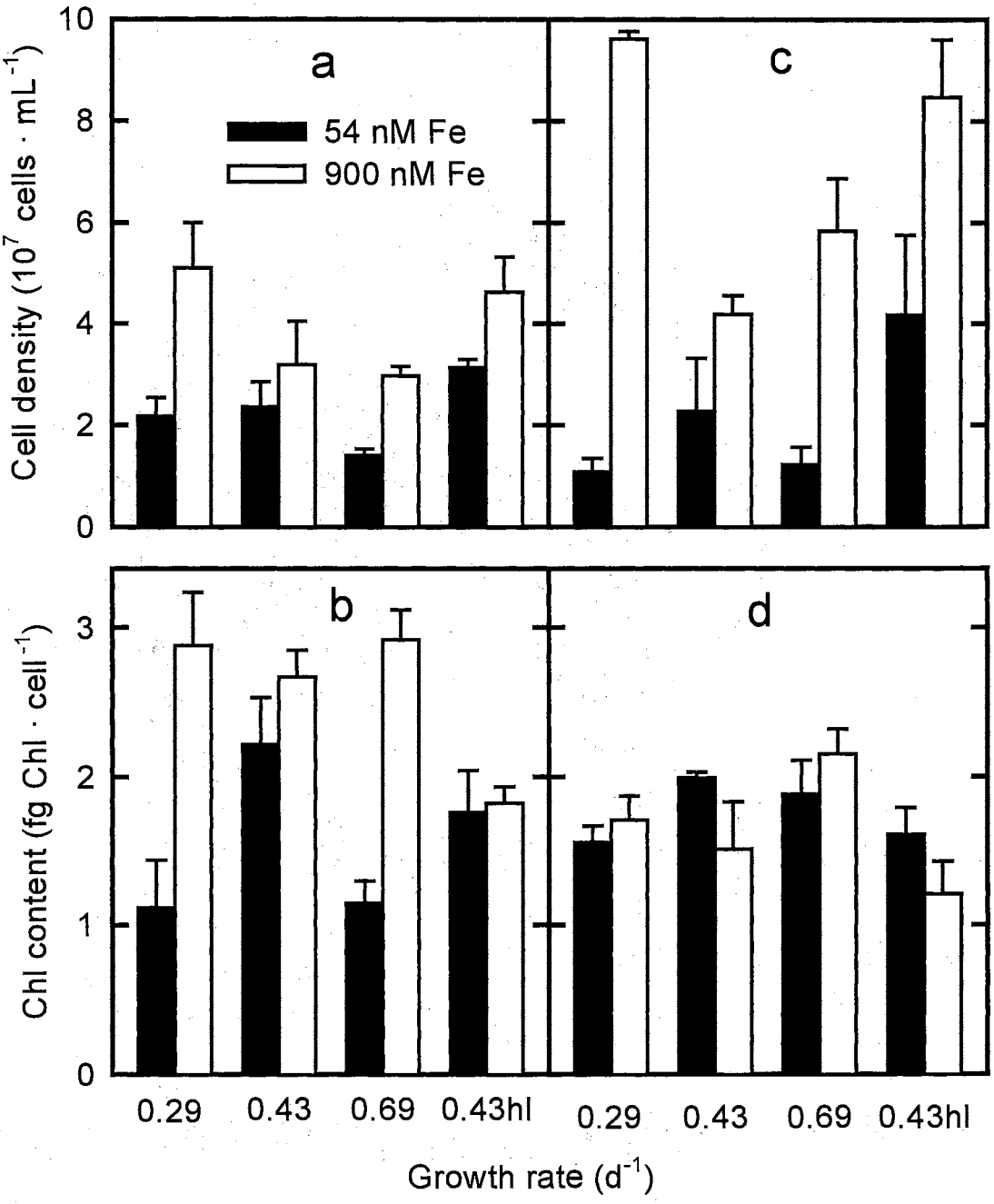

Fig. 13. Cell density (upper) and Chl content (lower) during semi-continuous growth of Synechococcus A2169 (left) and WH7803 (right) in Aquil at various growth rates; mean ± SD, n = 3-5. Panel a, c are cell density, panel b, d are Chl content. Cultures were grown at 50 µmol photons·m^{-2}·s^{-1}, except for 0.43hl, which was 0.43 d^{-1} cultures grown under 100 µmol photons·m^{-2}·s^{-1}.

Iron stress causes a peak shift in absorption spectrum

The room temperature absorption spectra of equilibrated semi-continuous Synechococcus cultures are exemplified by cultures at 0.43 d^{-1} growth rate (Fig. 14). Chlorophyll (Chl) had an absorption peak in the red region at about 678 nm and another absorption peak at about 435 nm, and phycoerythrin (PE) had an absorption peak at about 545 nm. The absorption spectra at other growth rates were similar (not shown). The PE : Chl ratio was higher in Fe-limited cultures in both strains.

The absorption λ_{max} of Chl and PE of all tested growth rates are presented in Table 6. In semi-continuous A2169 at each growth rate, Chl λ_{max} of different iron treatments were significantly different ($p<0.05$, n = 3-5; Tukey multiple comparison). The λ_{max} of low Fe cultures exhibited a blue shift > 3 nm. Interestingly, WH7803 showed no such blue shift of Chl λ_{max} at all tested growth rates.

However, PE λ_{max} was independent of iron concentration for both strains, except for 0.43 d^{-1} A2169. At each Fe concentration, growth rate did not significantly affect Chl and PE λ_{max}.

Based on the absorption ratio of PE to Chl ($A_{\lambda PE} : A_{\lambda Chl}$), the PE:Chl ratio of Fe-limited cells was higher than that of corresponding Fe-replete cells for both strains (Table 7). Grown under higher light further decreased the ratio regardless of Fe treatments. The results implied that Chl decreases more that PE under Fe stress and high light. Alternatively, the decrease of pigment content per cell may cause a relative increase in scattering that nonspecifically increases apparent absorption at shorter λ.

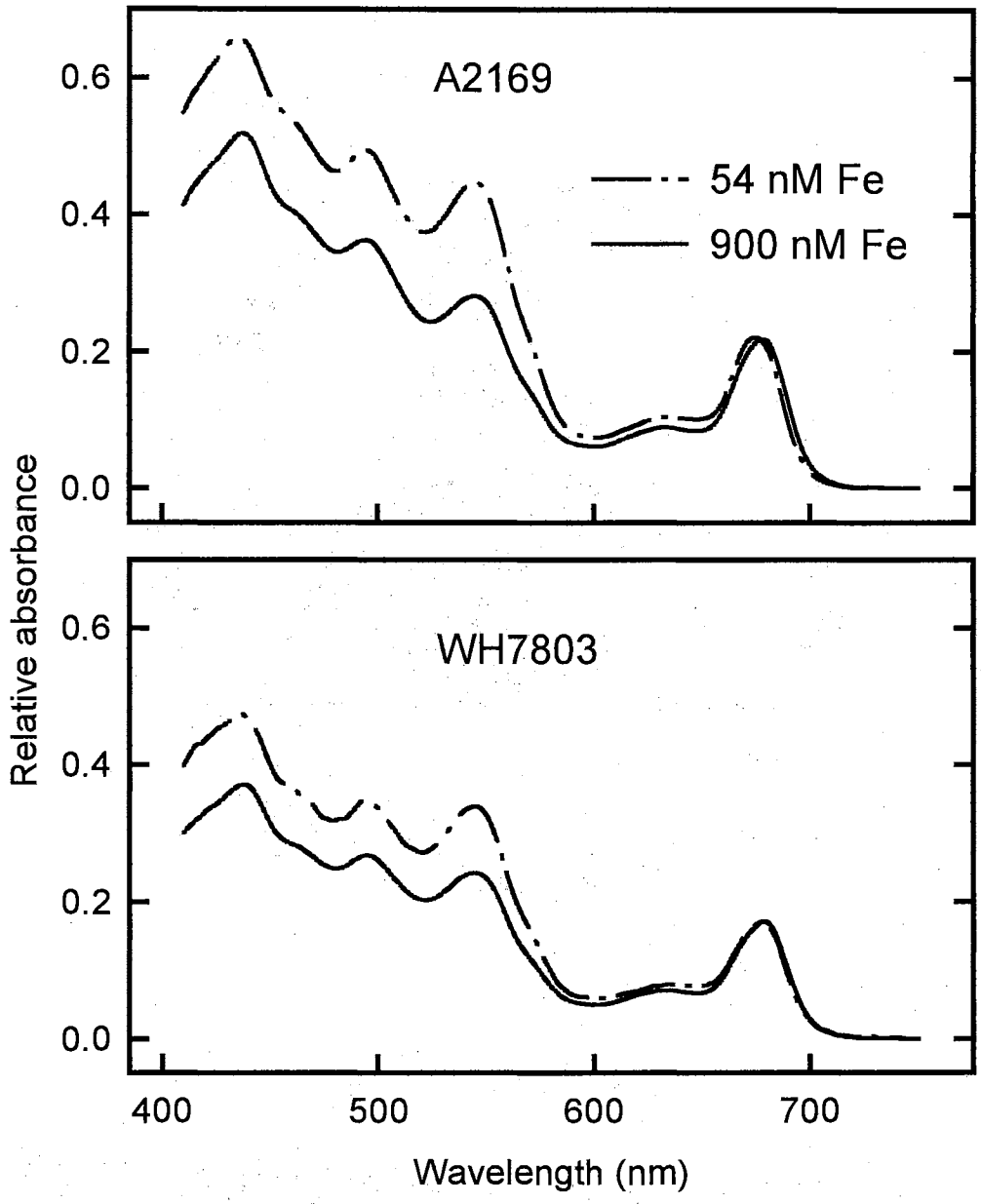

Fig. 14. In vivo (whole cell) absorption spectra of Synechococcus A2169 and WH7803 in semi-continuous cultures at 0.43 d^{-1}. Absorption peaks at about 435 and 678 nm are due to Chl a. The peaks at about 550 and 500 nm are due to phycoerythrobilin and phycourobilin, respectively. Spectra of 54 nM Fe cultures have been normalized to 900 nM Fe cultures at Chl peak; n = 3. Cells collected on GF/F filter were scanned against a blank filter to correct for scattering.

Table 6. Chlorophyll λ_{max} and phycoerythrin λ_{max} (nm) of Synechococcus A2169 and WH7803 in semi-continuous cultures. Values are means ± SD of 3-4 cultures. Cells collected on GF/F filter were scanned against a blank filter to correct for scattering.

		A2169		WH7803	
μ	nM Fe	PE λ_{max}	Chl λ_{max}	PE λ_{max}	Chl λ_{max}
0.29 d^{-1}	54	545.2±0.8	674.7±0.8	544.7±1.0	678.8±0.8
	900	546.2±0.6	678.2±0.3	545.7±0.6	678.5±0.0
0.43 d^{-1}	54	543.6±3.4	675.8±1.5	545.6±0.8	678.3±1.2
	900	546.0±0.0	678.9±0.2	545.5±0.0	678.8±0.3
0.69 d^{-1}	54	546.0±0.4	675.4±1.8	543.3±5.1	678.2±2.4
	900	546.2±0.3	679.0±0.0	545.0±0.9	679.0±0.0
0.43 d^{-1}, hl	54	544.3±0.6	676.3±0.3	545.2±0.3	677.7±0.8
	900	544.3±1.2	678.0±0.0	544.6±0.5	678.8±0.3

Table 7. $A_{\lambda PE} : A_{\lambda Chl}$ ratio of marine Synechococcus A2169 and WH7803 in semi-continuous cultures. Values are means ± SD of 3-4 cultures.

nM Fe	0.29 d^{-1}	0.43 d^{-1}	0.69 d^{-1}	0.43 d^{-1}-hl
		A2169		
54	2.14±0.18	1.83±0.21	2.21±0.16	1.63±0.29
900	1.48±0.11	1.55±0.17	1.97±0.02	1.16±0.10
		WH7803		
54	1.69±0.23	2.48±0.66	2.48±0.23	1.71±0.65
900	1.51±0.06	1.62±0.05	1.63±0.02	1.26±0.04

Effects of iron-stress and acute high light stress on photosynthesis and respiration

P-I curves with or without acute high light stress, as well as the trend of O_2 exchange during high light treatment is exemplified by WH7803 grown at 0.29 d^{-1} (Fig. 15). Controls (left panels) were measured after 5 min dark-adaptation. Fe-limited cells had lower net photosynthesis but higher dark respiration than Fe-replete cells. When cells were exposed to high light (middle panels), photosynthetic induction occurred during the first 10 min, then net photosynthesis declined, as in batch cultures (Fig. 9). P-I curves (Fig. 15, right panels) were measured immediately after the high light exposure to assess photoinhibition. Results for both strains at several growth rates are summarized in Figs. 16-21.

For both strains A2169 and WH7803, cells from Fe-replete cultures demonstrated higher gross P_m^{cell} in comparison to Fe-limited cultures at the same growth rate, except for A2169 at 0.43 d^{-1} (Fig. 16). There was not significant interaction between Fe concentrations and growth rates ($p > 0.05$, two-way ANOVA). Exposure to high light for 30 min did not obviously affect gross P_m^{cell}. At 0.43 d^{-1}, cells grown under higher light (0.43 d^{-1}-hl) exhibited decreased gross P_m^{cell} for both strains regardless Fe concentration.

Fe limitation affected gross P_m^{Chl} differently in the two strains (Fig. 17). Gross P_m^{Chl} of Fe-limited A2169 cells was either slightly (but non-significantly) higher (0.43 d^{-1}) or significantly higher (0.29 and 0.69 d^{-1}) than corresponding Fe-replete cells. In contrast, gross P_m^{Chl} of Fe-limited WH7803 cells was either significantly lower (0.43 d^{-1} and 0.69 d^{-1}) or not different (0.29 d^{-1}) than that of Fe-replete cells. Exposure to high light for 30 min did not significantly affect gross P_m^{Chl} for both strains ($p > 0.05$, two-

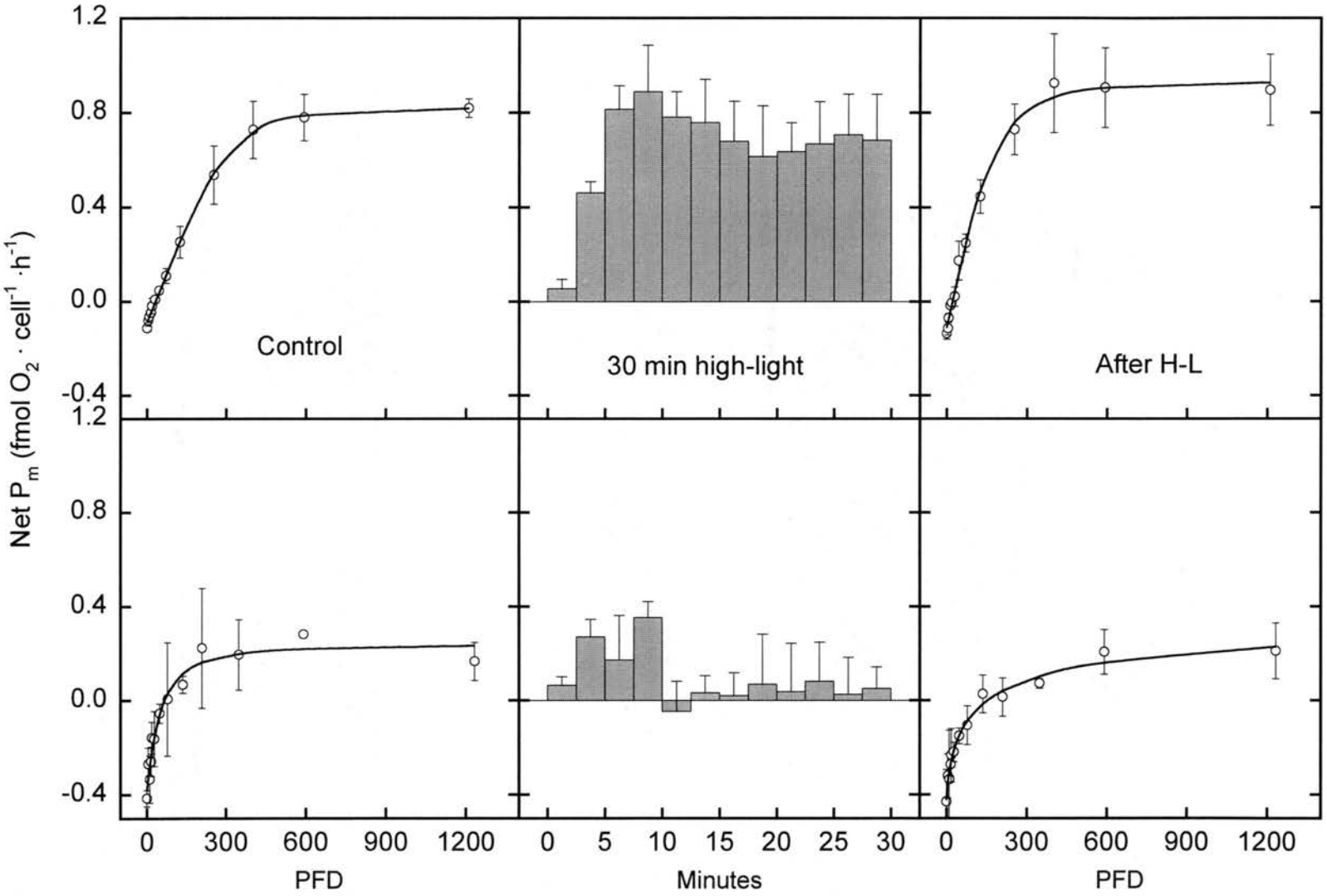

Fig. 15. Semi-continuous Synechococcus WH7803 at 900 nM (upper) and 54 nM Fe (lower) grown at 0.29d^{-1} : photosynthesis-irradiance curves with (right) and without (left) prior exposure to 30 min at 1000 µmol photons$m^{-2}s^{-1}$. Middle panels show net photosynthetic performance during the exposure to high light. Mean±SD, n=3.

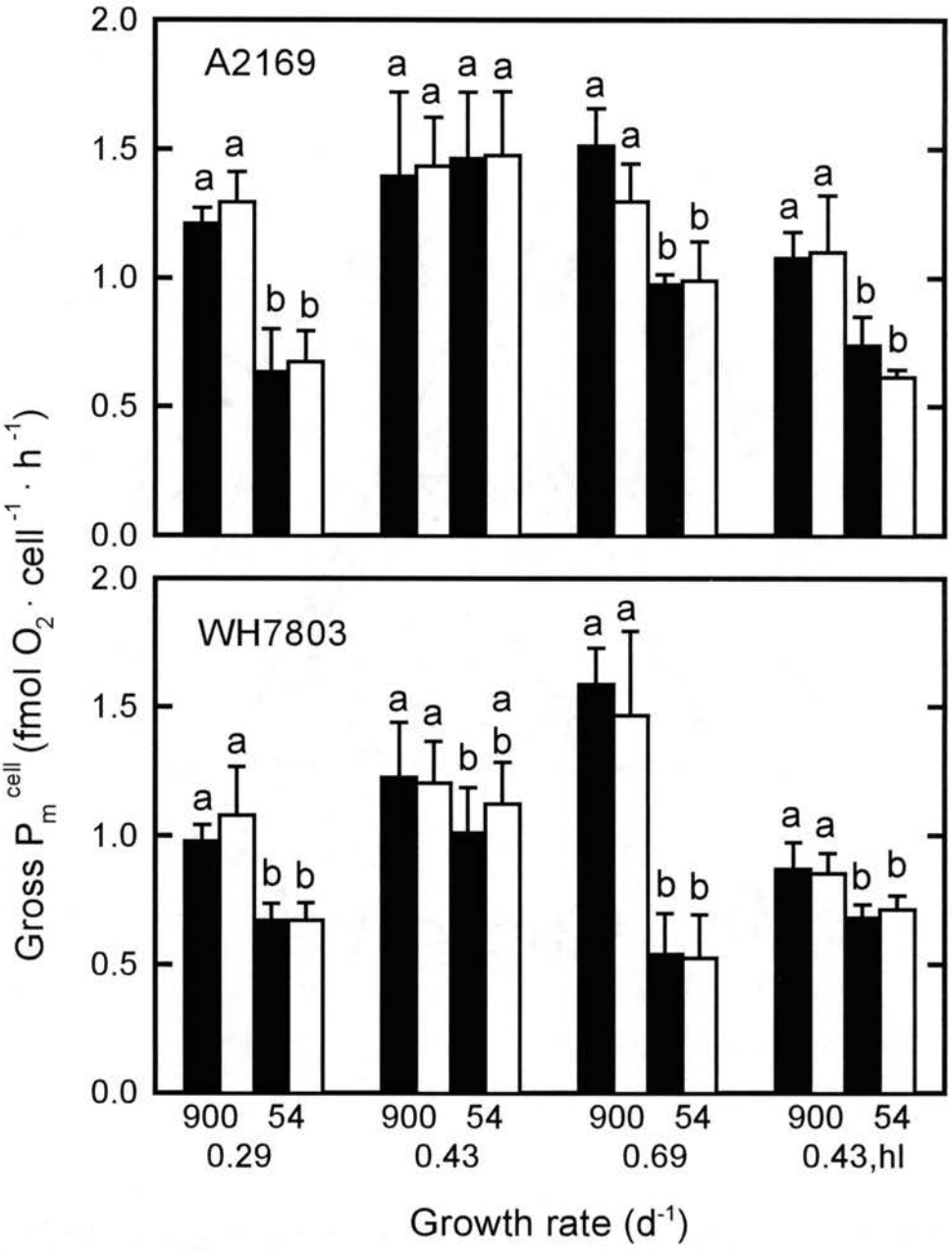

Fig. 16. Cell normalized gross photosynthetic capacity (fmol $O_2 \cdot cell^{-1} \cdot h^{-1}$) of A2169 and WH7803 in semi-continuous cultures with (white bar) or without (black bar) exposure to 1000 µmol photons·m^{-2}·s^{-1} prior to measurement; mean ± SD, n = 3-4. Within each growth rate, the left two bars are 900 nM Fe cultures, the right two bars are 54 nM Fe cultures, and treatment having different letters differ significantly ($p < 0.05$; Tukey multiple comparison).

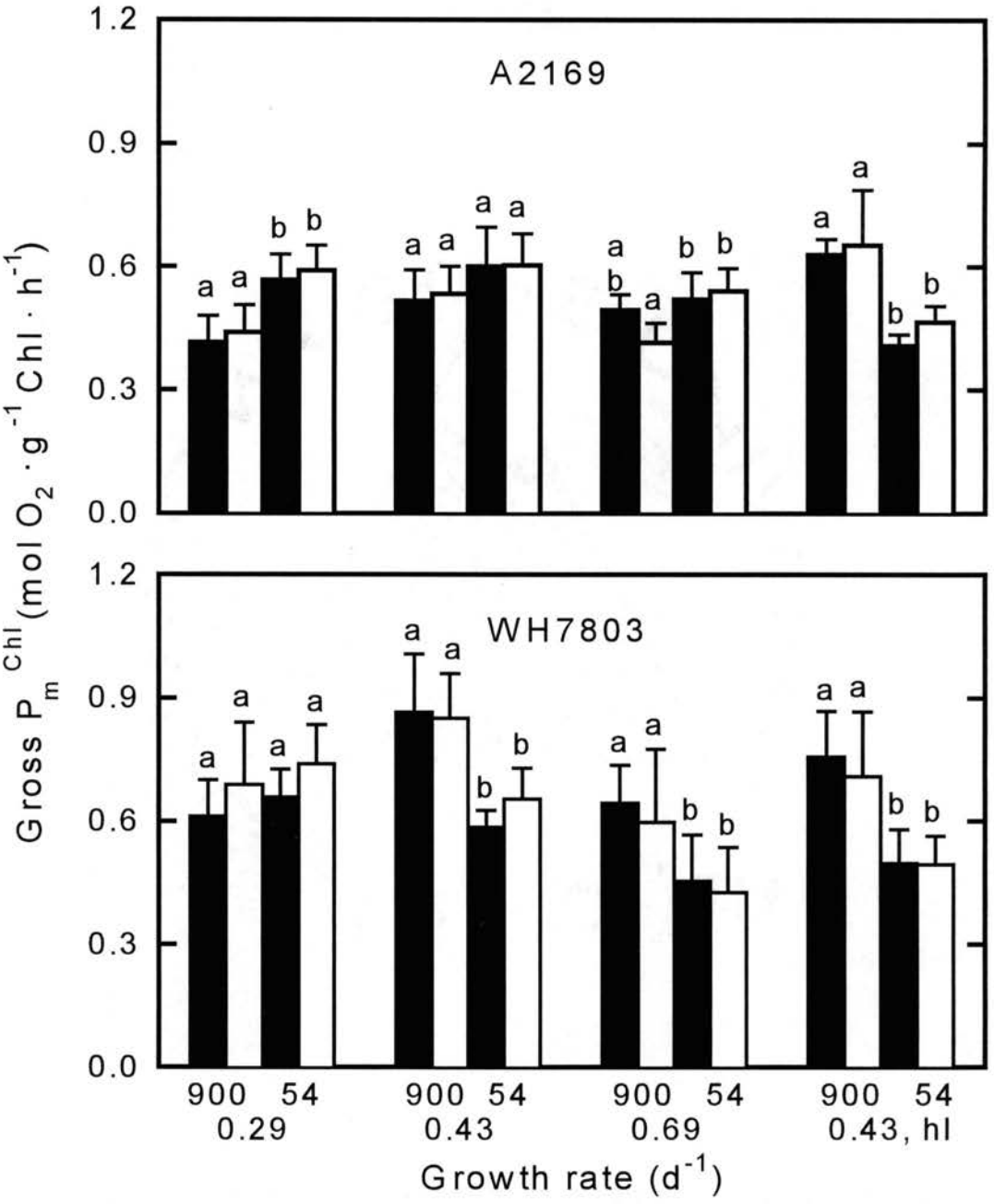

Fig. 17. Chl normalized gross photosynthetic capacity (mol $O_2 \cdot g^{-1}$Chl $\cdot h^{-1}$) of A2169 and WH7803 in semi-continuous cultures with (white bar) or without (black bar) exposure to 1000 μmol photons·m^{-2}·s^{-1} prior to measurement; mean ± SD, n = 3-4. Within each growth rate, the left two bars are 900 nM Fe cultures, the right two bars are 54 nM Fe cultures, and treatment having different letters differ significantly ($p < 0.05$; Tukey multiple comparison).

way ANOVA). At 0.43 d^{-1}, growth under higher light (0.43 d^{-1} hl) significantly decreased gross P_m^{Chl} of Fe-limited but not Fe-replete A2169 cells, and slightly decreased gross P_m^{Chl} of both Fe-limited and Fe-replete WH7803 cells.

As in batch cultures (Table 2), the cell normalized initial slope (α^{cell}) was quite variable (Fig 18). α^{cell} of Fe-limited cells were not significantly different from corresponding Fe-replete cells for all tested growth rates of both strains, except 0.29 d^{-1} WH7803. Exposure to high light for 30 min significantly reduced α^{cell} of Fe-replete cells of A2169 at 0.43 d^{-1}, and WH7803 at 0.43 d^{-1} and 0.69 d^{-1}. At 0.43 d^{-1} to, cells grown under high light (0.43 d^{-1}-hl) had lower α^{cell} for both strains. However, the difference was not statistically significant.

As with α^{cell}, Chl normalized initial slope (α^{Chl}) was quite variable (Fig 19). α^{Chl} of Fe-limited cells was either higher (0.29 and 0.69 d^{-1}) or lower (0.43 d^{-1}) than Fe-replete cells for both strains. Exposure to high light for 30 min did not significantly affect α^{Chl}, except for WH7803 at 0.43 d^{-1}. At 0.43 d^{-1}, cells grown under high light (0.43 d^{-1}-hl) had slightly (but non-significant) lower α^{Chl} for both strains, and Fe-replete cells were affected more than Fe-limited cells.

R_d^{cell} was significantly related to Fe concentrations for both strains at all tested growth rates, except for 0.43 d^{-1} (both strains) and 0.43 d^{-1}–hl (A2169 only; Fig. 20). In these two exceptional cases, R_d^{cell} was still higher in Fe-limited cells, but not significantly. Compared to Fe-replete cells at the same growth rate, R_d^{cell} of Fe limited cells was about 2.3- and 1.6-fold higher at 0.29 and 0.69 d^{-1} A2169, and 3.5- and 1.9-fold higher at 0.29 and 0.69 d^{-1} WH7803, respectively. Exposure to high light treatment for

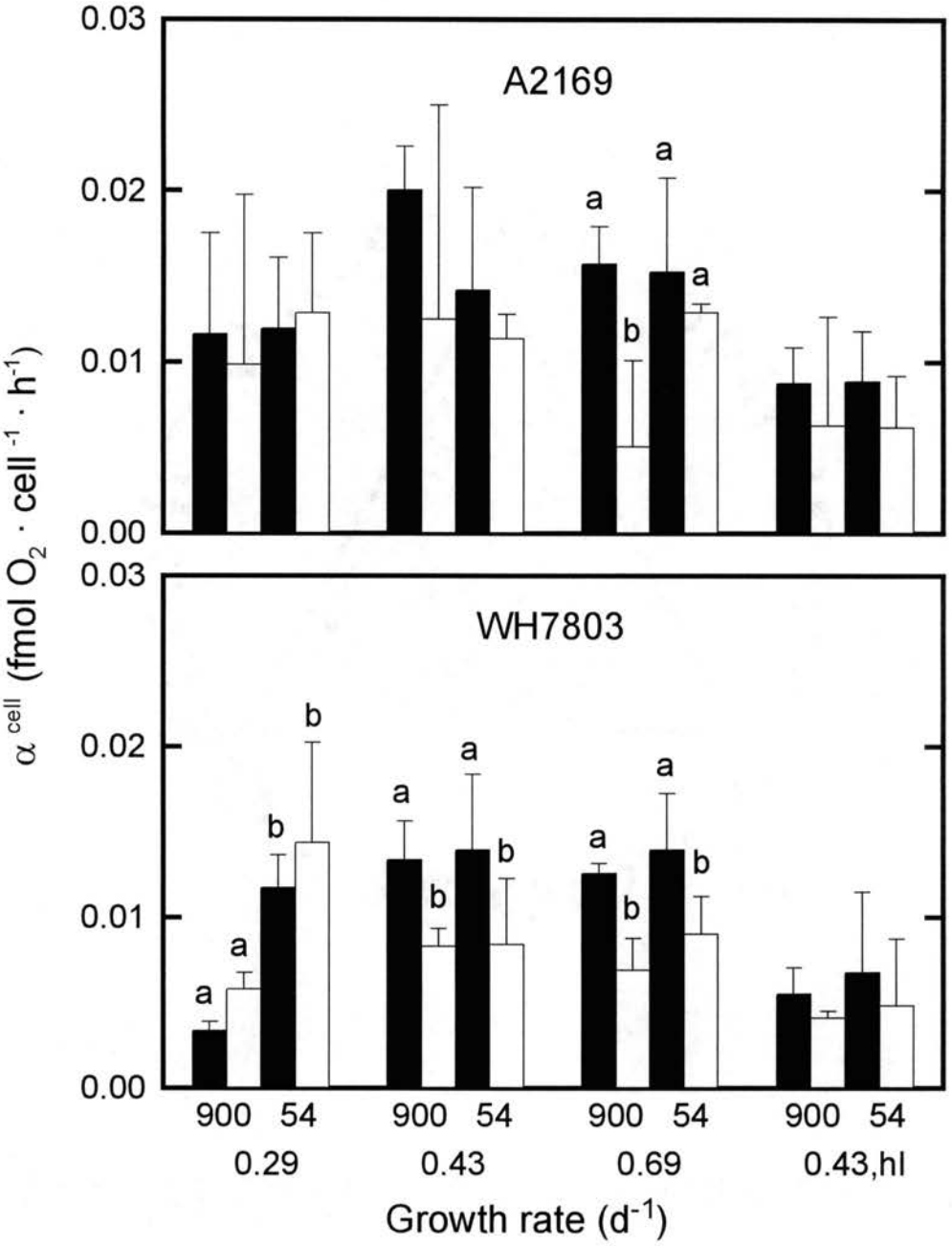

Fig. 18. Cell normalized initial slope α (fmol $O_2 \cdot cell^{-1} \cdot h^{-1}$) of A2169 and WH7803 in semi-continuous cultures with (white bar) or without (black bar) exposure to 1000 μmol photons$\cdot m^{-2} \cdot s^{-1}$ prior to measurement; mean, n = 3-4. Within each growth rate, the left two bars are 900 nM Fe cultures, the right two bars are 54 nM Fe cultures, and treatment having different letters differ significantly ($p < 0.05$; Tukey multiple comparison). No letter means no difference in the four values for that growth rate.

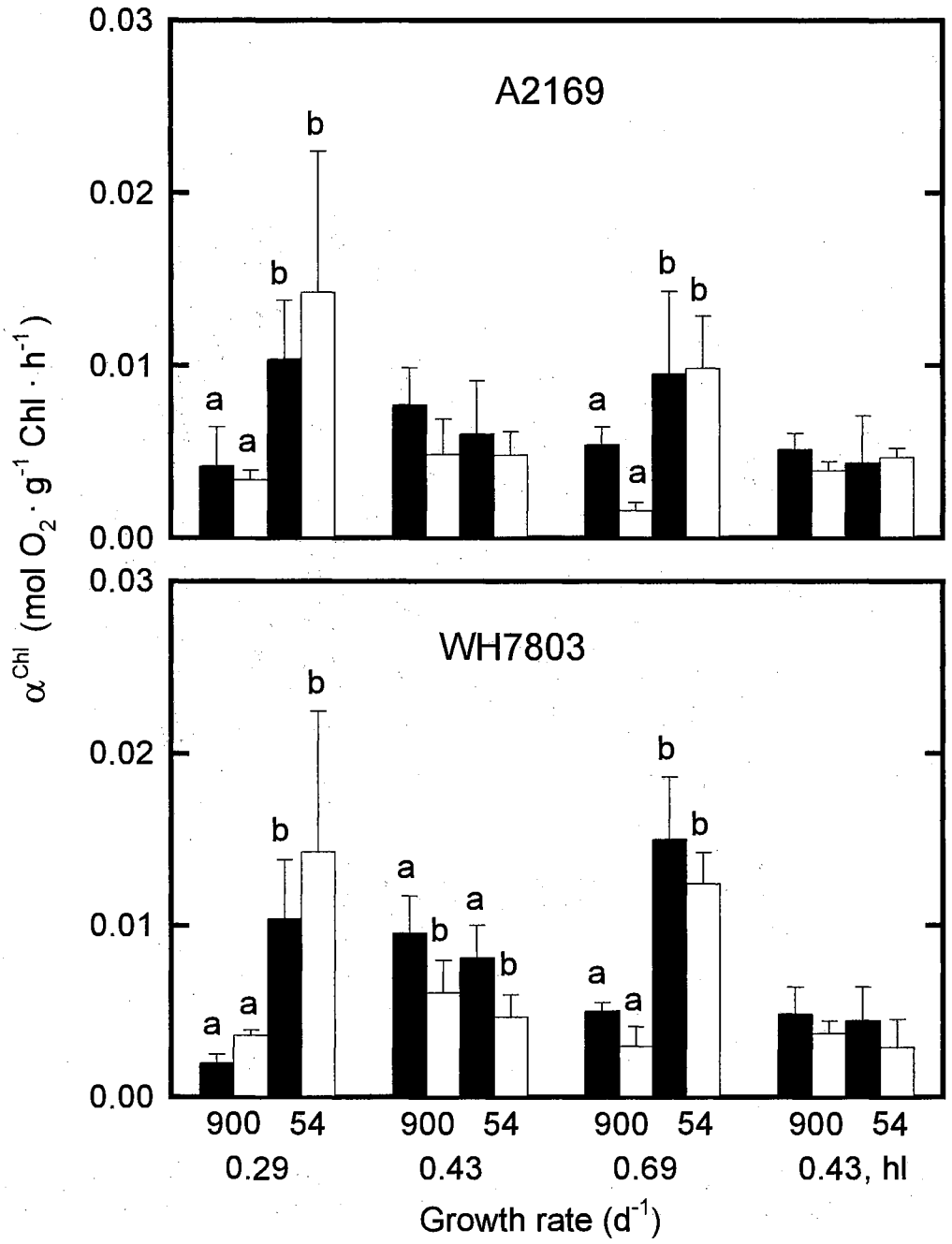

Fig. 19. Chl normalized initial slope α (mol $O_2 \cdot g^{-1}$Chl $\cdot h^{-1}$) of A2169 and WH7803 in semi-continuous cultures with (white bar) or without (black bar) exposure to 30 min 1000 μmol photons·m^{-2}·s^{-1} prior to measurement; mean ± SD, n = 3-4. Within each growth rate, the left two bars are 900 nM Fe cultures, the right two bars are 54 nM Fe cultures, and treatment having different letters differ significantly ($p < 0.05$; Tukey multiple comparison). Treatment without letters are not significantly different for that growth rate.

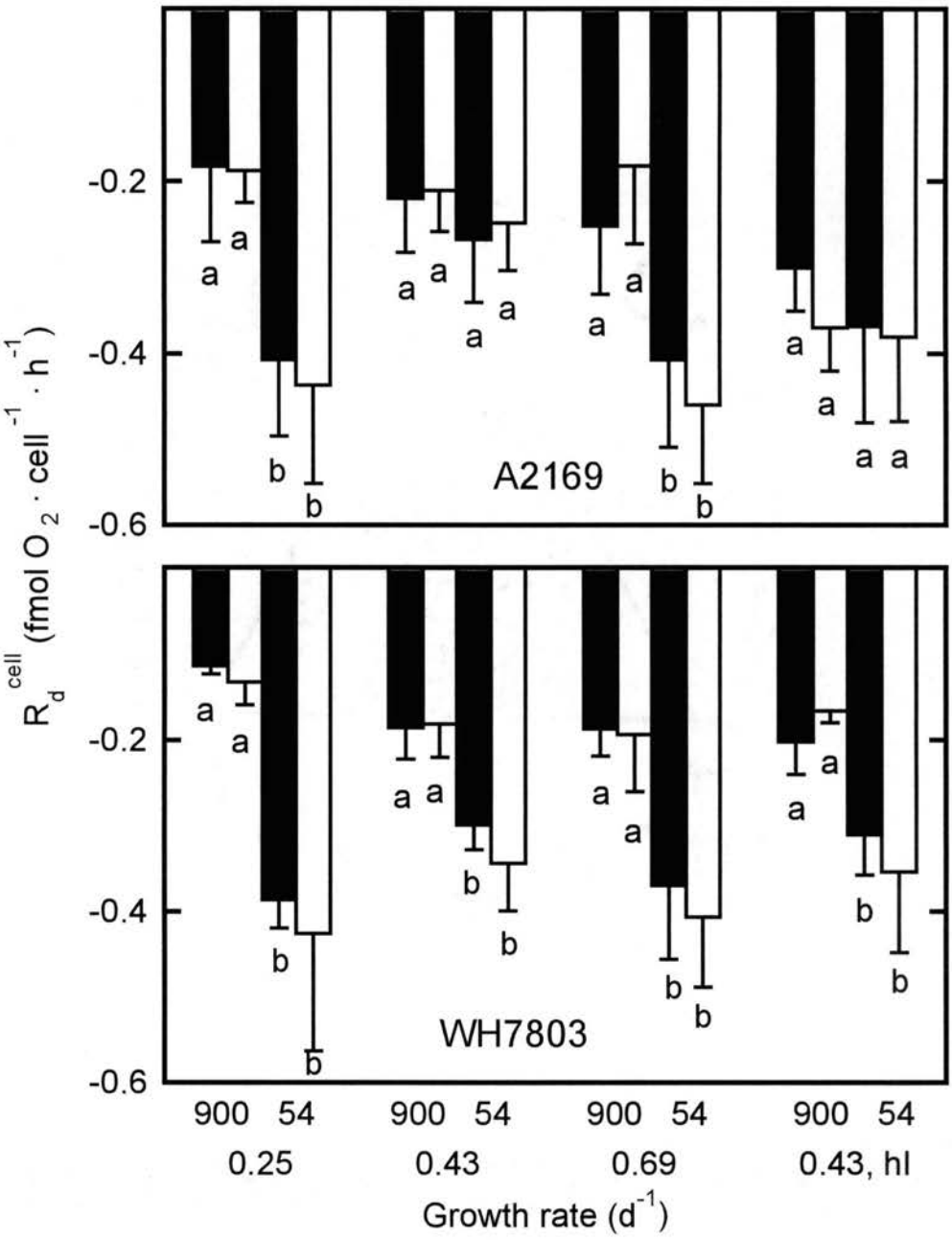

Fig. 20. Cell normalized dark respiration (fmol O_2 · cell^{-1} · h^{-1}) of A2169 and WH7803 in semi-continuous cultures with (white bar) or without (black bar) exposure to 1000 µmol photons·m^{-2}·s^{-1} prior to measurement; mean ± SD, n = 3-4. Within each growth rate, the left two bars are 900 nM Fe cultures, the right two bars are 54 nM Fe cultures, and treatments having different letters differ significantly ($p < 0.05$; Tukey multiple comparison).

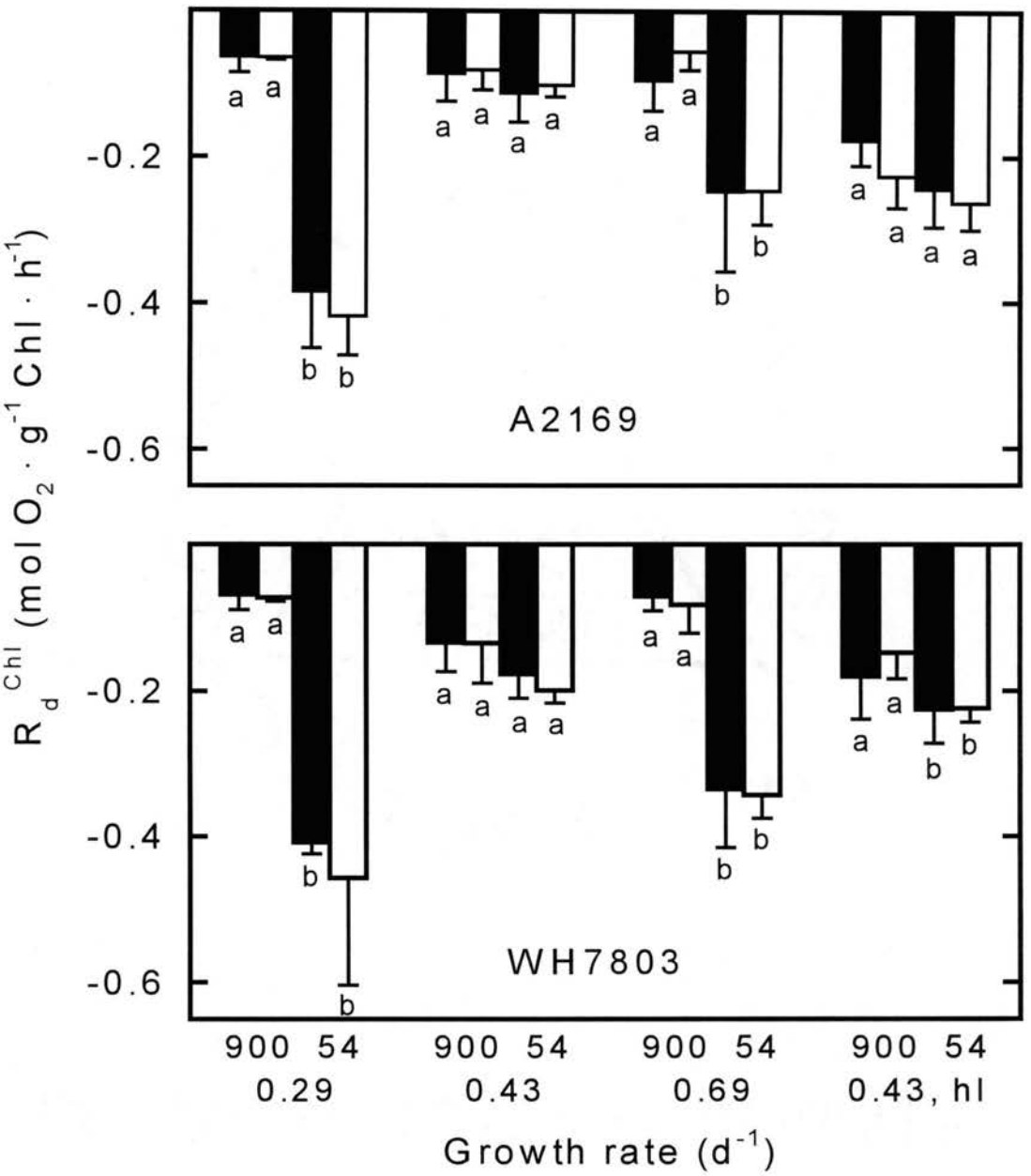

Fig. 21. Chl normalized dark respiration (mol $O_2 \cdot g^{-1}$Chl $\cdot h^{-1}$) of A2169 and WH7803 in semi-continuous cultures with (white bar) or without (black bar) exposure to 1000 µmol photons·m^{-2}·s^{-1} prior to measurement; mean ± SD, n = 3-4. Within each growth rate, the left two bars are 900 nM Fe cultures, the right two bars are 54 nM Fe cultures, and treatments having different letters differ significantly ($p < 0.05$; Tukey multiple comparison).

30 min resulted in slight (but non-significant) increase in R_d^{cell} of Fe-limited cells of both strains at all growth rates, except for 0.43 d^{-1} A2169. At 0.43 d^{-1}, growth under higher light (0.43 d^{-1}–hl) did not significantly affect R_d^{cell}.

Chl normalized dark respiration (R_d^{Chl}) was higher at low Fe concentrations for both strains, but the difference was only significant at 0.29 and 0.69 d^{-1} for both strains (Fig. 21). Compared to Fe-replete cells at the same growth rate, R_d^{Chl} of Fe-limited cells was about 6.3- and 2.8-fold higher for A2169 at 0.29 and 0.69 d^{-1}, and 5.9- and 4.9-fold higher for WH7803 at 0.29 and 0.69 d^{-1}. Even at 0.43 d^{-1} for both strains, R_d^{Chl} was still higher in Fe-limited cells, but not significantly. Exposure to high light for 30 min did not significantly affect R_d^{Chl} of Fe-limited cells of both strains at all growth rates. At 0.43 d^{-1}, growth under higher light (0.43 d^{-1}–hl) increased R_d^{Chl} significantly in Fe-limited WH7803, but only slightly in Fe-limited A2169.

Because R_d^{cell} increased while gross P_m^{cell} decreased at low Fe concentrations, Fe-replete cultures had higher net P_m^{cell} than corresponding Fe-limited cultures for both strains at all tested growth rates, except for A2169 at 0.43 d^{-1} (Table 8). Net P_m^{cell} of Fe treatments at the same growth rate differed by 1.2- to 4.5-fold for A2169, and by 1.6- to 8.2-fold for WH7803. For 0.29 d^{-1} A2169 and 0.29 and 0.69 d^{-1} WH7803, R_d^{cell} exceeded net P_m^{cell} in Fe-limited cells.

Net P_m^{Chl} of Fe-limited cultures was lower due to high R_d^{Chl} (Table 9), although in some cases gross P_m^{Chl} of Fe-limited cultures was higher than corresponding Fe-replete cultures (Fig. 17). Net P_m^{Chl} of Fe-replete cultures was significantly higher than corresponding Fe-limited cultures ($P < 0.05$, $n = 3$-4) for both strains at all tested growth

Table 8. Cell normalized net photosynthetic capacity (fmol $O_2 \cdot cell^{-1} \cdot h^{-1}$) of Synechococcus A2169 and WH7803 in balanced semi-continuous cultures. Values are means ± SD of 3-4 cultures. For each strain, values with different superscript letters are significantly different ($p < 0.05$, Tukey multiple comparison).

nM Fe	0.29 d^{-1}	0.43 d^{-1}	0.69 d^{-1}
		A2169	
54	0.23±0.09[b]	1.12±0.19[a]	0.57±0.06[b]
900	1.03±0.14[a]	1.30±0.31[a]	1.26±0.19[a]
		WH7803	
54	0.28±0.10[b]	0.71±0.16[b]	0.17±0.08[b]
900	0.86±0.06[a]	1.13±0.07[a]	1.40±0.15[a]

Table 9. Chl normalized net photosynthetic capacity (mol $O_2 \cdot g^{-1}$ Chl $\cdot h^{-1}$) of Synechococcus A2169 and WH7803 in balanced semi-continuous cultures. Values are means ± SD of 3-4 cultures. For each strain, values with different superscript letters are significantly different ($p < 0.05$, Tukey multiple comparison).

nM Fe	0.29 d^{-1}	0.43 d^{-1}	0.69 d^{-1}
		A2169	
54	0.18±0.05 [b]	0.47±0.06 [a]	0.27±0.07 [b]
900	0.35±0.08 [a]	0.47±0.04 [a]	0.40±0.06 [a]
		WH7803	
54	0.25±0.08 [b]	0.41±0.02 [b]	0.12±0.05 [b]
900	0.54±0.07 [a]	0.78±0.07 [a]	0.57±0.10 [a]

rates, except for A2169 at 0.43 d^{-1}, which also had unexpectedly high Chl content (Fig. 13). The difference of net P_m^{Chl} was not as large as net P_m^{cell}.

Chlorophyll fluorescence signals: onset and recovery from photoinhibition

Chlorophyll fluorescence at 77K of Synechococcus A2169 and WH7803 semi-continuous cultures at 0.43 d^{-1} growth rate is showed in Table 10. Variable fluorescence (F_v) was calculated as ($F_m - F_0$), whereas F_m was the maximal fluorescence. F_v/F_m of Fe-replete cultures was significantly higher than Fe-limited cultures for both strains. Exposure to high light (1000 μmol photons·m^{-2}·s^{-1}) for 30 min significantly decreased F_v/F_m for both strains regardless Fe concentration. Adding the protein synthesis inhibitor chloramphenicol (CAP) 40 μg·mL^{-1} immediately prior to high light exposure exaggerated the extent of photoinhibition of both Fe-limited and Fe-replete cells.

Table10. Effects of high light (exposure to 1000 μmol photons·m^{-2}·s^{-1} prior to measurement) or high light + chloramphenicol (CAP, 40 μg/mL) on F_v/F_m of Synechococcus A2169 and WH7803 in semi-continuous cultures at 0.43 d^{-1} growth rate. Values are means ± SD, n = 3, except for WH7803 at 54 nM Fe, n = 4. For each strain, values with different superscript letters are significantly different ($p < 0.05$, two-way ANOVA, followed by Tukey multiple comparison).

nM Fe	Control	High-light	High-light+CAP
		A2169	
54	0.26 ± 0.03^{d}	0.18 ± 0.03^{c}	0.11 ± 0.03^{a}
900	0.38 ± 0.02^{f}	0.31 ± 0.01^{e}	0.15 ± 0.02^{b}
		WH7803	
54	0.20 ± 0.05^{c}	0.14 ± 0.04^{b}	0.10 ± 0.02^{a}
900	0.30 ± 0.03^{d}	0.23 ± 0.02^{c}	0.14 ± 0.02^{b}

DISCUSSION AND CONCLUSION

Iron-limitation of μ, yield, and photosynthetic pigments

Nutrient limitation may affect phytoplankton in two independent ways: reduced rate processes (photosynthesis and μ) and/or biomass yield (Cullen 1991, Falkowski et al. 1992, Wells et al. 1994, Wilhelm 1995). In this study I have demonstrated that Fe concentration can limit both the cell yield and μ of marine Synechococcus, with onset of yield-limitation at higher Fe than for μ-limitation. Cell yield of the Pacific strain A2169 was slightly less readily Fe-limited than the Atlantic strain WH7803 (Figs. 4, 5). Strain differences in μ response to Fe were less clear, because absolute μ was equal or greater in WH7803 than in A2169 at intermediate and higher Fe concentrations, but A2169 may grow faster (Fig. 5) or slower (Table 3) at low Fe concentrations. Even small differences in exponential μ could quickly translate to large differences in species dominance in natural communities. The results confirm previous observations that Synechococcus A2169 had the lowest subsistence Fe requirement of several strains tested, including WH7803, perhaps related to the low Fe tropical Pacific origin of A2169 (Brand 1991).

WH7803 cell yields at 90 nM Fe/150 μM N (Figs. 4, 5) were less than the 3×10^8 cells · mL^{-1} reported by Chadd et al. (1996) for WH7803 grown in ASW medium, but were about five-fold higher than the 2×10^7 cells · mL^{-1} reported by Kudo and Harrison (1997) for WH7803 grown at 100 nM Fe/30 μM N, which might be N-limited. The absence of an increase in μ at the lowest Fe concentration in the experiment suggests that Fe-scavenging siderophores were not induced as they were in the coastal strain

Synechococcus PCC 7002 (Wilhelm and Trick 1994, Wilhelm 1995, Trick and Wilhelm 1995, Wilhelm et al. 1996). Siderophores have not yet been reported in A2169, WH7803 (Rueter and Unsworth 1991, Chadd et al. 1996), nor any other oceanic Synechococcus strain.

As cells become progressively more Fe-limited, they are predicted to increase the maximum Fe uptake rate and to decrease the cell quota (Q_{Fe}) by at least 10-fold, which allows μ to remain near maximal at lower Fe concentrations than would occur without acclimation (Morel 1987). This is evident in the results in that μ and yield varied <3- and <20-fold, respectively, over the 250-fold range of Fe in Aquil, consistent with previous reports (Rueter and Unsworth 1991, Wilhelm and Trick 1995, Wilhelm et al. 1996). The Q_{Fe} reduction was not fully compensatory because cell yields and μ were reduced at low Fe concentrations. Q_{Fe} at the end of batch 2 cultures in Aquil can be estimated, assuming that all added Fe was incorporated into or adsorbed onto cells, by simply dividing Fe concentration by final cell density. The respective values at 900, 90, 18 and 3.6 nM Fe were 5.5, 0.8, 0.4 and 0.3 amol Fe·cell^{-1} (1 amol = 10^{-18} mol) for A2169 and 7.0, 1.1, 1.6 and 0.5 amol Fe·cell^{-1} for WH7803. Measured concentrations in WH7803 grown with 30 μM nitrate were similar to my estimates, at about 1.0 and 0.8 amol Fe·cell^{-1} at 10 and 1 nM Fe (calculated from Figs. 3 and 4 in Kudo and Harrison 1997). Thus, the Pacific isolate Synechococcus A2169 seems to have a greater capacity than the Atlantic strain WH7803 to reduce Q_{Fe} while continuing to grow, although it is possible that it could simply reflect cell size. By comparison, the oceanic isolate Synechococcus WH8018 in similar batch yield experiments contained ~50 and 1.8 amol·cell^{-1} at 1 μM and 10 nM Fe

(Rueter and Unsworth 1991). The coastal isolate Synechococcus PCC 7002 contained from ~0.4–54 amol·cell^{-1} depending on influent Fe concentration in chemostat (Trick and Wilhelm 1995, Wilhelm and Trick 1995) or initial Fe concentration in log phase batch cultures (Wilhelm et al. 1996).

Both strains needed only a short period to acclimate to the respective Fe concentrations in batch cultures (Figs. 1, 4). The batch 3 experiment definitively showed that growth rates and yields are already acclimated in batch 2 cultures. Photosynthetic parameters in batch 3 (Table 4) were also similar to those in batch 2 cultures (Table 1), providing additional evidence of acclimation to respective Fe treatment in batch 2 cultures.

It has been argued that batch cultures are not natural and cannot reflect the situation of natural communities because batch cultures never reach steady-state growth. In batch culture the growth of the cells constantly changes the environment (nutrient availability). It is important to maintain cells in balanced growth before examining photoadaptation and/or photoinhibition. Ideally, chemostat culture should be used to study nutrient limitation because steady-state growth can only be reached in continuous culture. However, maintaining Fe-limited chemostat cultures for marine phytoplankton is very difficult, as indicated by the existence of only one publication using this technique (Wilhelm and Trick 1995). Semi-continuous culture is similar to chemostat culture, except that periodic dilutions are performed manually rather than continuously by a pump. It is possible to maintain cells in nearly steady-state growth in both nutrient-sufficient and nutrient-limited conditions in semi-continuous cultures (Brand et al. 1981,

Thomas and Carr 1985). Thus, I further studied Synechococcus in semi-continuous cultures.

From the batch culture results, I found that the two Synechococcus strains showed symptoms of Fe-stress when grown in Fe concentrations < 90 nM. Preliminary semi-continuous data showed that 18 nM Fe cultures were too low to reliably measure P-I curves (data not shown), so I used 900 nM Fe Aquil as the Fe-replete medium and 54 nM Fe as the Fe-limited medium for semi-continuous culture experiments.

In continuous culture, the growth rate equals the dilution rate when phytoplankton are grown at steady state (Cullen et al. 1992). Both marine Synechococcus strains acclimated to a range of growth rate from 0.29 to 0.69 d^{-1}. Cells acclimated to the growth rate in two to three doubling times (9, 7, and 5 days for 0.29, 0.43, and 0. 69 d^{-1}, respectively) after daily dilution at the corresponding dilution rate, which is confirmed by the relatively steady cell density (Fig. 12).

The steady-state cell density of Fe-limited cultures was significantly lower than corresponding Fe-replete cultures at every tested growth rate for both strains (Fig. 13). Within Fe treatments, there was considerable variability in cell densities at different growth rates, but there was no obvious relationship between cell density and growth rate. The steady-state cell densities of semi-continuous Fe-replete and Fe-limited cultures were comparable to their counterparts in batch 2 cultures at middle exponential phase (Fig. 4). This result also indirectly confirms that batch 2 cultures are already acclimated to the corresponding growth conditions.

I did not see any obvious relationship between growth rate and Chl content in semi-continuous cultures (Fig. 13b,d). Cell Chl contents in semi-continuous cultures are

similar to batch 2 cultures at middle exponential phase (Fig. 6). The two marine Synechococcus strains, one (A2169) from the Pacific and the other (WH7803) from the Atlantic, responded differently in the production of photosynthetic pigments under Fe stress. In both batch and semi-continuous cultures, Fe-replete A2169 cells had higher Chl content than Fe-limited cells. However, the difference of Chl content between Fe-replete and Fe-limited WH7803 cells was not significant ($p < 0.05$, $n = 3$-5). Also, I found that the Chl red λ_{max} exhibited a blue shift > 3 nm in semi-continuous A2169 but not in WH7803. The results suggest that changes in cellular pigments may not be uniform in all marine Synechococcus strains. Assuming that the spectral shift indicates an adaptation to Fe stress, it supports my previous suggestion that the Pacific isolate A2169 has a greater capacity than the Atlantic strain WH7803 to adapt to Fe limitation (reduce Q_{Fe}).

This is the first study that reports the shift of Chl peak in oceanic Synechococcus. A blue shift of 6-7 nm was also observed in Fe-stressed freshwater (Öquist 1974) and coastal Synechococcus (Wilhelm and Trick 1995).

Oceanic Synechococcus may also differ from coastal strains in the response of light-harvesting pigments to Fe limitation. A shift in both Chl λ_{max} and phycocyanin (major light-harvesting pigment for coastal strains) λ_{max} was found in coastal Synechococcus under Fe limitation (Wilhelm and Trick 1995). In this study I did not find a shift in PE λ_{max} in the two marine strains. However, the PE:Chl ratio is higher in Fe-limited semi-continuous cultures than corresponding Fe-replete cultures at the same growth rate (Table 7), implying less decrease of PE than Chl. There is a possibility that the change in PE:Chl peak heights could be due to change in cell size (less likely) and

scattering properties. In this study I did not have direct evidence to prove that PE decreases less than Chl under Fe-stress. Other study showed that PE concentration does decrease in Fe-limited marine Synechococcus (Chadd et al. 1996), but possibly decreases less than Chl concentration. PE delivers 95% of absorbed light to the reaction center of PS II, whereas Chl also serves as light-harvesting pigment for PS I (Bryant 1986), and the number of Chl molecules in a PS I complex is greater than in PS II complexes (Fujita et al. 1994). The amount of PS I and/or PS I:PS II ratio may decrease under Fe limitation, leading to a marked decrease in cellular photosynthetic activity (Hardie et al. 1983). When Fe availability is limited in their environment, cyanobacteria must actively concentrate Fe into cells by a pumping system. The required extra energy for such pumping system may be met by changing the proportion of non-cyclic versus cyclic electron transport in thylakoids (Fujita et al. 1994). Thus increased PE:Chl ratio may imply that marine Synechococcus can adapt to changes in Fe availability by adjusting light harvesting pigments and cellular content of photosystems.

Growth under higher light decreased cellular Chl content (Fig. 13b, d; 0.43 d^{-1}-hl) and PE:Chl ratio (Table 7) of Fe-replete cells and Fe-limited cells in both strains. Marine Synechococcus can adapt to changes in light intensity by adjusting their amounts of light harvesting pigments and the cellular amount of photosystems, which may affect efficiency of electron transport and thus their photosynthetic efficiency (see following section). High light (1000 μmol photons·m^{-2}·s^{-1}) was reported to cause considerable reduction in the energy flow form phycobilisomes to Chl in WH7803 (Hassidim et al. 1997).

My estimate of PE:Chl ratio may be inaccurate because it is based on in vivo relative PE and Chl peak heights, instead of comparing extracted PE content to Chl content. I could not measure cellular PE content due to technique difficulty. However, the apparent changes of PE:Chl ratio (Table 7) is reversibly related to the changes of Chl content (Table 6), which indirectly confirms that the PE:Chl ratio reflects a relative change of phycobilisomes and reaction center complexes.

Based on these results, I suggest that the growth and synthesis of photosynthetic pigments of semi-continuous Synechococcus cultures are similar to the middle exponential phase of batch cultures, and the two marine strains respond differently in synthesis of photosynthetically active pigments under Fe limitation.

Effects of Fe-stress on photosynthesis and respiration

Changes in gross P_m^{Chl} and α^{Chl} in response to changed growth conditions are mediated through adjusting the photosynthetic pigments, (on P_m^{Chl} level) enzyme activities, and electron transport (Zevenboom, 1986). While the strategy of changing P_m^{Chl} with constant α^{Chl} seems to be important in marine species growing in nature under relatively high light conditions, adaptation in α^{Chl} with constant P_m^{Chl} is thought to be crucial in low-light environments (Glover 1980, Glover et al. 1986). I did not find any clear trend of change in α^{Chl} and P_m^{Chl} in either strain of marine Synechococcus under Fe limitation.

Chl-specific, light-saturated gross photosynthesis (P_m^{Chl}) remained relatively unchanged in response to Fe limitation, while respiration rate (R_d^{Chl}) exhibited a dramatic increase under Fe limitation. In comparison, cell-specific P_m^{cell} decreased significantly and R_d^{cell} increased to a smaller extent than R_d^{Chl} with decreasing Fe for both strain, thus the increase R_d^{Chl} can only be partly attributed to decreasing Chl content. On a cell basis, the overall result is that Fe-stress decreases gross photosynthesis to a very low level compared to Fe-replete cells, accompanied by increased respiration. Fe-stress induced decrease in gross P_m^{cell} with no change in P_m^{Chl} indicates a proportional decrease in photosynthetic apparatus components, consistent with results for the higher plant Beta vulgaris (Terry 1980, 1983, Nishio et al. 1985, Winder and Nishio 1995).

The elevated respiration in Fe-stressed cells may provide energy to enhance Fe-scavenging ability, as it does for N-uptake in N-starved dinoflagellate cells (Paasche et al. 1984). The results suggest that marine Synechococcus can regulate respiration, in addition to photosynthesis, to supply extra energy beyond that minimally required for survival. Respiration data are generally unavailable for Synechococcus under any growth conditions, because photosynthesis is usually measured as ^{14}C uptake (Kana and Gilbert 1987b, Rueter and Unsworth 1991), and R_d was not reported by Barlow and Alberte (1985) who measured O_2-exchange. The results of this project show that Fe-stress elicited large physiological changes in marine Synechococcus A2169 and WH7803 (Table 1): proportional decrease in Chl·cell^{-1} and gross P_m^{cell}, and an increase in R_d^{cell}. Consequently, net P_m^{cell} decreased to very low levels ($<R_d^{cell}$) in the lowest Fe cultures. Inspection of R_d^{Chl} and Chl·cell^{-1} data for the halotolerant coastal Synechococcus PCC 7002 grown in an Fe-limited chemostat (Wilhelm and Trick 1995) also suggests an

increase in R_d^{cell} at low Fe. A disproportionate loss of photosynthetic capacity relative to respiratory capacity is a well known response to Fe-stress in freshwater cyanobacteria (Guikema and Sherman 1983, Sandmann and Malkin 1983). An absolute increase in R_d^{cell} under Fe-stress is contrary to the general pattern in phytoplankton: R_d^{cell} is positively related to light- or nitrogen-limited μ (Falkowski et al. 1985, Osborne and Geider 1986, Herzig and Falkowski 1989, Laws and Chalup 1990, Levy and Gantt 1990). Iron-stressed Phaeodactylum tricornutum exhibited reduced R_d^{cell}, although P_m^{cell} decreased by a three-fold higher proportion (Greene et al. 1991). The physiological advantage of the low Fe-induced changes is unclear, but they reflect large shifts in energy metabolism and possibly in Fe-allocation and -acquisition patterns. Photosynthesis and respiration in cyanobacteria share some electron transport components (Mi et al. 1994, Schmetterer 1994, Vermaas 1994), and conceivably changes in one pathway necessarily affect the other. The problem is analogous to (in fact may be the evolutionary origin of) chlororespiration in higher plants (Garab et al. 1989) and algae (Peltier and Schmidt 1991). In Chlamydomonas chlororespiration increases under N-deficiency, perhaps contributing to ATP synthesis and $NADP^+$ regeneration (Peltier and Schmidt 1991). Similarly, (chloro)respiration increased temporarily in Chlamydomonas and Synechococcus (Lajkó et al. 1997).

The ratio of dark respiration to gross photosynthesis measured under optimal growth conditions is generally taken to be about 0.10. However, there are wide variations among species and algal class. Based on oxygen measurement, the optimal R_d^{Chl} : gross P_m^{Chl} ratios range from 0.043 to 0.630 for phytoplankton (Langdon, 1993), and near 0.1

or less for cyanobacteria (Geider and Osborne, 1989). My data indicate that the optimal R_d^{Chl} : gross P_m^{Chl} ratios were about 0.1 for both marine Synechococcus A2169 and WH7803, very similar to the value of 0.095 for WH7803 observed by Grande et al. (1989). There was a trend for the R_d^{Chl} : gross P_m^{Chl} ratio to be increased under Fe-limitation. For both strains, there was about a 3-fold increase in the R_d^{Chl} : gross P_m^{Chl} ratio when grown under Fe-limitation. The change in R_d^{cell} : gross P_m^{cell} ratio was similar to that of R_d^{Chl} : gross P_m^{Chl} ratio (Fig. 6). Elevated R_d : gross P_m ratio is a good indicator of Fe-limitation.

Ideally, the photosynthesis-irradiance curves would be measured for cultures in middle exponential phase. However, the O_2 electrode system (essential for measuring respiration) is too noisy at $< 10^7$ cells·mL^{-1}, which requires very long incubations to measure P-I curves in exponential phase for low Fe cultures due to their low cell density. To be consistent, P_m and R_d in all cultures were measured in late exponential (approaching stationary) phase. Table 4 showed that gross P_m^{cell} in particular dropped sharply on approach to stationary phase, at least in high Fe cultures. The difference in gross P_m^{cell} between 900 and 18 nM Fe cultures on batch 3 days 5 and 6 was either nonexistent or smaller than observed in the original batch 2 experiment (Table 1). This seems to validate the significant difference in the experiment when the WH7803 900 nM Fe cultures were close to stationary phase. In other words, presumably the difference would have been even larger earlier in the growth curve.

Similar to results of batch cultures, Fe-limited semi-continuous Synechococcus cultures showed a decrease in gross P_m^{cell} (Fig 16) and an increase in R_d^{cell} (Fig. 20) for both strains. Consequently, net P_m^{cell} decreased to very low levels in Fe-limited cultures

(Table 8). As pointed out in the previous section, photosynthesis and respiration in cyanobacteria share some electron transport components (Mi et al. 1994, Schmetterer 1994, Vermaas 1994, Lajkó et al. 1997). My semi-continuous data once again indicate that respiratory electron transport increases at the expense of photosynthetic electron transport in marine Synechococcus under Fe stress. Acclimation of the cells to Fe-limited growth appears to involve differential regulation of photosynthesis and respiration.

Semi-continuous cultures respond in photosynthetic capacity to Fe-stress similarly to batch cultures. Gross P_m^{cell} of Fe-replete and Fe-limited semi-continuous cultures are almost the same as their counterparts in batch cultures (Table 1), except for 0.43 d^{-1} A2169, but R_d^{cell} of Fe-replete and Fe-limited semi-continuous A2169 is higher than R_d^{cell} of batch A2169. R_d^{cell} of Fe-replete semi-continuous WH7803 is also higher than R_d^{cell} of Fe-replete batch WH7803, while R_d^{cell} of Fe-limited semi-continuous WH7803 is comparable to their batch counterparts. The difference between batch cultures and semi-continuous may be partially due to the fact that the samples were harvested in different phases: semi-continuous cultures could be regarded as in middle exponential phase, while batch cultures were in late exponential to stationary phase. Both gross P_m^{cell} and R_d^{cell} of batch cultures would have been higher if the samples were in middle exponential phase, based on results of Table 4 and Fig. 3a. However, I cannot rule out the possibility that semi-continuous cultures actually have higher gross P_m^{cell} and R_d^{cell} than batch cultures in middle exponential phase.

The results show that gross P_m^{Chl} in semi-continuous cultures is largely independent of Fe-limited growth rate (Fig. 18), as in nitrogen limited eucaryotic

phytoplankton (Herzig and Falkowski 1989). Quite surprisingly, gross P_m^{Chl} of Fe-limited semi-continuous A2169 was higher than the corresponding Fe-replete cells at all three growth rates but the opposite was true in WH7803 (Fig. 17). Likewise, both batch WH7803 and batch A2169 exhibited equal or higher gross P_m^{Chl} in high Fe (Table 1). Cullen et al. (1992) pointed out that P_m^{Chl} may remain unchanged in continuous cultures maintained at low, but balanced, nutrient-limited growth rates, although a decrease of P_m^{Chl} is typically observed in batch cultures. My results suggest that P_m^{Chl} sometimes may be higher in Fe-limited semi-continuous cultures. Many aspects of metabolism appear to be modified in Fe-limited semi-continuous A2169. Fe-limited A2169 cells have lower gross P_m^{cell} (Fig. 16) but higher gross P_m^{Chl} (Fig. 17) than Fe-replete cells, because Fe-limited cells have less Chl content (Fig. 13b). I suggest that A2169 has the capability to adapt to Fe limitation by increasing photosynthetic efficiency at saturating light. The possible means of adaptation to low-Fe conditions for oceanic algae are reduction in size or cell Fe requirement (Sunda et al. 1991). A reduction in size was not observed in this research, and oceanic cyanobacteria (including Synechococcus A2169 and WH7803) are thought to be unable to evolve a low Fe quota like the oceanic eukaryotes (Brand 1991). Thus A2169 must have some other way to meet their cell Fe requirement in order to maintain high gross photosynthetic capacity under Fe stress (although it is reduced relative to Fe-replete cells). WH7803 does not display typical ferredoxin/flavodoxin response to Fe limitation (Chadd et al. 1996). Inability to replace ferredoxin with flavodoxin may partially contribute to the low photosynthetic capacity in Fe-limited WH7803. It would be very interesting to see if A2169 is capable of expression flavodoxin under Fe stress, considering that A2169 has the lowest

Fe:macronutrient ratio among the marine Synechococcus strains investigated (Brand 1991). Further careful investigations are required to draw a more definitive conclusion.

The gross P_m^{Chl} of semi-continuous cultures in general is much higher that gross P_m^{Chl} of batch cultures at middle exponential phase (Table 4), especially for Fe-limited A2169 and Fe-replete WH7803. One possible explanation is that the longer exponential time of semi-continuous culture allows the cells to adapt better to the growth conditions. The gross P_m^{Chl} in semi-continuous cultures is higher although the Chl (and possibly also PE) content in semi-continuous cells is similar to batch cells (see previous section). R_d^{Chl} is also very high in semi-continuous cultures (Fig. 21), especially in 0.29 and 0.69 d^{-1} growth rates, compared to batch cultures (Table 1). R_d^{Chl} in Fe-limited cells is higher than corresponding Fe-replete cells in both strains at all tested growth rates.

In the ocean, even at the surface, phytoplankton are in darkness about half of each day. During this time, phytoplankton cells continue to respire, thereby losing some unknown fraction of their diurnal production. Maximum diel carbon balances (assuming saturating growth irradiance, which probably was not the case) were calculated as follows, with assumed photosynthetic and respiratory quotients of 1.2 and 1.0:

$$\sum P = (14\ h \cdot \text{net}\ P_m) / 1.2 + (10\ h \cdot R_d)$$

Based on my data, the calculations indicate a net C gain (10.1 ± 2.6 and 8.9 ± 0.7 fmol $O_2 \cdot cell^{-1} \cdot h^{-1}$ for A2169 and WH7803, respectively) for the 900 nM Fe cultures but a net C loss (-1.4 ± 0.7 and -0.6 ± 1.5 fmol $O_2 \cdot cell^{-1} \cdot h^{-1}$ for A2169 and WH7803, respectively; probably not significantly different from zero) for 54 nM Fe cultures, assuming the high R_d persists throughout the night. However, 54 nM Fe cultures might still have a net C

gain if the high R_d is only a temporary phenomenon following illumination. Thus, for further research it will be necessary to study changes of R_d over a long period (at least one hour) of time.

Effects of Fe-stress on the P-I response to acute high light stress

During the 30-min exposure to acute high light, marine Synechococcus (A2169 shown in Fig. 9, WH7803 similar) showed no reduction in P_m^{Chl} in the first 10 min. Photoinhibition or down-regulation only occurred after exposure longer than 10 min. This confirms the importance of incubation time during measurement of P-I curve (Henley 1993). Long incubation (> 7 min) at each PFD level during measurement of P-I curve, resulting in the commonly observed negative slope at high PFDs, indicates photoinhibition incurred during the incubation period rather than photoinhibition prior to incubation, the latter being more ecologically important.

In response to the high light treatment, in batch cultures gross P_m^{Chl} remained unchanged in Fe-stressed cells, but increased to some degree in Fe-replete cells. R_d^{Chl} increased significantly in Fe-starved cells but remained basically unchanged in Fe-replete cells. Consequently Fe-stress further increased the already high respiration rates compared to Fe-replete cells. Acute high light stress is known to increase respiration at least temporarily in eucaryotic algae (Falkowski et al. 1985). Kana (1992) demonstrated that Synechococcus WH7803 grown under nutrient-replete conditions had very low O_2 consumption in darkness or limiting PFD, but it increased markedly at saturating PFD. However, the increase was attributed to the Mehler reaction, which occurs only in the

light and therefore cannot explain the higher R_d rates that we observed. The Fe stress dependence of the HL-induced increase in R_d has implications for susceptibility to photoinhibition and photoprotection under low Fe conditions. In Anacystis nidulans IU625, HL-enhanced R_d appears to reduce photoinhibition and enhance recovery, possibly by maintaining the ATP supply for protein (e.g. D1) synthesis (Shyam et al. 1993). The decrease of net P_m during the 30 min HL exposure (Fig. 9), which was observed at all Fe concentrations, could simply reflect increased light respiration rather than a photoprotective mechanism or damage to photosystem II. If R_d initially increased in all treatments, the rates remained elevated after the end of the acute high light exposure in Fe-stressed but not Fe-replete cells (Table 5). One possible explanation of this phenomenon is that cyanobacteria use increasing respiration as a protective mechanism to provide energy for repair processes under high light stress as well as Fe-stress. While photosystems may already be decreased under Fe-stress, cells may rely heavily on respiration to supply energy for repair processes under high light stress. My results support the possibility of a more specific function for respiration in cyanobacteria under certain ecological conditions (Schmetterer 1994). The general idea is that the function of respiration in plants is the generation of a minimum amount of energy necessary for survival in the dark. This needs to be evaluated carefully in cyanobacteria because of the interaction of their photosynthetic and respiratory electron transport.

Fe-stressed cells of the two Synechococcus strains showed no symptom of photoinhibition after high light treatment, except for an increase of respiration (Table 5). The gross photosynthetic capacity in Fe-limited cells was unaffected and there was no

obvious trend of change in photosynthetic efficiency (initial slope α). Based on photosynthetic data in batch cultures, Fe-limited Synechococcus cells are not more susceptible to photoinhibition, although we are cautious of extrapolating observations on strains maintained in the laboratory to mixed assemblages growing under natural conditions.

I also assessed the effects of Fe-stress on acute high light in semi-continuous cultures for both strains. Aliquots of the same semi-continuous cultures were used to measure P-I curves and assess effects of acute high light treatment. Thus the results of semi-continuous cultures are more robust than the batch data, in which different cultures were used for controls and high light exposure.

Exposure to 30 min high light treatment did not significantly affect R_d in semi-continuous cultures. However, grown under higher light (100 μmol photons·m^{-2}·s^{-1}) increased R_d, especially in strain A2169 (Figs. 20-21). It was originally argued that marine Synechococcus, including isolate WH7803, are adapted to low photon flux densities (Barlow and Alberte 1985). However, later studies (Kana and Gilbert 1987a, b) showed that they can actually tolerate full sunlight. They are probably exposed to high light for at least a brief period in their native environment. Compared to batch-grown cells, semi-continuous cells increase R_d less under high light stress, which may simply be because semi-continuous cells were in middle exponential phase and adjusted their metabolism better. A protective role of dark respiration in photoinhibition of photosynthesis has been reported in freshwater Synechococcus (Shyam et al. 1993).

I do not agree that Chl is a poor parameter for data normalization for Fe-limited populations (Rueter and Unsworth 1990, Wilhem and Trick 1995), even though we still do not fully understand it. Chl normalized data are widely available in both procaryotic and eucaryotic algae (Glover 1980, Barlow and Alberte 1985, Herzig and Falkowski 1989, Grande et al. 1991, Cullen et al. 1992, Kana 1992) and provide an useful indicator for interspecific comparison. The synthesis of Chl and other light-harvesting pigments under Fe limitation may be a key issue to understand the adaptation of marine Synechococcus to Fe limitation and deserves further investigation.

The initial slope (α) of the P-I curve is used as an indication of photosynthetic efficiency (Barlow and Alberte 1985, Henley 1993). α is the product of the specific absorption coefficient for Chl and the quantum yield for photosynthesis (Cullen et al. 1992). The maximum photosynthesis is a function of the maximum substrate flux through CO_2-fixing enzymes, which may be limited by enzymatic reactions of the Calvin cycle or by diffusion and transport processes but is generally not limited by electron transfer processes (Richardson et al. 1983, Henley 1993). The shape of a culture's P-I curve changes as the cells adapt to the growth conditions. In this study, there was no obvious trend of α^{cell} and α^{Chl} either among different growth rates or between Fe-limited and Fe-replete cells, indicating that α may be independent of the nutrient-limited growth (Cullen et al. 1992). However, in general, both α^{cell} and α^{Chl} decreased when grown under higher light or under acute high light stress, consistent with earlier reports for WH7803 (Kana and Glibert 1987a, b). As pointed out by Cullen et al. (1992), the initial slope is difficult to measure accurately. My results show that both α^{cell} and α^{Chl} are very

variable in batch cultures and semi-continuous cultures, which could only be possibly overcome by using much longer incubations for greater sensitivity. The variation among replicate cultures is so high that it is impossible to see any clear Fe-dependent trend. Barlow and Alberte (1985, Tables 1 and 2) did not show standard deviation for Synechococcus α data, so the variability in their data is unclear. Based on the above discussion, α may not be a good diagnostic of Fe limitation, as in the case of nitrogen limitation (Herzig and Falkowski 1989, Cullen et al. 1992), unless it is carefully measured. For future investigation of P-I curves, I suggest longer measuring time (e.g. 10 min) at low PFDs (<100) and more than 5 PFDs in the linear initial slope region.

Based on the above discussion, it can be concluded that Fe limitation significantly enhances respiratory electron transport, which may be exaggerated by acute high light stress or growth under higher light. My result is in agreement with a recent study in heat-stressed freshwater Synechococcus (Lajkó 1997). Dark respiration data are generally unavailable because most phytoplankton photosynthesis studies use the ^{14}C technique. R_d also was not reported in O_2 exchange measurements of marine Synechococcus (Alberte et al. 1984, Barlow and Alberte 1985). I have produced the first complete data set of dark respiration under Fe limitation and high light stress. Some of the high respiration observed in this study could represent bacterial respiration since the cultures were not axenic (especially A2169). However, the fact that batch and semi-continuous cultures gave similar results suggests that bacterial contamination is not a likely explanation. I could predict greater relative bacterial contamination (thus higher R_d) in

late batch cultures, as nutrient-stressed cells began to stop growing and possibly leached organic substrates for bacterial growth.

With regard to the cyanobacterial respiratory chain, "...not all reactions or components are present in all strains or cell types and there must be regulatory mechanisms that activate or deactivate certain reactions under different external conditions" (Schmetterer 1994). To my knowledge, this is the first report that cyanobacteria increase their respiration under Fe-stress and high light conditions. Clearly much more work is necessary to understand the molecular mechanism regulating distribution of energy between photosynthesis and respiration in oceanic cyanobacteria under nutrient and light stress.

It is the general concept that excess light inhibits the photochemical efficiency of PS II. My photosynthetic data do not provide evidence of photoinhibition when Synechococcus were exposed to high light stress. It would be interesting to study the effects of the high light treatment on the fluorescence kinetics and to see if the F_v/F_m ratio changes when Synechococcus are exposed to high light stress.

Chlorophyll fluorescence: an indicator of photosynthetic efficiency

In batch cultures, I found that exposure to acute high light stress induced a further increase in R_d^{cell} in Fe-limited but not Fe-replete Synechococcus. In contrast, acute high light stress did not significantly affect either R_d^{cell} or R_d^{Chl} in semi-continuous cultures of either marine Synechococcus strain. However, I did observe a slight increase in R_d^{cell} in Fe-limited cells for both strains at all tested growth rates, except for 0.43 d^{-1} A2169

(Fig. 20). Chlorophyll fluorescence was used to unambiguously diagnose the effect of acute high light stress on photosynthetic efficiency of semi-continuous cultures.

Immediately after a dark-adapted sample is illuminated the fluorescence emission increases to an initial level F_0. This initial fluorescence is thought to be due to emission from excited antenna chlorophyll prior to the migration of excitation energy to the reaction center (Geider and Osborne 1992). The F_0 should only be determined when all of the reaction centers are open and Q_a is fully oxidized. In this investigation, F_0 was determined with background far-red light in order to fully oxidize the intersystem carriers and maintain all of the reaction centers in the open state. At high irradiances, the fluorescence emission rises sharply to the peak fluorescence F_m, which is generally thought to reflect the initial rapid reduction of Q_a by PS II as excitation energy is transferred from closed to open reaction centers (Geider and Osborne 1992). Thus F_v/F_m can be regarded as the maximum fluorescence observed when all PSII reaction centers have been closed. The difference between F_0 and F_m is called variable fluorescence, F_v. F_v/F_m is related to the maximum quantum efficiency of PSII photochemistry (designated ϕ_{II}; Butler, 1978).

Two major factors cause changes in fluorescence yield: the rate of photochemical energy conversion and the rate of nonradiative energy dissipation. At the temperature of liquid nitrogen (77K) only primary photochemical reactions can take place, and the fluorescence emission characteristics are dependent almost entirely on the redox state of Q_a. No appreciable reoxidation of Q_a occurs during induction and temperature-dependent nonphotochemical processes are eliminated so that much simpler induction kinetics are

observed (Geider and Osborne 1992). Thus changes in F_0, F_m and F_v/F_m at 77K are attributed solely to variation in the rate constants for photochemistry. Measurements of chlorophyll fluorescence at 77K have been particularly useful as an indicator of environmentally induced damage to the photosynthetic apparatus despite the fact that the reoxidation of Q_a is blocked (Geider and Osborne 1992). With healthy nonstressed cells, there are only small differences in F_v/F_m measured at 77K , and variations in the F_v/F_m ratio due to environmental perturbations are often correlated with measurements of the maximum light-limited photon yield of O_2 evolution (Björkman and Demmig 1987).

For both marine Synechococcus strains, P-I curves of 0.43 d^{-1} semi-continuous cultures showed little effect of Fe limitation among all tested growth rates (Figs. 16-21). However, measurement of chlorophyll fluorescence at 77K suggested Fe limitation on cells in 54 nM Fe for both strains. F_v/F_m of Fe-limited cells was significantly lower than Fe-replete cells in both strains at 0.43 d^{-1} growth rate (Table 10), confirming that nutrient limitation can result in damage or alteration to the PSII reaction center (Greene et al. 1991, Geider and La Roche 1994). Exposure to acute high light stress further decreased F_v/F_m of both Fe-limited and Fe-replete cells, indicating a decrease in PS II photosynthetic efficiency. One possible explanation to the difference between P-I and fluorescence data is that enhanced R_d keeps plastoquinone (PQ) in a more reduced state such that F_v/F_m is lower. This hypothesis could be tested by adding respiratory inhibitors that do not affect photosynthesis, and determining if the F_v/F_m decrease in low Fe and/or high light is at least partly blocked.

Despite the apparent insensitivity of P-I parameters (particularly α) to acute high light stress in semi-continuous Synechococcus, the decrease of F_v/F_m indicates that photosynthetic efficiency is affected in marine Synechococcus under high light stress. Changes in chlorophyll-fluorescence characteristics appear more rapidly and with larger amplitude than changes in oxygen evolution. Although measurements of 77K fluorescence on whole plants largely confirm the prediction that photon yield of PSII photochemistry measured indirectly by O_2 exchange techniques should be related to the F_v/F_m ratio (Schafer and Björkman 1989), there are few measurements with algae (Geider and Osborne 1992). My results suggest that F_v/F_m is a more sensitive indicator of high light stress than P-I parameters. It provides information on the state of the photosynthetic apparatus that is not available from measurements of chlorophyll a. Based on a low F_v/F_m in situ, with recovery in F_v/F_m following iron addition, Greene et al. (1994) suggested that the dominant picoplankton were iron-limited.

Chloramphenicol (CAP), an inhibitor of 70S ribosome function, had significant effects on photoinhibition in marine Synechococcus. 40 $\mu g \cdot mL^{-1}$ CAP exaggerated the extent of photoinhibition of cells exposed to acute high light stress of both strains at 0.43 d^{-1} growth rate (Table 10). This result implies that high light stress induces rapid, substantial damage to the photosynthetic apparatus, and recovery from photoinhibition involves de novo chloroplast protein synthesis which is blocked by CAP. This process may reflect photoinhibitory damage to the structure of PSII, perhaps caused by unbalanced dynamics of the D1 proteins of the reaction center. D1 protein is degraded during photoinhibition (Kyle et al. 1984, Chow et al. 1989) and repaired and/or

resynthesized during recovery (Ohad et al. 1984, Ohad et al. 1990, Aro et al. 1993). If the ATP supply is restricted due to Fe limitation, the process of repair of D1 protein from photoinhibition may be slowed down, resulting in a longer period of recovery. Another photoprotection mechanism used by marine Synechococcus (including WH7803) is the dissipation of a substantial fraction of the light energy collected by phycoerythrin as autofluorescence under high light (Barlow and Alberte 1985). As discussed above, Fe stress decreased PE content, thus limiting the cell's ability to efficiently transfer absorbed light energy. The results suggest that Fe limitation and photoinhibition act synergistically in marine Synechococcus. In higher plants and eucaryotic algae, cells have an active xanthophyll cycle, which is important in protection from photoinhibitory damage in addition to turnover of D1 protein in eucaryotic cells (Demming-Adams 1990, Franklin et al. 1992, Falkowski et al. 1994). Cyanobacteria do not have a xanthophyll cycle, although they have large amounts of zeaxanthin that can perhaps dissipate energy. Thus dynamics of chloroplast protein (D1 protein) is more important in recovery from photoinhibitory damage in cyanobacteria than eucaryotic cells, as pointed out by Öquist et al. (1995).

The maximum quantum efficiency of photosynthesis (ϕ_m) should be proportional to that of photosystem II (ϕ_{II}), although changes in the ratio of cyclic to linear electron flow could potentially contribute to uncoupling variations in ϕ_{II} from variations in ϕ_m (Geider and Osborne 1992). Empirical relations between ϕ_m and the fluorescence parameter F_v/F_m have been established in higher plants (Björkman and Demmig 1987) and correlation of ϕ_m and F_v/F_m has been demonstrated in the macroalga Ulva rotundata

(Henley et al. 1991). However, a comparison of changes in ϕ_m and F_v/F_m in phytoplankton is limited to observations for a diatom (Greene et al. 1991). Unlike physiological diagnostics which show profound interspecific variability in absolute magnitudes, F_v/F_m appears to have an absolute upper bound that is independent of the taxon under consideration (Falkowski et al. 1992, Greene et a. 1994). Thus, submaximal values of F_v/F_m could be taken as strong evidence of physiological stress (Geider and La Roche 1994). There are few publications that employed (semi)continuous cultures to study Fe stress in marine phytoplankton. This study therefore provides important new data concerning changes in respiration, photosynthesis, and chlorophyll fluorescence in Fe-limited semi-continuous cultures of marine Synechococcus. Further testing of the use of F_v/F_m to assess ϕ_m is clearly desirable.

Batch cultures vs. continuous cultures

My results showed that semi-continuous data were similar to batch culture data. Cell yield, Chl content, and P-I parameters in semi-continuous cultures were comparable with batch cultures in middle exponential phase. (Semi-)continuous cultures are closer to balanced growth, thus are ideal for study on physiological regulation and photosynthesis under nutrient-limitation. Growth and biochemical composition in batch culture are thought to be unbalanced (Cullen et al. 1992). However, changes in growth rate in response to changing growth conditions can only be studied in batch cultures. My data indicated that batch cultures could acclimate to the Fe condition after two transfers at the

end of exponential growth. In the past, reports used either batch culture or continuous cultures, and the results of batch culture and continuous culture were not interchangeable (Cullen et al. 1992). My results suggest that it is useful to study nutrient limitation in batch cultures in addition to (semi-)continuous cultures.

CONCLUSIONS

Prokaryotic picoplankton such as marine Synechococcus are relatively abundant in putatively Fe-limited high nutrient, low chlorophyll (HNLC) regions of the oceans. The physiology of Synechococcus under Fe-stress has been studied less than eucaryotic algae. Recent evidence suggests that, although biomass and growth rates of Synechococcus are not typically Fe-limited in situ, cells may still exhibit symptoms of Fe-stress. I grew marine Synechococcus A2169 and WH7803 in laboratory batch cultures and semi-continuous cultures in the artificial medium Aquil.

In batch cultures, cell yields, and in some experiments exponential specific growth rate (μ) were more readily Fe-limited in the Atlantic isolate WH7803 than the equatorial Pacific isolate A2169. In both strains, final cell yields spanned about an order of magnitude and decreased continuously with Fe concentration from 900 to 3.6 nM (150 μM N, 10 μM P), whereas μ decreased much less and only at Fe concentrations below 90 nM. Synechococcus yield and μ were limited primarily by low Fe concentration. Under severe Fe-stress, cellular chlorophyll (Chl) content and light-saturated gross photosynthetic capacity (P_m^{cell}) decreased proportionately and dark respiration (R_d^{cell}) increased, such that net P_m^{cell} was extremely low, but gross P_m^{Chl} was unchanged. There was a further increase in R_d following acute high light exposure in Fe-stressed but not in Fe-replete Synechococcus. For both strains, there was about a 3-fold increase in the R_d^{Chl} : gross P_m^{Chl} ratio when under Fe-limitation. The change in R_d^{cell} : gross P_m^{cell} ratio was similar to that of R_d^{Chl} : gross P_m^{Chl} ratio.

Semi-continuous cultures were similar to batch cultures. Growth and synthesis of photosynthetic pigments of semi-continuous Synechococcus cultures were comparable with the middle exponential phase of batch cultures. A 3 nm blue shift in red Chl absorption peak was observed in Fe-stressed semi-continuous A2169 but not in WH7803. Photosynthetic parameters in semi-continuous Synechococcus were largely independent of Fe-limited growth rate. Similar to results of batch cultures, Fe-limited semi-continuous Synechococcus cultures showed a decrease in gross P_m^{cell} and increase in R_d^{cell} for both strains. Consequently, net P_m^{cell} decreased to very low levels in Fe-limited Synechococcus. Fe-limited cells had lower gross P_m^{cell} but higher gross P_m^{Chl} than Fe-replete cells in A2169, because Fe-limited cells had less Chl content. Exposure to acute high light did not significantly affect R_d in semi-continuous Synechococcus. However, growth in higher light increased R_d in semi-continuous cultures. Cyanobacteria may use increasing respiration as a protective mechanism to provide energy for repair process under high light stress as well as Fe-stress.

Chlorophyll fluorescence (F_v/F_m) of Fe-limited semi-continuous cells was significantly lower than in Fe-replete cells in both marine Synechococcus strains at 0.43 d^{-1} growth rate. Exposure to acute high light stress caused a further decrease in F_v/F_m of both Fe-limited and Fe-replete Synechococcus, indicating decrease in PS II photosynthetic efficiency.

SUGGESTIONS FOR FURTHER STUDY

Typical Aquil medium, which has a Fe background about 2 nM (Anderson and Morel 1982), was used in this study. The relatively high background Fe made it impossible to test effect of Fe concentration comparable to open ocean regions (< 0.5 nM). For further study on effect of lower and more realistic Fe concentration, it will be necessary to use polycarbonate or teflon vessels in each step of preparing medium. It will be better to use glass fiber or Teflon column to pack Chelex 100, or use 8-hydroxyquinoline-immobilized resin instead of Chelex 100 resin to remove trace metals from Aquil medium (Kudo and Harrison 1997).

In this study, I assumed that dark respiration rate is the same whether in light or in darkness. This may or may not be true. Dark respiration in cyanobacteria is assumed to be very low or absent in the light. However, Kana (1992) showed that gross oxygen consumption in WH7803 increased at high light, although it was attributed to the Mehler reaction. Respiratory oxygen uptake in freshwater Synechococcus and Anabaena may be inhibited in the light (for reference see Scherer et al. 1987). However, another study pointed out that respiration rate in freshwater Synechococcus is enhanced and then stabilized after 60-90 min exposure to low or moderately high light intensity (Shyam et al. 1993). It is important to study if the high respiration persists throughout the night or if it is only a temporary phenomenon following illumination. In the future, further experimental work will also be needed to verify the respiration rate in light by combining O_2 evolution measurement with the ^{18}O technique (Grande et al. 1989).

It will also be necessary to confirm that the elevated rates of dark of respiration under Fe-limitation observed in this study is not mainly due to bacterial contamination.

The first step should be finding a method to distinguish Synechococcus from bacteria using either epifluorescence microscopy or flow cytometry. The second step will be working with definitively axenic Synechococcus cultures.

Based on my results of semi-continuous cultures, marine Synechococcus should be able to grow at low residual Fe concentration in Fe-limited chemostat. Chemostat theory assumes residual Fe in the medium is negligible relative to the influent concentration. Recently, Synechococcus A2169 was successfully maintained in an Fe-limited cyclostat at 0.3 d^{-1} for over six weeks (William Henley, unpublished data). For further study on the effects of Fe limitation on chronic photoinhibition, it will be possible to grow Synechococcus in chemostat cultures at two Fe concentrations but identical dilution (growth) rate.

Photosynthesis (P-I curve) measurement based on oxygen evolution contains instructive information on photosynthetic efficiency, photosynthetic capacity, and the susceptibility of the organism to photoinhibition (Cullen et al. 1992). O_2–evolution measurement has the advantage of allowing direct and accurate measurement of respiration. However, changes in O_2 concentration in Fe-limited cultures are generally too small to be precisely detected with a polarographic electrode (Falkowski 1994). It would be almost impossible to measure O_2 evolution in Fe-limited cultures grown in medium containing Fe comparable to that in open ocean (< 0.5 nM), due to low cell density and cell Chl content. An alternative method would be measurement of changes in the quantum yield of fluorescence induced by stimulating flashes (Falkowski 1994), perhaps in confunction with selective inhibitors to resolve respiratory and photosynthetic electron transport. The results of active fluorescence by a pump and probe or fast

repetition rate fluorometer can provide information about biophysical parameters of photosynthesis controlled by nutrient and light conditions. A pump-and-probe-based fluorescence method for estimating the PS II photochemical efficiency has a precision of about 10% (Falkowski 1994). Fluorescence signals will be useful for diagnosing mechanisms of photoinhibition and response to iron-limitation. This method can also be used to measure horizontal and vertical profiles of F_v/F_m in the ocean to study the maximum quantum efficiency of natural phytoplankton (Falkowski and Woodhead 1992, Falkowski et al. 1994).

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