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Department of Botany

The University of British Columbia
Vancouver, Canada

Date April 18, 2002
Abstract

Light and iron (Fe) Co-limitation occur in temperate, Fe-limited regions in seasons where days are short and the mixed layer is deeper than the euophotic zone. In the first part of this study, the effect of light and Fe co-limitation on *Pseudo-nitzschia granii* isolated from the NE Subarctic Pacific was investigated. This was one of the first studies to investigate the effect of Fe and light on the physiology of oceanic, pennate diatoms in artificial seawater. Fe-limited *P. granii* grew at slower rates than Fe-replete cells, and the former showed signs of chlorosis and silica deficiency. *P. granii* was efficient at utilizing light, especially low light. Light and Fe were found to affect different aspects of photosynthesis. Whereas Fe affected the quantum yield of photosynthesis, light affected photochemical efficiency. Despite low growth rates, *P. granii* was able to survive and grow under Fe-limitation. This was possibly because Fe limitation increased the activity of non-photochemical quenching mechanisms, allowing the cells protection against photodamage. Overall, growth rates were directly related to the rate of linear electron transport through the cells.

In the second part of this thesis, interaction of light and temperature on the physiology of *P. granii* was investigated. Studies on the combined effects of light and temperature, especially in temperate phytoplankton, are rare. Both light and temperature were found to influence growth rates. Temperature dependency decreased with decreasing light. Cellular chlorophyll displayed a modal response to temperature and light. Photosynthetic yield and efficiency were generally depressed at low and high temperatures. Unlike in green algae and some cyanobacteria, low-temperature acclimation in *P. granii* was not similar to high light acclimation.
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Chapter 1
General Introduction

Aquatic carbon assimilation (primary productivity) accounts for approximately 40% of global carbon fixation (Falkowski and Raven, 1997), a considerable percentage when the implications of carbon dioxide removal in terms of global warming are considered. This increases the need for scientists to understand what controls primary production of marine phytoplankton in the ocean. Traditionally, scientists have investigated how macronutrients, such as nitrate and phosphate, affect marine phytoplankton physiology, with the general consensus that nitrate is the limiting nutrient in most regions of the ocean (Falkowski and Raven, 1997).

However, there are many, paradoxical regions in the ocean where the concentration of macronutrients is high, but the photosynthetic biomass (chlorophyll) is low (Hutchins, 1995). Many hypotheses were proposed to explain the limitation of algal growth rates in High nutrient- low chlorophyll (HNLC) regions. These included active grazing by meso- and microzooplankton, low light levels, strong wind mixing, or unavailability of essential micronutrients (trace metals), most specifically iron (Fe) (Martin et al., 1990). The latter was difficult to discern because of high levels of background contamination and poor trace metal sampling techniques. However, in 1990, trace metal clean shipboard bottle experiments provided evidence to support the role of Fe as a limiting factor of algal growth and biomass in the NE subarctic Pacific (a HNLC region) (Martin et al., 1990). In the decade that followed, further refinement of trace metal clean techniques, numerous shipboard experiments and three large-scale Fe fertilizations unequivocally confirmed Fe as the limiting factor of algal growth in two
HNLC regions, namely the Equatorial Pacific (Iron EX1 and 2) and the Southern Ocean (SOIREE) (Boyd et al., 2000; Martin et al., 1994). In these experiments, the addition of Fe stimulated primary productivity and nutrient drawdown, and increased photosynthetic efficiency and phytoplankton biomass, especially for large diatoms (> 20 μm) (Boyd et al., 2000; Martin et al., 1994). It was estimated that over 30% of the world's oceans is Fe-limited, and one of the most important HNLC, Fe-limited regions was Ocean Station P in the NE subarctic Pacific (Boyd and Harrison, 1999; Harrison, et al. 1999).

A brief History Ocean Station P (OSP):

Ocean station P (OSP; formerly ocean station Papa), located in the Alaskan gyre (145°W 45°N) was the site of the longest running series of oceanographic data (Harrison et al., 1999). Observation at OSP started in 1950's with the weathership program as implemented by the Canadian government (Boyd and Harrison, 1999; Whitney et al., 1998; Freeland et al., 1997). Initial measurements included temperature, salinity, chlorophyll and nutrients. In 1956, hydrographic casts were conducted on a daily bases and primary productivity as well as zooplankton data were collected. A series of 13 stations were sampled on the way to OSP; they ranged from coastal stations (PO4) to offshore stations (OSP, also known as P26) (Boyd and Harrison, 1999). This transect, known as line P, provided a valuable opportunity to compare oceanic and offshore regions. The weather ship program was cancelled in the early 1980's (Boyd and Harrison, 1999). Sampling at line P was continued by the Institute of Ocean Sciences (IOS, Sidney, BC) through the world ocean current exchange program (Whitney et al., 1998 and Freeland, et al., 1997). The number of stations increased to 26 and the frequency of sampling decreased to three times a year (spring, summer and winter). Proceeding the world current exchange program, sampling at
OSP is characterized by a permanent shallow pycnocline (40 m in the summer, and 140 m in the winter because of storm activity) (Whitney et al., 1998 and Freeland et al., 1997). The concentration of macronutrients is high throughout the year, chlorophyll low and, unlike coastal regions, there is no spring bloom of phytoplankton in the spring (Boyd and Harrison, 1999; Harrison et al., 1999). Dominant phytoplankton species are less than 5 μm and consist of mainly flagellates (prymnesiophytes such as *Emiliania huxlyii* and *Phaeocystis* sp.) and cyanobacteria (*Synechococcus* sp.) (Boyd and Harrison, 1999; Harrison et al., 1999). The absence of larger phytoplankton (specifically diatoms) is due to Fe limitation (Boyd et al., 1996; Boyd et al., 1995a; 1995b; Martin et al., 1990). OSP was the first oceanographic station where trace metal clean techniques were employed in shipboard experiments (Martin et al., 1990). Sources of Fe to OSP in order of significance are dust deposition from the Gobi Desert in China, recycling by bacteria and micrograzers, horizontal advection of water from the coast of the Aleutian Islands and vertical mixing (Young et al., 1991).

In the July of 2002, OSP will be the site of a large-scale Fe fertilization experiment akin to those conducted in the Equatorial Pacific and the Southern Ocean. The emphasis of the project is to investigate the effect of *in situ* Fe addition on the flux of biogenic gases (such as carbon dioxide and dimethyl sulfide) from and to the ocean (Harrison, pers. commun.).

**The Role of Fe in Cellular Physiology:**

Though it is the fourth most abundant terrestrial element, Fe is found in trace concentrations in seawater, from nM (10^{-9}, in Fe-replete, or coastal areas) to pM (10^{-12},
in Fe-deficient, oceanic areas) (Rue and Bruland, 1997). This d-block element has high redox potential \((\text{Fe}^{3+} + e^- \leftrightarrow \text{Fe}^{2+}, E_{\text{cell}} = -0.244)\) within the limits of biological molecules, making it a favorable electron carrier in many metalloproteins and enzymes. Fe is a required micronutrient for all living organisms and it is especially important in photosynthesis, where it is an integral component of a variety of electron carriers, such as heme proteins (cytochromes) (Glover, 1977), or Fe-sulfur proteins such as ferredoxin (Doucette et al., 1996; La Roche et al., 1996; 1995). The function of electron transport in these proteins depends on the redox transition between ferric \((\text{Fe}^{3+})\) form and ferrous \((\text{Fe}^{2+})\) form. Fe is also involved in the chlorophyll synthesis pathway and Fe limited cells are often chlorotic (Geider and La Roche, 1994). Fe is also required for the structural integrity and function of photosynthetic reaction centers, photosystem 1 and photosystem 2 (PS 1 and PS2, respectively) (Geider and La Roche, 1994).

The effect of Fe stress on the physiology of coastal phytoplankton is well studied (Doucette et al., 1996; La Roche et al., 1995; Greene et al., 1992; 1991; Glover, 1977). Phytoplankton follow a combination of two strategies to relieve Fe stress, including the depletion of Fe-dependent photosynthetic electron carriers, or their replacement by functionally equivalent molecules that do not require Fe (Doucette et al., 1996; Glover, 1977). In general, photosynthetic enzymes and components constitute the largest Fe quota in diatoms (Raven et al., 1999). The details of photosynthesis in diatoms, as well as most chlorophyll-c containing organisms are poorly understood compared to terrestrial plants and green algae.

**Light and Photoacclimation:**
In addition to Fe limitation, phytoplankton communities in temperate HNLC regions are exposed to low, sub-saturating levels of light, especially in the winter season (Timmermans et al., 2001; Maldonado et al., 1999). Photosynthetic cells acclimate to low light by producing more chlorophyll and more photosynthetic components to maximize light absorption (Sunda and Huntsman, 1997). Low light acclimation increases Fe requirements considerably, and if Fe is limiting in the environment, the cells will be under a double stress. In the spring and summer, Fe addition (shipboard experiments) at OSP results in a bloom of large diatoms (Boyd et al., 1995a). This bloom is not as pronounced in the winter unless irradiance is also increased during the Fe addition (Maldonado et al., 1999). During winter at OSP, light levels are low because days are short, cloud cover is high and the pycnocline is often deeper than the euphotic zone due to storm-induced mixing (Maldonado et al., 1999).

Photoacclimation involves changes in pigment concentration, cellular composition, cellular volumes and carbon fixation within the genetic limitation of the organism (Anning et al., 2000). It is thought to be a relatively fast process occurring within the span of one generation or less depending on the organism’s growth rate (Anning et al., 2000). There are two strategies for photoacclimation in marine algae: altering the number of photosynthetic units available without affecting the absorption cross-section of the photosynthetic unit, or altering the cross-section by changing the composition of the photosynthetic unit (Greene et al., 1992).

The Ecology and Physiology of Diatoms:

Diatoms are ubiquitous in freshwater and marine environments. Unique to them is a siliceous, decorative frustule that resembles petri dishes. In the coastal environment, diatoms (Bacillariophyceae) account for 20% of new production, and on the west coast of Canada,
fast growing diatoms (such as *Skeletonema costatum*) dominate the spring bloom (Taylor and Haigh, 1996). Diatoms are grazed upon by mesozooplankton (such as the calanoid copepod *Neocalanus plumchrus*), which are then grazed upon by pelagic fish (Mackas et al., 1998). Therefore, the dynamics of diatom blooms on the west coast relate indirectly to fluctuations in fish populations (Mackas et al., 1998). In the open ocean, diatoms are small and less abundant than phytoflagellates and cyanobacteria (Boyd and Harrison, 1999). Unlike flagellates and cyanobacteria, diatoms have the ability to sink and are an important source of carbon flux in the open ocean, and are also important in the geochemical cycle of silica.

Diatoms are divided into two groups, centric and pennate, depending on the symmetry of their silica frustules, radial and bilateral respectively. Each can exist as single cells or colonies. Centric diatoms are non-motile and planktonic, whereas pennate diatoms are predominantly motile (through raphes) and benthic. Several genera of pennate diatoms (such as *Pseudo-nitzschia*) exist planktonically in the coastal and offshore environments (Haigh and Taylor, 1996).

Both pennate and centric diatoms reproduce asexually until they reach a certain size at which gamete production is triggered and sexual reproduction between different strains commences (Hiltz et al., 2000). The gametes of centric diatoms are morphologically different; certain cells become large sessile gametes (eggs) while others produce small, flagellated cells (sperm) in a process known as oogamy. In pennate diatoms, gametes are indistinguishable; both are small, and the overall process is known as isogamy (Davidovich and Bates, 1998). Sexual reproduction is triggered in the laboratory by nitrate depletion or by varying the length and intensity of the light cycle (Hiltz et al., 2000). Pennate diatoms are
also thought to require a substrate on which to mate, as well as the presence of a different clone from the same species (Davidovich and Bates, 1998).

Diatoms have chlorophyll a, c1, c2, fucoxanthin as well as two xanthophyll pigments, didinoxanthin and diatoxanthin, that are restricted to them (Olaizola and Yamamoto, 1994). Diatoms are easier to grow in the laboratory than other phytoplankton. Therefore, a wealth of information about their physiology exists in the scientific literature. Most of these studies have focused on so called “weed” species that are easy to grow in the laboratory but are not true representatives of all species. In addition, most studies so far have focused on coastal or estuarine species of diatoms, which have been shown to be physiologically quite different than oceanic species (Sunda and Hunstman, 1995). Pennate diatoms are more difficult to isolate and grow in the lab compared to centric diatoms, and are studied less frequently than their centric relatives. Most studies available on the physiology of pennate diatoms have focused on the diatom *Phaeodactylum tricornutum*, a weedy, coastal species (for examples, see Geider et al., 1993; Johansen, 1991; Osborne and Geider, 1987; Glover, 1977).

**The Genus *Pseudo-nitzschia***:

The two most famous genera of Nitzschoid diatoms are *Nitzschia*, a genus of single, benthic cells encompassing marine and freshwater species, and *Pseudo-nitzschia*, a genus of thinly silicified, planktonic and chain-forming marine species (Hasle 1995, 1994). The split between *Nitzschia* and *Pseudo-nitzschia* was suggested in the early 1900's by Paragallo on the basis of chain formation, raphe structure and motility (Hasle 1995, 1994). The split between these genera is widely debated and will not be discussed any further. Nitzschoid diatoms are the most common pennate diatoms on the coast of British Columbia, especially in the Strait of Georgia (Harris, 2001). Some diatoms are thought to be transported to the
west coast of Vancouver Island via the Vancouver Island Coastal Current (VICC), which is driven by the input of the Fraser River into the Strait of Georgia (Taylor and Haigh, 1996).

Certain members of the genus *Pseudo-nitzschia* gained a measure of notoriety over the past decade because of their association with domoic acid, a causative agent of amnesic shellfish poisoning (Horner and Postel, 1993; Bates et al., 1989). In 1987, many people were poisoned in Prince Edward Island, Canada, after ingesting shellfish that had accumulated domoic acid in their tissues (Bates et al., 1989). Later studies and examination of water samples revealed a bloom of *Pseudo-nitzschia* in the area where the shellfish were cultured and harvested. In the early 1990s, sea lions and pelicans died because of domoic acid contaminated shellfish off the coast of California (Scholin et al., 2000).

The threat of *Pseudo-nitzschia* is responsible for fuelling a flurry of scientific research that was specifically aimed at monitoring blooms of the genus and the physiological causes of domoic acid production (Bates and Leger, 1992). Most of this research has focused on identifying toxic species of *Pseudo-nitzschia* and monitoring blooms before they occur. Classifying species of *Pseudo-nitzschia* depends on minor morphological features indistinguishable under light microscopy, and requires careful examination under the scanning electron microscope (Hasle, 1994). Before samples are examined, careful techniques are applied to dissolve the organics that obscures fine features on the siliceous frustule without damaging it (Hasle, 1995; 1994). Attempts have been made to develop an RNA-based, fluorescent marker to distinguish between toxic and non-toxic strains. This has been met with little success because RNA degrades quickly and because of cross-reaction of RNA markers between different strains and species (Scholin et al., 2000).
The exact rates of domoic acid production are unknown. Domoic acid is a nitrogenous compound, which recently has been shown to have a high Fe-binding constant (Maldonado, unpublished results). This feature may assist the cells of *Pseudo-nitzschia* to acquire Fe at OSP by acting as an Fe-chelators similar to those in bacteria and fungi (Trick et al., 1983). Studies on the Fe-sequestering nature of domoic acid are still in their infancy. Some oceanic isolates of this genus, such as *Pseudo-nitzschia granii*, have been shown to produce trace amounts of domoic acid (Marchetti, A. and Trainer, V., unpublished results). It is unknown whether they have the ability to produce domoic acid at harmful levels, or whether this process is affected by Fe nutrition.

**Summary:**

Iron (Fe) limits the growth of phytoplankton in many high nutrients low chlorophyll regions of the oceans. In high latitude, Fe-limited regions such as the NE subarctic Pacific (Ocean Station P, OSP) and the Southern Ocean, Fe-limitation is confounded with light limitation in the winter. Light and Fe interact through the process of photosynthesis, and cells under light limitation have a larger Fe quota and are often Fe deficient. Large diatoms are scarce in HNLC regions but when Fe is added, they grow efficiently and dominate the Fe-enriched assemblages. For example, species of the chain-forming, pennate diatoms, *Pseudo-nitzschia* dominate Fe-enriched phytoplankton community in the summers at OSP. In addition, *Pseudo-nitzschia* is an important genus of diatoms in coastal temperate areas because its blooms are sometimes associated with domoic acid production, a causative agent of amnesic shellfish poisoning. Studies on Fe and light co-limitation, as well as studies on the physiology of pennate, oceanic diatoms are scarce.
Chapter 2
Pulse Amplitude Modulated Chlorophyll Fluorescence

Chlorophyll Fluorescence and The Kautsky Effect:

Chlorophyll Fluorescence is a highly exploited property of photosynthetic cells, especially in biological oceanography and limnology, where it is used as a proxy for phytoplankton biomass (Falkowski et al. 1999). Light absorbed by photosynthetic material encounters one of three, competing fates (Maxwell and Johnson, 2000): Under normal conditions, the majority (97%) is used in photochemical processes (photosynthesis and linear electron transport), a fraction is directed towards non-photochemical reactions (xanthophyll cycle, state transitions and photoinhibition) and finally a small fraction (up to 3%) is remitted as long wavelength (~690 nm), red fluorescence (Maxwell and Johnson, 2000; Hall and Rao, 1999; Krause and Weis, 1991). Photochemical quenching, non-photochemical quenching and fluorescence compete with each other, and, the accurate quantification of one or two of these processes can indicate the efficiency of the remaining process(es) (Krause and Weis, 1991).

Fluorescence can be easily quantified using modulated and non-modulated fluorometers.

On a molecular level, when chlorophyll molecules absorb light, they acquire energy that elevates them from a ground level to a high-energy, excited level (Trissle and Lavrange, 1994), and if this energy is not dissipated via photochemical and non-photochemical processes, it is remitted as fluorescence. The difference in wavelength between absorbed and re-emitted fluorescence allows the differentiation and subsequent quantification of both the light source and the fluorescence signal (Maxwell and Johnson, 2000). The intensity of fluorescence depends on the amount of light (Watts, Einsteins, Quanta...etc) per unit area,
and on the wavelength of the source light and the concentration of chlorophyll (Maxwell and Johnson, 2000). If fluorescence intensity remains constant, the change in fluorescence yield, defined as the change in the amount of fluorescence at the same intensity over time, can be calculated (Maxwell and Johnson, 2000, Falkowski and Raven, 1997, Schreiber et al., 1995a; 1995b).

When a sample of solvent-extracted chlorophyll is transferred from dark into light, the fluorescence signal from the extract is constant over the period of illumination. However, when a sample of intact photosynthetic cells is transferred from dark into light, the fluorescence signal has a distinct kinetic shape, which is directly related to the physiological status of the cell (Hall and Rao, 1999). When dark-adapted samples are transferred to actinic (photosynthetic) light, chlorophyll fluorescence rises from a basal level (F0) to a maximal level (FM) and then it decays (Govindjee, 1995, Schreiber et al 1986, Bradbury and Baker, 1981). The rise and decay of fluorescence is polyphasic, and a brief steady state level is reached after FM (Schreiber, 1994). These fluorescence kinetics were first observed in 1931 by Kautsky and co-workers. Therefore, the kinetic change of fluorescence yield over time when dark-adapted samples are illuminated is called the Kautsky effect (Fig 2.1) (Govindjee, 1995).

The shape and various phases of the induction curve are directly related to electron transfer through the photosynthetic unit and to the physiology of the organism involved. At room temperature, most of plant fluorescence originates at photosystem 2 (PS2) (Falkowski and Raven, 1997; Krause and Weis 1991). Unlike photosystem 1 (PS1), which is quite efficient at capturing light energy, PS2 is a shallow electron trap; the core complexes do not contain the excitation energy well, and some of that energy is returned to the antennae.
pigments resulting in fluorescence (Falkowski and Raven, 1997). The total fluorescence of plant cells increases at 77K (-80°C) due to increased contribution of the core complexes of PS1, and the contributions of PS1 fluorescence may also be taxa specific (Hall and Rao 1999).

When a photon is absorbed by PS2, P680 (the core of PS2) becomes excited and an electron is transferred from the electron carrier, phaeophytin and then to the plastoquinone pool through the carrier QA. At this stage, PS2 is closed, which means that it cannot accept any electrons until QA is reoxidised. This leads to the rise in fluorescence to the peak level F_M. The subsequent quenching of fluorescence is due to a combination of photochemical quenching (the re-oxidization of QA by QB) and non-photochemical quenching (Govindjee, 1995, Krause and Weis, 1991).

The difference between F_o and F_M, referred to as variable fluorescence, F_V, indicates the potential efficiency of PS2. Variable fluorescence is normalized to F_M (Equations 1, 2) (Schreiber et al. 1986), and the maximum, theoretical efficiency of PS2 is expressed in terms of F_V/F_M. Under optimal conditions, F_V/F_M ranges from 0.83 for higher plants to 0.37 for some species of algae, and the differences between taxa are dependent on their physiological makeup (Maxwell and Johnson, 2000; Büchel and Wilhelm, 1993). Within each group of organisms, conditions that decrease photosynthetic efficiency of the cell will lower F_V/F_M (Caspi et al., 1999; Genty et al. 1989).

\[
F_V = F_M - F_o \quad (1)
\]

\[
\frac{F_V}{F_M} = \frac{F_M - F_o}{F_M} \quad (2)
\]
Fig 2.1: A schematic of the Kautsky effect illustrating the kinetics of fluorescence observed when plants are transferred from darkness to high light. $F_o$ is basal fluorescence, $P$ is the fluorescence peak after the sample is removed from the dark into actinic light, $F_M$ is maximal fluorescence, $F_v$ is variable fluorescence and $F_s$ is steady-state fluorescence.
Measuring $F_V/F_M$ in dark-adapted samples is simple. It requires a measuring light beam to measure basal fluorescence level $F_0$, and is identical to the measuring beam in most non-modulated fluorometers. $F_0$ should be adequate to provide a relatively strong signal without inducing any actinic effects (Ting and Owens 1993; 1992). Maximal fluorescence, $F_M$, is obtained by applying a saturating pulse to the dark-adapted sample (Schreiber et al., 1986). $F_V/F_M$ can also be determined using the toxin 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU), which artificially closes reaction systems by blocking the activity of PS2 (Equation 3) (Satoh and Fork, 1983).

$$Q_AQ_B^- + DCMU \rightarrow Q_A^-DCMU + Q_B$$ (3)

**PAM fluorometry:**

As mentioned previously, quenching kinetics of $F_M$ contains valuable information on photochemical and non-photochemical processes. The potential importance of this has led to the development of various techniques to decipher the fluorescence induction curve to express these processes in mathematical terms. The most significant of these techniques is the light doubling method introduced by Bradbury and Baker (1981). They propose which photosynthetic processes are responsible for the observed fluorescence signals when the sample is illuminated with photosynthetic "actinic" light, and their work serves as the foundation for Schreiber's Pulse Saturation method and the development of the first commercial Pulse Amplitude Modulated (PAM) fluorometers (Schreiber, 1994; Schreiber et al., 1986). This is comparable to measuring $F_V/F_M$ except that samples are illuminated with an actinic light source, so they are actively photosynthesizing (while fluorescence is at a steady state level $F_s$) while the saturating pulses are applied (Fig 2.2). Therefore, the maximum fluorescence peaks ($F'_M$)
Fig 2.2. The fluorescence trace generated using Pulse Amplitude Modulated fluorescence in dark-adapted *Pseudo-nitzschia granii*. \(F_0\) is basal fluorescence after dark-adaptation, \(F_M\) is maximum fluorescence, \(F_S\) is steady-state fluorescence under actinic conditions, \(F'_M\) is maximum fluorescence under steady-state conditions, \(F'_0\) is the basal fluorescence after steady-state.
obtained during actinic illumination provide information on non-photochemical processes when normalized to the maximal fluorescence of dark-adapted samples. Normalizing variable fluorescence to the steady state level fluorescence provides valuable information about the actual photosynthetic yield of the cell (the quantum yield of PS2, \( \phi_{PS2} \), equation 5) (Schreiber et al., 1995a; 1995b; Genty et al., 1990; 1989; Schreiber et al., 1986).

\[
\phi_{PS2} = \frac{F'_M - F'_S}{F'_M} \quad (5)
\]

Under most physiological conditions, the quantum (or operational) yield of PS2 is linearly correlated with the yield of carbon assimilation, expressed as moles of atmospheric CO\(_2\) fixed per unit light absorbed (equation 6) (Genty et al. 1990; 1989). \( \phi_{PS2} \) is also a measurement of the rate of charge separation in PS2 (Bradbury and Baker, 1981). This parameter contrasts nicely with the \( F_V/F_M \) ratio, which indicates theoretical photosynthetic efficiency.

\[
\phi_{CO_2} = \frac{\text{Moles } CO_2 \text{ Taken up}}{\text{Irradiance}} \quad (6)
\]

A relative rate of electron transport (\( J_e \)) can be derived from \( \phi_{PS2} \) taking into account incident light, fractionation between PS1 and PS2 and other electron sinks within the cells (Maxwell and Johnson, 2000). For terrestrial plants, the relationship between \( \phi_{PS2} \) and the rate of carbon assimilation is strongly linear (Genty et al., 1989) and varies from linearity under extreme conditions, such as abnormally high light. In marine phytoplankton, the linear rate of electron transport competes with non-photosynthetic enzymes that use electrons, such as nitrate reductase (Fleming and Kormkamp, 1998; Büchel and Wilhem, 1993). It is also affected by heterogeneity in PS2 centers and the contribution of PS1 to fluorescence signals.
Therefore, absolute values of electron transport rates cannot be estimated accurately from \( \phi_{PS2} \) in marine phytoplankton.

There have been a few attempts to test the correlation between aquatic primary productivity derived from fluorescence with that derived using \( ^{14}C \). Boyd et al. (1997) tested this correlation in the North Atlantic using a Fast Repetition Rate Fluorometer (FRRF). They found that the trend in primary productivity derived from fluorescence (using FRRF measured optical cross-section values) correlated well with those derived from \( ^{14}C \). Spectral normalization explained a 3-fold difference between the absolute values derived from both techniques. Hartig et al. (1998) compared primary productivity derived from a PAM fluorometer with those derived from \( ^{14}C \) in benthic, freshwater communities. They measured optical cross-sections using a dual wavelength spectrophotometer. The trends between both techniques correlated well, yet absolute values of primary productivity (derived from fluorescence) were overestimated at high irradiance and underestimated at low irradiance. No spectral normalization was performed in their experiments.

Other parameters calculated from chlorophyll fluorescence include photochemical quenching (\( P_{q} \), which measures light capture efficiency, Chapter 3) (Belkhodja et al., 1998), redox pressure on PS2 (1- \( P_{q} \), which measured the accumulation of electrons on the acceptor side of PS2, Chapter 3) (Maxwell et al., 1994; 1995a; 1995b) and non-photochemical quenching (\( Q_{n} \)). Several components of non-photochemical quenching can be resolved depending on their relaxation kinetics. The fast component of \( Q_{n} \) (\( Q_{E} \)) indicates the re-energization of thylakoid membranes due to the build up of the proton gradient across thylakoid membranes (Maxwell and Johnson, 2000). This triggers heat dissipation of absorbed light via the activation of the xanthophyll cycle, and is though to be the most
important mechanism of photoprotection in chlorophyll-c containing organisms; in diatoms, excess light triggers the de-epoxidation of the carotenoid didinoxanthin into diatoxanthin (Olaziola and Yamamoto, 1994). This cycle is unique to diatoms. In higher plants, the xanthophyll cycle is based on the rapid de-epoxidation of violaxanthin into zeaxanthin (Olaziola and Yamamoto, 1994).

The middle component of $Q_n$, $Q_T$, relates to the poorly understood phenomenon of state transition, which has not been documented in diatoms (Maxwell and Johnson, 2000). The last and slowest component of $Q_{PH}$ is due to photoinhibition. The inclusion of a component of photoinhibiton in the definition of $Q_{PH}$ is confusing because whereas $Q_E$ and $Q_T$ are a product of protective mechanisms, $Q_{PH}$ is an indicator of damage.

**Technical concerns regarding PAM fluorescence:**

The technical and mathematical articulation PAM fluorescence fostered a boom in the application of the saturation pulse method (Maxwell and Johnson, 2000; Govindjee, 1995). Modulated fluorometers can distinguish between excitation beams, saturation pulses, emitted fluorescence signals and background light. They are equipped with a light emitting diode adequately sensitive to differentiate between the signals, without being saturated. In addition to this, a wide variety of commercial, modulated fluorometers are available for field application, where background sunlight is a problem for non-modulated fluorometers.

Considerations regarding applying chlorophyll fluorescence in the field and laboratory arise from the following: instrumentation (sensitivity and settings), organismal physiology, nomenclature and the interpretation of induction curves.
The excitation beam and determination of $F_0$

The basal level of fluorescence $F_0$, to which almost all measured parameters are normalized, requires an excitation beam that is low in intensity to avoid actinic effects, yet high enough to generate a strong signal (Schreiber et al., 1995a; Ting and Owens, 1992; 1993). This dictates that the frequency of the beam is low (usually around 1.6 KHz), because high frequencies of even the lowest light intensities may induce photosynthesis. To a certain extent, this depends on the physiological status and photosynthetic system of the organism probed. Highly concentrated samples will require a low intensity excitation beam to give a strong signal (Ting and Owens, 1992), while low chlorophyll samples need a higher intensity beam than high chlorophyll cells, increasing the chance of photosynthetic effect (Schreiber et al., 1995a; Büchel and Wilhelm, 1993). The wavelength for the excitation beam is important as well because PS2 is excited at 680 nm and PS1 is excited at 700 nm (Hall and Rao, 1999), therefore, PAM measurements made with light above 700 nm may lead to significant errors because of PS1 fluorescence (Genty et al., 1989).

Dark-adapting the samples is crucial for measuring $F_v/F_m$ because it allows the reaction centers to re-oxidize and open (Büchel and Wilhelm, 1993). If this is not the case, $F_0$ will be overestimated and $F_v/F_m$ will be underestimated (Maxwell and Johnson, 2000). The length of the dark adaptation period should be sufficient to allow the full re-oxidization of sample and to allow all photochemical and non-photochemical reactions in the cell to cease (Ting and Owens, 1993; 1992). This period usually ranges between 10 minutes to an hour depending on the physiology and the growth conditions (Büchel and Wilhelm, 1993). For example, chlorophyll-c containing organisms may require a longer dark adaptation time than chlorophyll-b containing organisms (Schreiber et al., 1995a; Ting and Owens, 1992; 1993).
Dark adaptation should not be long enough to induce dark respiration because anaerobic conditions change the fluorescence signals in some marine algae (Schreiber et al., 1995a; Schreiber and Neubauer, 1990). Ample care and time should be invested in the correct determination of $F_0$, and once these conditions are determined, they should be kept constant for all measurements performed for a single species to allow valid comparison between treatments.

**Terminology and Nomenclature:**

In the years that followed the introduction of the light doubling method and the pulse saturation method, there was a boom in the application of this technique, and various research groups modified the original technique to suit their application and organisms of choice. The nomenclature of the parameters calculated was not uniformly used, and many researchers used different terminology to describe what is essentially the same thing, therefore rendering the early literature on fluorescence rather confusing for the inexperienced reader (Maxwell and Johnson, 2000; van Kooten and Snell, 1990). Van Kooten and Snell (1990) successfully unified the terminology applied in fluorescence studies. Almost all papers, with a few minor exceptions, published on chlorophyll fluorescence in the past decade had used the normalized nomenclature.

**The Physiological status of the cell:**

The successful interpretation of fluorescence induction curves requires sufficient knowledge of the physiology of the organism involved. The growth conditions of the organism may affect the shape of the induction curve and the strength of the signal received. The same organism will give a different signal if it is grown under continuous light rather than a regimented light: dark cycle (Strasser et al., 1999). In some marine algae, younger,
faster growing cells (middle exponential) are healthier and have a higher F$_{v}$/F$_{m}$ ratio than older cells (in exponential phase), which have a lower F$_{v}$/F$_{m}$ ratio. In the course of an experiment, PAM fluorescence should be measured in the middle of exponential growth for all treatments to eliminate ontological effects.

Nutritional status also affects fluorescence induction curves, especially nutrients that affect photosynthesis (such as trace metals and nitrogen) (Parkhill et al., 2001; Caspi et al., 1999, Lippemeier et al., 1999). These effects depend on the acclimation of the cells (balanced vs. unbalanced growth) (Parkhill et al., 2001). Unless experimental design involves testing the effect of varying nutrient concentrations, nutrients and media should be kept constant for all treatments and samples in an experiment.

**Specific concerns for phytoplankton samples:**

Kautsky's initial observations were made using a solution of the green, freshwater algae *Chlorella* (Maxwell and Johnson, 2000; Govindjee, 1995). The studies preceding Kautsky's work mostly dealt with higher plants, while the development of PAM fluorometry for algal suspensions was relatively ignored, based on the rather naive assumption that algae are miniature plants and they function in very much the same way (Schreiber et al., 1995a; Schreiber, 1994; Ting and Ownens, 1992; 1993). In 1994, the first cuvette assembly PAM attachment was introduced, with a highly sensitive detection system made specifically for low chlorophyll algal suspensions (at μM levels) and attempts were made to remodel PAM technique used in higher plant to suit phytoplankton research (Schreiber, 1994).

Even with the introduction of this highly sensitive system, many reasons exist as to why PAM fluorometry is difficult to apply to algal cultures and field assemblages. Firstly,
the current scientific understanding of the photosynthetic process in algae is lacking, which hinders the interpretation of chlorophyll fluorescence. In addition to physiology, there is a requirement for sufficient biomass. High concentrations of chlorophyll increase the signal to noise ratio allowing for a stronger, more reliable fluorescence signal (Ting and Owens, 1993), and most oceanic concentrations of chlorophyll are too low and noisy for reliable PAM signals. Under laboratory conditions, producing enough biomass to perform reliable PAM measurements is not problematic. Coastal and freshwater specimens often reach a high concentration of chlorophyll without much effort. Open ocean species are difficult to grow in the lab, and do not often reach high chlorophyll levels, in which case, the samples are concentrated either by filtration and resuspension or by centrifugation and resuspension. Concentration techniques are criticized because they may sometimes result in damaged cells if they are performed too vigorously or if the cells are fragile, which results in the underestimation of $F_V/F_M$ (Bücher and Wilhelm, 1993, Schreiber, 1994). Highly concentrated samples may also result in self-shading which also underestimates $F_V/F_M$ (Bücher and Wilhelm, 1993). It is best, therefore, to avoid concentration procedures, and if this is not possible, it is recommended to develop a non-damaging concentration technique for each organism used (Ting and Owens, 1992).

**Summary:**

Chlorophyll fluorescence provides an easy and non-invasive mechanism of investigating photosynthesis *in vivo*. Research in the area is fuelled by the availability of highly sensitive, user friendly and portable modulated fluorometers. Recently, the introduction of a modulated fluorometer that measures photosynthetic efficiency using a cuvette system sparked interest in using chlorophyll fluorescence to investigate
photosynthesis in marine phytoplankton. The interpretation of fluorescence signals is complicated and requires sufficient knowledge of physiology as well as instrumentation. However, PAM fluorescence remains a promising tool for oceanographic research.

**Thesis Objectives:**

1. To isolate *Pseudo-nitzschia granii* from the NE Subarctic Pacific, and to optimize its growth under laboratory conditions.

2. To develop a protocol for measuring the photosynthetic efficiency of *P. granii* using Pulse Amplitude Modulated (PAM) Fluorescence.

3. To investigate the effects of light and Fe co-limitation on the physiology of *P. granii*.

4. To investigate the effects of light and temperature on the photosynthetic efficiency and growth of *P. granii* under ecologically relevant irradiances and temperatures.
Chapter 3
Effect of Light and Fe on the Physiology of
Pseudo-nitzschia granii Isolated From the NE Subarctic Pacific

Introduction

Phytoplankton photosynthesis (primary production) is a significant carbon sink from the atmosphere into the ocean. One of the most important chemical factors limiting primary production in ~30% of the oceans is iron (Fe) (Boyd et al., 2000; Hutchins, 1995; Geider and La Roche, 1994; Martin et al., 1990). In high latitude Fe-limited areas, such as the NE subarctic Pacific and the Southern Ocean (Timmermans et al., 2001; Maldonado et al., 1999), primary production may also be limited by the availability of light, especially in the winter season where surface irradiance is low, days are short and the mixed layer is deep. Fe nutrition and light absorption are strongly coupled through the process of photosynthesis. In the photosynthetic unit, Fe is required as a structural building block in photosynthetic reaction centers (Photosystems 1 and 2 (PS1, PS2)) and as an electron carrier in cytochromes and ferredoxin because of its redox versatility (Geider and La Roche, 1994). In order to maximize light absorption, cells grown under low light increase the concentration of photosynthetic pigments and reaction centers, thereby increasing their Fe quota (i.e. requirement). Sometimes light limitation drives organisms to the point of Fe limitation (Falkowski et al., 1985). Chlorophyll synthesis is dependent on Fe availability, and Fe-limited cells are often chlorotic, which hinders their ability to absorb light (Kudo and Harrison, 1997; Greene et al. 1992; 1991). Therefore, cells grown under low light and low Fe are considered to be under a "double stress". The individual effects of photoacclimation and Fe limitation on the photosynthesis of various phytoplankton are well documented in the
scientific literature (Langdon, 1988). However, the combined effects of both light and Fe on the physiology of oceanic phytoplankton are rarely studied (Van Leeuwe and De Baar, 2000).

There is preliminary evidence to Fe and light co-limitation in the field. During the winter season at Ocean Station P (OSP, 145°N 45°W), increasing irradiance along with a Fe addition in shipboard experiments results in a dramatic increase in chlorophyll concentrations and carbon uptake, more than when each factor is added separately (Maldonado et al., 1999). Similar results have been obtained in the Southern Ocean (Timmermans et al. 2001).

It is especially important to study the effect of Fe and light co-limitation on the physiology of oceanic diatoms. Large diatoms are rare in Fe-limited regions (<6% of biomass, Harrison et al., 1999) because of their low surface area to volume ratio, which is thought to hinder their ability to compete with smaller, more efficient cells for Fe (Harrison et al., 1999). In Fe-limited regions, when Fe is added during shipboard experiments, large diatoms grow quickly and dominate the enriched assemblage (Boyd et al., 1995a; 1995b; Martin et al. 1994; 1990). The ability to survive Fe stress under limiting conditions, as well as opportunistic growth when Fe is added, indicates that diatoms are physiologically well adapted to Fe stress. Studies on the physiology of diatoms often focus on coastal, centric diatoms, which are easier to culture and maintain in the laboratory than either pennate or oceanic diatoms.

Pennate diatoms account for 30% of diatomaceous carbon at OSP (Boyd and Harrison, 1999), and the genus *Pseudo-nitzschia* dominates Fe-enriched assemblages, especially in the summer (Boyd et al., 1995a; 1995b; M. Lipsen, pers. comm.). Some coastal strains of *Pseudo-nitzschia* produce domoic acid, which causes amnesic shellfish poisoning.
and is responsible for the death of humans and wildlife on the eastern coast of Canada and the coast of California respectively (Bates et al., 1999; Horner and Postel, 1993; Bates et al., 1989). It is unknown whether *Pseudo-nitzschia granii* or any other oceanic *Pseudo-nitzschia* sp. produce domoic acid at ecologically relevant levels.

This study examines the effect of light and Fe on the oceanic diatom, *Pseudo-nitzschia granii* (isolated from OSP in June of 2000). In addition to physiological and biochemical parameters (growth rates, chlorophyll, particulate organic carbon, particulate organic nitrogen and biogenic silica), photosynthetic efficiency was measured using pulse amplitude modulated (PAM) chlorophyll fluorescence. This technique is widely used in terrestrial plant physiology, and has been only recently adapted for liquid phytoplankton samples (Schreiber, 1994). The efficacy of fluorescence-based techniques in measuring primary production is widely debated, but the possibility of employing them to measure photosynthetic efficiency in the oceans is desirable because they are easy, inexpensive and non-invasive (Hartig et al., 1998; Boyd et al., 1997). Chlorophyll fluorescence provides molecular information about the physiological status of the cell without the use of invasive, biochemical techniques. In this study, maximum photosynthetic efficiency (F_v/F_m), quantum photosynthetic efficiency (ΦPS2), photochemical quenching (P_q) and non-photochemical quenching (Q_n) were measured using PAM fluorescence (see Chapter 2 for review).
Materials and Methods

Diatom isolation:

Chains of Pseudo-nitzschia granii were isolated from Fe-enriched (2 nM FeCl$_3$ in 10% HCl stock) OSP samples collected from Go-Flo® bottles on board the CCGS J.P. Tully in June of 2000. The samples were kept at low light and temperature (4°C, 12:12 L:D cycle at 50 μmol photons m$^{-2}$ s$^{-1}$) and enhanced with ESAW vitamins (Harrison et al., 1980) and AQUIL nutrients (Price et al., 1988/1989; Appendix 1) until the time of isolation (Chemicals purchased from Sigma-Aldrich). The cells were 60 μm in length and 5 μm in width at the time of isolation, and each chain contained 5-6 cells. The isolation medium consisted of AQUIL nutrients (metal stocks at half the concentration of the recipe to avoid metal toxicity) in a base of filtered (0.2 μm, trace metal clean) OSP seawater. The chains were isolated using two techniques: picking individual chains (to isolate monoclonal cultures) and dilution (to isolate polyclonal cultures). Experimental cultures were polyclonal to facilitate sexual reproduction (Hiltz et al., 2000). The isolated cultures were maintained at the above conditions until growth rates stabilized (almost a month). Growth rates were monitored using fluorescence (Turner AU-10 fluorometer). The species of Pseudo-nitzschia was identified by Dr. Vera Trainer's group (University of Washington, Seattle) using scanning electron microscopy.

Culture Conditions:

Semi-continuous batch cultures were maintained in Aquil/SOW medium (Appendix 1). There were two Fe treatments: Fe-replete (control, pFe 19.4, where pFe = - log [Fe$^{3+}$])
and Fe-deficient (Fe-limited or Fe-deplete, with no added Fe, background contamination was <2 nM (total Fe), pFe = 24-25). Nutrient stocks and SOW were treated with Chelex 100 ion exchange resin (Bio-Rad, Richmond, CA) to remove traces of Fe (Price et al., 1988/89). Cultures were grown in polycarbonate containers (30 ml), which were acid-washed to avoid Fe contamination. The containers were first incubated in 10% HCl for one week, rinsed with Milli-Q® water, incubated in 10% Seastar® HCl (Seastar Chemicals, Sidney, BC) for one week, rinsed with Milli-Q® water again, and finally incubated in 0.1 N Seastar® acetic acid (Price et al., 1988/1989). Culturing tubes were microwave-sterilized (Keller et al., 1983) in Milli-Q® water before usage. There were 6 light treatments (166, 90, 60, 30, 15 and 7 µmol photons m⁻² s⁻¹, continuous light), supplied by cool white Vita-lite lamps (110 W) and attenuated using neutral density screening. The experiments were conducted at 11.5°C. Cultures were grown in triplicate, and acclimated to their respective treatments before sampling (between 10 and 20 generations depending on the treatment). Acclimation was achieved when growth rates stabilized. Cells were enumerated using a Coulter Counter (Model Z). Growth rates (µ, doublings day⁻¹) were calculated as the slope of the natural log of fluorescence (in vivo chlorophyll) values over time (Guillard, 1973). Sampling was conducted during exponential growth.

**Chlorophyll (Chl cell⁻¹), non-modulated chlorophyll fluorescence (FSU) per unit chlorophyll (FSU Chl⁻¹):**

Basal, non-modulated chlorophyll fluorescence (in fluorescence unit, FSU) was measured using 10 ml of intact sample in the Turner AU-10 fluorometer. Chlorophyll was extracted according to the standard, fluorometric method of Parsons et al. (1984); a 10 ml sample was filtered through a GF/F filter, which was then extracted in 90% acetone for 24 h.
in the dark in the freezer. The samples were then thawed for half an hour and the fluorescence before and after acidification was measured to correct for phaeopigments. These chlorophyll measurements accounted for chlorophyll \( a \), and chlorophyll \( c \) was not measured. Chlorophyll per cell (Chl cell\(^{-1}\)) was calculated by normalizing chlorophyll to cell number. Basal fluorescence was normalized to extracted chlorophyll to calculate FSU chl \(^{-1}\).

**Photosynthetic Efficiency Using DCMU (\( F_{V}/F_{M} \text{DCMU} \))**: 

The fluorescence of a 10 ml sample was measured to obtain basal fluorescence, \( F_{o} \). The fluorescence was measured again after the addition 50 \( \mu \)l of 40 \( \mu \)M 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) to obtain maximal fluorescence, \( F_{M} \). \( F_{V}/F_{M} \) was calculated according to the equation in Table 3.1.

**Biogenic Silica (BSi)**: 

A 10 ml sample was filtered through a 0.6 \( \mu \)m polycarbonate filter. Biogenic silica was dissolved then measured spectrophotometrically following Parsons et al. (1984).

**Particulate Organic Carbon and Particulate Organic Nitrogen (POC and PON)**: 

A 10 ml sample was filtered through a combusted (450°C for 4.5 h) GF/F Whatman® filter. Ample care was taken not to contaminate the samples with extraneous carbon or nitrogen. The filters were dried at 60°C for 24 h and stored until analysis using a Carlo Erba NCS elemental analyzer NA1500. Filter blanks and container blanks were subtracted from the samples.

**Modulated Chlorophyll Fluorescence**: 

Chlorophyll fluorescence kinetics were measured using a 101 PAM fluorometer (Walz, Germany) complete with a cuvette system (Schreiber, 1994). Fluorescence was
measured at mid-exponential growth with chlorophyll concentrations between 20-40 μg L⁻¹ to minimize noise without affecting the integrity of the signal (Ting and Owens, 1993; 1992). A 2 ml sample was acclimated for 15 min in the dark before measurements commenced (Ting and Owens, 1992). Basal light intensity was chosen so that it was strong enough to give a signal (basal fluorescence, $F₀$ between 0.30 -0.4) without inducing a photosynthetic effect (otherwise known as an actinic effect). A pulse of saturating light (> 2000 μmol photons m⁻² s⁻¹ for 700 ms, Cole-Palmer lamp) was applied to measure maximum fluorescence, $F_M$, which is analogous to $F_{M, DCMU}$). Actinic light was applied to the sample at the same intensity as the growth light (Hanstech lamp, attenuated using neutral density screening) (see Fig. 2.2 for trace kinetics of PAM fluorescence). Once steady state fluorescence was achieved ($F_S$), saturating pulses (at 2000 μmol photons m⁻² s⁻¹, 300 ms) were applied every 20 s to measure $F'_{M}$. $F'_{0}$ was measured after the fluorescence signal stabilized when the actinic light was turned off (see Chapter 2 for details, Fig. 2.2).

Theoretical photosynthetic efficiency ($F_V/F_M$), quantum photosynthetic efficiency ($\phi PS2$), linear rate of electron transport ($J_e$), photochemical quenching ($P_q$), redox poise (1-$P_q$) and non-photochemical quenching ($Q_n$) were measured and calculated according to equations in Table 3.1.

**Statistical Analysis:**

An analysis of variance (ANOVA), followed by a Student’s t-test was used to determine the statistical differences between treatments ($\alpha = 0.95$) (assuming data were normally distributed). When specified, a linear regression was also used to determine the degree of correlation between parameters ($r^2$). All statistical analyses followed Zar (1984).
Table 3.1: Equations of photosynthetic parameters calculated from chlorophyll fluorescence kinetics. F$_v$ is variable fluorescence, F$_{Mv}$ is maximal fluorescence, F$_o$ is basal fluorescence, F$_s$ is steady state fluorescence, F'$_M$ is variable fluorescence during steady state, F'$_o$ is basal fluorescence following steady state, Q$_A$ is the phaeophytin A, I is incident light intensity and DCMU, is 3-(3,4- dichlorophenyl)-1,1dimethyl urea. References listed are examples of how these parameters are applied.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Equation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum Photosynthetic Efficiency (DCMU)</td>
<td>F$_v$/F$_M$</td>
<td>$\frac{F_{MDCMU} - F_o}{F_{MDCMU}}$</td>
<td>Parkhill et al. (2001)</td>
</tr>
<tr>
<td>Maximum Photosynthetic Efficiency</td>
<td>F$_v$/F$_M$</td>
<td>$\frac{F_M - F_o}{F_M}$</td>
<td>Schreiber et al. (1995a; 1995b); Bradbury and Baker (1981)</td>
</tr>
<tr>
<td>Quantum Yield of PS2</td>
<td>$\phi_{PS2}$</td>
<td>$\frac{F'_M - F'_s}{F'_M} = \frac{\Delta F}{F'_M}$</td>
<td>Genty et al. (1989)</td>
</tr>
<tr>
<td>Rate of Linear Electron Transport</td>
<td>$J_e$</td>
<td>$\phi_{PS2} * I$</td>
<td>Maxwell and Johnson (2000); Genty et al. (1989)</td>
</tr>
<tr>
<td>Photochemical Quenching</td>
<td>$P_q$</td>
<td>$\frac{(F'_M - F'_s)}{(F'_M - F'_o)}$</td>
<td>Buschmann (1999)</td>
</tr>
<tr>
<td>Redox Poise</td>
<td>$1 - P_q$</td>
<td>$\frac{Q_{ARED}}{Q_{ARED} - Q_{AOXD}}$</td>
<td>Maxwell et al. (1995a;1995b)</td>
</tr>
<tr>
<td>Non-photochemical Quenching</td>
<td>$Q_n$</td>
<td>$1 - \frac{(F_M - F'_o)}{(F'_M - F'_o)} = \frac{F_v - F'_v}{F_v}$</td>
<td>Maxwell and Johnson (2000); Buschmann (1999)</td>
</tr>
</tbody>
</table>
Results

Diatom Isolation:

_Pseudo-nitzschia granii_ maintained its chain-forming ability in the isolation medium. The cells were gradually acclimated to an AQUAL/Synthetic Ocean Water (SOW) medium (Price et al., 1988/1989), whereby the number of cells per chain decreased to 3 per chain. Abnormalities in the shape of the cell appeared in this medium shortly after transfer. However, the cells did resume their natural shape two months after acclimation (Figs. 1 and 2). This is not unusual in cultures of nitzschoid pennate diatoms (Suba Rao and Wohlgeschaffen, 1990) and is thought to be the result of metal concentrations higher than natural oceanic levels. The length of cells decreased to 25 μm (length) and ~ 3.5 μm (width) by the time of the experiment.

Growth Rate and Biochemical Parameters:

The growth rate of Fe-limited _Pseudo-nitzschia granii_ (Figs. 1 and 2) was 10-50% (average 30%) lower than Fe-replete cells (Fig. 3.3A). Over the range of irradiances studied, the greatest differences between Fe treatments occurred in the middle range of irradiance (Fig. 3.3B). In general, growth rates decreased with decreasing irradiance; at the two lowest light treatments (7 and 15 μmol photons m\(^{-2}\) s\(^{-1}\)), growth rates stabilized at 0.35 for Fe-replete cells, and 0.2 for Fe-limited cells. The initial slope (□) of the growth irradiance (μ-I) was higher under Fe-replete conditions than under Fe limitation (6.5 x 10\(^{-3}\) and 2.2 x 10\(^{-3}\) respectively). Whereas Fe-replete cells reached a μmax of 0.81 day\(^{-1}\) at 166 μmol photons m\(^{-2}\) s\(^{-1}\), Fe-limited cells did not plateau under the range of irradiance used in this study.
Under Fe-replete conditions, chl cell$^{-1}$ at 7 μmol photons m$^{-2}$ s$^{-1}$ was 3-fold higher than at 166 μmol photons m$^{-2}$ s$^{-1}$ (Fig. 3.4A). Chl cell$^{-1}$ was inversely related to light (Fig. 3.4A). Under Fe-limited conditions, the relationship between chl cell$^{-1}$ and light was not as pronounced as for Fe-replete cells, as chl cell$^{-1}$ at 7 μmol photons m$^{-2}$ s$^{-1}$ was only 2-fold higher at low light than at high light. However, cells under Fe limitation were severely chlorotic compared to Fe-replete cells (P <0.05). Chlorosis means chlorophyll deficiency, or bleaching mostly due to nutrient stress or extreme physical stress.

Normalizing chl to particulate organic carbon removes the effect of differing cellular volumes between treatments and allows a comparison of the response of Fe limitation between various organisms. C chl$^{-1}$ was significantly higher under Fe limitation compared to Fe-replete conditions and chl cell$^{-1}$ increased with increasing irradiance (Fig. 3.4b).

Basal non-modulated fluorescence is a good indicator of physiological stress in phytoplankton. Normalizing FSU to chlorophyll allows comparison between organisms of differing chlorophyll concentrations. FSU chl$^{-1}$ was generally higher for Fe-limited cells compared to Fe-replete cells (P< 0.05), and was inversely related to irradiance in Fe-limited cells (Fig. 3.5).

The C:N ratio is a useful indicator of nitrogen stress in marine phytoplankton (see discussion). In this experiment, the C:N ratio ranged between 7 and 10, and was generally higher, though not significantly (P >0.05) under Fe replete conditions as compared to Fe-limited conditions, and C:N was inversely related to irradiance (Fig. 3.6A). C cell$^{-1}$ was not significantly higher in Fe-replete cells (P>0.05) compared to Fe-limited cells (Fig 3.6B). Cells under Fe limitation also exhibit a lower N cell$^{-1}$ than Fe replete cells (P< 0.05) (Fig. 3.6B). There was no relationship between either cellular POC or PON with light.
In the field, the uptake ratio of Si to N is measured to assess Fe limitation. The premise of the Si:N ratio as an indicator of Fe limitation is that Fe limitation decreases nitrogen uptake (due to decreased activity of nitrate reductase), but does not affect Si uptake (Hutchins et al., 1998; Takeda, 1998). Therefore, Fe-limited cells have a higher uptake ratio of Si to N than Fe-sufficient cells. However, the molar ratio of biogenic Si to N is rarely studied. Furthermore, the behavior of this ratio has not been examined under conditions of balanced growth. In this study, the molar ratio of BSi to PON (BSi:N) under balanced (acclimated) growth was measured. The BSi: N ratio was at least 2-fold higher in Fe-replete cells compared to Fe-deficient cells and the ratio displayed a hyperbolic relationship with light (Fig. 3.7A).

BSi cell\(^{-1}\) was significantly higher (P <0.05) in control cells compared to Fe-deficient cells, and like BSi:N, it displayed a hyperbolic relationship with light (Fig. 3.7b). It is commonly hypothesized that the proteinaceous matrix for silica deposition in diatoms is composed of mainly serine and glycine, two by-products of photorespiration. Therefore, cells with higher levels of photorespiratory activity will have a thicker silicate frustule.

**Chlorophyll Fluorescence Kinetics:**

\(F_V/F_M\) was measured using the PAM fluorometer (results not shown) and by DCMU treatment. Using both measurements, \(F_V/F_M\) was significantly higher under Fe-replete conditions compared to Fe-limited conditions (P <0.05) (Fig. 3.8). \(F_V/F_{M\text{(DCMU)}}\) was not significantly affected by light in either Fe treatments. Results from both methods correlated strongly (\(r^2 = 0.69, P< 0.05\)) (Fig. 3.9).

Over the entire range of treatments in this experiment, the relationship between chl cell\(^{-1}\) and \(F_V/F_{M\text{(DCMU)}}\) was non-linear (Fig. 3.10); when chl cell\(^{-1}\) was higher than 5 pg cell\(^{-1}\),
Fv/Fm stabilized at ~0.6 (as seen in Fe replete cells). At concentrations below this threshold level, as seen in Fe-limited cells, Fv/Fm decreased linearly with decreasing chl cell\(^{-1}\).

The quantum yield of photosynthesis, \(\varphi_{PS2}\) was much higher under Fe-replete conditions than under Fe limitation (\(P < 0.05\)), and was inversely related to irradiance (Fig. 3.11).

In this study, the relative rate of linear electron transport, \(J_e\), was calculated by multiplying \(\varphi_{PS2}\) with incident light without accounting for fractionation between the photocenters, the photosynthetic cross-section, or the fraction of electrons donated to non-photosynthetic enzymes. \(J_e\) was significantly higher in Fe-replete cells than under Fe limitation (\(P < 0.05\)) (Fig 3.11B). Under both Fe treatments, \(J_e\) had a strong, positive relationship with growth rate (\(r^2 = 0.95 - 0.96\)) (Fig. 3.12).

Photochemical efficiency, \(P_{q}\) was not significantly different between Fe treatments (\(P > 0.05\)), and was inversely related to irradiance (Fig. 3.13A). The redox pressure on photosystem 2, \(1-P_{q}\), was not significantly different between Fe treatments (\(P > 0.05\)), and was positively related to irradiance (Fig. 3.13B). Non-photochemical quenching, \(Q_n\), was higher, though not significantly under Fe-limited conditions than under Fe-replete conditions (\(P > 0.05\)). Under Fe-replete conditions, \(Q_n\) was significantly lower at low light than high light (\(P < 0.05\)) (Fig. 3.14).
Figure 3.1: A) Scanning electron micrograph of *Pseudo-nitzschia grani*. Arrow points to diatoms (bar = 10 μm). B) Deformed cell after three months of isolation. Arrow points to deformation (both bars = 10 μm), and C) close-up of surface features (bar =1 μm; Photos were taken by Brian D. Bill, University of Washington).
Figure 3.2: Light micrographs of *P. granii* (bar = 10 μm). Arrow points to diatom chain.
Figure 3.3: Growth rate ($\mu$) vs. irradiance for *P. granii* grown under Fe-replete conditions (black circles) and Fe-deplete conditions (white circles) (A) and, ratio of Fe-deplete to Fe-replete growth rates vs. irradiance (B). Error bars represent standard error from triplicate cultures and are smaller than the symbol when invisible.
Figure 3.4: Chl cell\(^{-1}\) vs. irradiance (A), and C chl \(^{-1}\) vs. irradiance (B) for *P. granii* grown under Fe-replete conditions (black circles) and Fe-deplete conditions (white circles). Error bars represent standard error from triplicate cultures and are smaller than the symbol when invisible.
Figure 3.5: FSU chl$^{-1}$ vs. irradiance for *P. granii* grown under Fe-replete conditions (closed circles) and Fe-deplete conditions (open circles) (n=1).
Figure 3.6: C:N molar ratios vs. irradiance (A), and (B) C cell\(^{-1}\) vs. irradiance and (C) N cell\(^{-1}\) vs. irradiance for *P. granii* grown under Fe-replete conditions (black circles) and Fe-deplete conditions (white circles).
Figure 3.7: BSi:N molar ratios vs. irradiance (a), and BSi cell$^{-1}$ vs. irradiance (b) for *P. granii* grown under Fe-replete conditions (black circles) and Fe-deplete conditions (white circles). Error bars represent standard error from triplicate cultures and are smaller than the symbols when invisible.
Figure 3.8: $F_v/F_M$ (DCMU) vs. irradiance for *P. granii* grown under Fe-replete conditions (black circles) and Fe-deplete conditions (white circles). Error bars represent standard error from triplicate cultures and are smaller than the symbol when invisible.
Figure 3.9: Linear regression between $F_v/F_M$ (DCMU) and $F_v/F_M$ measured using the PAM fluorometer.
Figure 3.10: The relationship between $F_{V}/F_{M(DCMU)}$ and Chl cell$^{-1}$ for $P$. granii grown under Fe-replete conditions (black circles) and Fe-deplete conditions (white circles). Solid lines are linear regression lines.
Figure 3.11: Quantum photosynthetic efficiency ($\phi$PS2) vs. irradiance (A), and relative electron transport ($J_e$) ($\mu$mol e$^{-1}$ m$^{-2}$ s$^{-1}$) vs. irradiance (B), for *P. granii* grown under Fe-replete conditions (black circles) and Fe-deplete conditions (white circles). Error bars represent standard error from triplicate cultures and are smaller than the symbol when invisible.
Figure 3.12: Linear regression of $J_e$ and $\mu$ for *P. granii* grown under Fe-replete conditions (black circles) and Fe-limited conditions (white circles).
Figure 3.13: Photochemical efficiency ($P_q$) vs. irradiance (A), and redox pressure (1-$P_q$) vs. irradiance (B), for *P. granii* grown under Fe-replete conditions (black circles) and Fe-deplete conditions (white circles). Error bars represent standard error from triplicate cultures and are smaller than the symbol when invisible.
Figure 3.14: Non-photochemical quenching ($Q_n$) vs. irradiance for $P. \text{granii}$ grown under Fe-replete conditions (open circles) and Fe-deplete conditions (closed circles). Error bars represent standard error and are smaller than the symbol when invisible (n=3).
Discussion

The overall goal of the experiment was to study the effects of light and Fe co-limitation on the physiology of *P. granii*. This study was one of very few on the physiology of an oceanic, pennate diatom grown under laboratory conditions, and one of the first to limit the growth rate of a native HNLC species using Fe-limited synthetic ocean water rather than enriched natural seawater. Fe limitation was attained by acclimating *Pseudo-nitzschia granii* to a synthetic ocean medium without added Fe. Though using natural seawater eliminated possible artificial effects from synthetic media, it did not allow full chemical control over the properties of the medium selected. In addition, most laboratory experiments on oceanic phytoplankton employed growth temperatures higher than the ecologically relevant temperatures. In this experiment, *Pseudo-nitzschia granii* was grown at a temperature relevant to temperatures measured at OSP (between 6 and 12°C) and under ecologically relevant irradiances. However, the experiment was conducted under continuous light to minimize circadian changes in physiology that have been shown to influence results, especially chlorophyll fluorescence (Anning et al., 2000; Strasser et al., 1999). For example, variations in the light:dark cycle have been shown to affect sexual reproduction in diatoms (Hiltz et al., 2000).

Growth rates:

The relationship between growth and irradiance (μ-I curves) is an important tool in modeling primary productivity in the ocean. The hyperbolic μ-I curve can be divided into two regions (Langdon, 1988): 1) light-limited growth rates characterized by the initial slope of the curve (α) and, 2) light-saturated growth characterized by the plateau (μmax). Light-
limited growth is affected by the ability of the cell to absorb and process light, whereas light-saturated growth is affected by factors which limit the carbon fixation pathway, such as respiration, excretion of waste and temperature (Greene et al., 1991; Langdon, 1988). The response of growth to light may differ interspecifically, primarily due to differences in pigment composition between different taxa of phytoplankton (Langdon, 1988). It is worth noting that diatoms are thought to use light more efficiently compared to other phytoplankton (Langdon, 1988).

As expected, Fe-limited cells grew much slower (between 10 and 50% of Fe-replete growth rates) than Fe-replete cells (Fig. 3.3A). The most pronounced differences between treatments were observed in the middle range of irradiance (Fig. 3.3B). Fe-limited coastal diatoms typically grew at 30% of Fe-replete growth rates (Greene et al., 1992; 1991; Hudson and Morel, 1990). It had been shown that Fe-limited oceanic diatoms, specifically those isolated from HNLC regions, have a higher growth rate (50% of Fe-replete) than their coastal relatives (Muggli et al., 1996). This was due to a difference in Fe quotas between the species; coastal cells had much higher Fe quotas than oceanic strains and were much more sensitive to Fe limitation under laboratory settings (Sunda and Hunstman, 1997). The fact that Fe-limited *P. granii* maintained relatively high growth rates was a testament to how well adapted these cells were to Fe limitation.

Fe-limited cells exhibited a 3-fold lower \( \alpha \) than Fe-replete cells (Fig. 3.3A). Fe limitation is thought to affect the initial slope of the \( \mu-I \) curve by decreasing the concentration of photosynthetic pigments and reaction centers (Greene et al., 1991; 1992), therefore decreasing the ability of the cell to absorb light. It indirectly affects the maximum rate of photosynthesis by limiting the supply of ATP and NADPH to carbon fixing enzymes,
or by increasing dark respiration rates (Greene, 1992; R. Strzepek, unpublished results). It has also been shown that pronounced changes in the initial slope of the μ-I curve might be due to the change of the optical cross-section of PS1 as compared to PS2 (Dubinsky et al., 1986).

_**P. granii** grew faster with increasing irradiance regardless of its nutritional status (Fig. 3.3a). High light resulted in a small compensation or increase in growth rate under Fe limitation. Under both Fe treatments, growth rates reached a low plateau under low light. Results obtained by Ivanov et al. (2000) demonstrated a similar trend in Fe-limited *Synechococcus* where shuffling of cytochrome f, due to Fe limitation, impaired electron transport, which then decoupled PS2 and PS2. This enhanced cyclic electron transport around PS1 despite a lower concentration of active PS1 centers, leads to higher growth rates. Since most photosynthetic cells grown under sub-saturating irradiance have large Fe quotas (Hudson and Morel, 1990), decoupling between PS1 and PS2 is plausible under low light as well as under Fe limitation. Unlike cyclic electron transport around PS2 (Falkowski et al., 1986), cyclic electron transport around PS1 has not been observed in diatoms. In addition, whereas cyclic electron transport around PS2 does not contribute to carbon fixation nor to growth, cyclic electron transport around PS1 produces ATP by contributing to the proton gradient (Ivanov et al., 2000). Interestingly, the same pattern of growth at low light was comparable under both Fe treatments, indicating that it might have been induced primarily by low light. Finally, Fe contamination was not a likely culprit in increasing growth rates at low light given that this increase was also observed under Fe-replete conditions in _P. granii_.

**Chlorophyll and chlorophyll fluorescence:**
Fe-limited cells had a significantly lower concentration of chl cell\(^{-1}\) than in Fe-replete cells (Fig. 3.4A). Fe is thought to play a central role in the formation of protoporphyrin, one of initial steps of chlorophyll synthesis (Falkowski and Raven, 1997; Geider and La Roche, 1994). In addition to chlorosis, Greene et al. (1991) reported a decrease in PS2 proteins (namely CP43, CP47 and D1) in response to Fe deficiency in the coastal pennate diatom *Phaeodactylum tricornutum*. However, nuclear encoded light harvesting proteins were not affected by Fe limitation. *P. granii* grown under low irradiance had high Chl cell\(^{-1}\), which was a common response to low irradiance in phytoplankton in order to optimize light absorbency (Hutchins, 1995, Geider and La Roche, 1994; Greene et al., 1992).

The C chl\(^{-1}\) ratio is important in estimating specific growth rates of phytoplankton *in situ* (Boyd and Harrison, 1999; Harrison et al., 1999), and is an important indicator of photoacclimation. The ratio is usually between 10 and 200 in the field, but higher values (up to 500) have been reported in the laboratory (Boyd and Harrison, 1999; Muggli and Harrison, 1996; Le Court et al., 1994). The values of C chl\(^{-1}\) obtained in this study fell within the range previously reported for open ocean diatoms and coccolithophores (between 80 and 200) (Muggli and Harrison, 1997; Muggli et al., 1996) (Fig. 3.4B). C chl\(^{-1}\) was primarily affected by the rate of chlorophyll synthesis compared to growth rate and therefore, was low under low light and high under Fe limitation. Genetic variability, nutrient status and factors influencing carbon fixation and linear electron transport are also affected by the C chl\(^{-1}\) ratio (Falkowski and Raven, 1997; Eppley et al., 1973).

Under Fe limitation, FSU chl\(^{-1}\) was higher than Fe-replete cells and it was also negatively related to light (Fig. 3.5). This agreed with previously published results for diatoms (Le Court et al., 1994), and in sugar beets (Belkhodja et al., 1998). The differences
in FSU chl¹ between high and low light intensities were less pronounced under Fe-replete conditions. Cells with impaired photosynthetic machinery, had a high basal fluorescence since photochemical processes were blocked and most of the light absorbed by the cells was re-emitted as fluorescence (Chapter 2). A similar effect was observed in fluorescence vs. depth traces in the field, where the fluorescence of photoinhibited cells in the upper layers of the euphotic zone is high compared to the actual chlorophyll biomass (Kolber et al., 1994). Normalizing fluorescence to chlorophyll facilitated comparisons between treatments of different cellular chlorophyll concentrations.

**Particulate Organic Carbon (POC) and Particulate Organic Nitrogen (PON):**

The ratio of POC to PON (C:N) is a useful indicator of nutrient stress and the physiological status of marine phytoplankton (Falkowski, 2000) (Fig. 3.6A). Because of differences in cellular composition and in nutritional stresses, field measurements of the C:N ratio of marine phytoplankton range between 4 and 40 (Geider, 1987) and differ slightly between taxa. The majority of carbon in marine algae is in carbohydrates, proteins and lipids, and the majority of nitrogen is in amino acids with a smaller fraction in photosynthetic pigments (1% of cellular mass) (Parsons et al., 1961). Physiological conditions that limit nitrogen metabolism (fixation or reduction) yield higher C:N ratios. The effect of Fe on the C:N ratio is complicated by the fact that Fe limitation affects carbon fixation (through linear electron transport) as well as nitrogen reduction through nitrate reductase, which has a high Fe requirement (~23 atoms per molecule) (Geider and La Roche, 1994). Other factors that affect cellular composition, such as temperature, also influence the C:N ratio (Gao et al., 2000).
In this study, C:N ratios were generally high under Fe-replete conditions. This effect was most likely due to limitations imparted on nitrate reduction due to Fe depletion (Muggli and Harrison, 1997; Doucette and Harrison, 1991). The average C:N was 8.7, which was slightly higher than what was previously reported for diatoms (~7) (Thompson et al., 1991; R. Strzepek, pers. comm). This was not unexpected since diatoms vary widely in cellular composition, and pennate diatoms have a higher concentration of carbohydrates and pigments than most diatoms (Parsons et al. 1961).

**Biogenic Silica (BSi):**

It has been hypothesized that silicate uptake, unlike nitrate uptake, does not require Fe (De La Roche et al., 2000; Takeda, 1998). The uptake ratio of silicate to nitrate is usually 2-fold higher in Fe-limited cells (~2.5) compared to Fe-replete cells (~1) (Hutchins et al., 1998; Takeda, 1998). The BSi:N ratio in this study was much higher in Fe-replete cells compared to Fe-limited cells (Fig. 7A). BSi:N ratios in Fe-replete cells were comparable to what has been reported in the literature (De La Rocha et al., 2000). As mentioned previously, the BSi: N ratio measured in this study is not an uptake ratio and it was measured during balanced growth as opposed to unbalanced growth. These results indicate that the interpretation of BSi:N as an indicator of Fe stress may be dependent on the conditions of the experiment, and that the BSi:N responds differently to Fe depending on whether it is an uptake ratio or a steady state ratio. The results of this study also provide preliminary evidence that silicate metabolism could be Fe-dependent.

The hyperbolic relationship observed between BSi cell\(^{-1}\) and irradiance in this experiment supported the hypothesis that silicate uptake is powered by photorespiration (Lippemeier et al., 1999) (Fig. 7B). Photorespiration was triggered by high light because of
an increase in respiration substrate provided by high rates of photosynthesis (Hall and Rao, 1999). Because photorespiration generated amino acids that acted as a matrix for the silica frustule (Lippemeier et al., 1999), diatoms grown under high light might be heavier than those grown under low light. Incidentally, depressed \( \varphi_{PS2} \) at high light might provide additional support for increased photorespiration activity at high light (see Fig. 3.11A and the discussion below). In addition, there was evidence that low rates of photosynthesis were correlated with decreased sinking rates, indicating that under energetic stress, cells ceased silicate uptake inducing dissolution of their frustules (Culver and Smith, 1989). The relationship between BSI cell\(^{-1} \) and irradiance could have been a result of the relationship between irradiance and light (Fig. 3.3), indicating that faster growing diatoms (at high light) required more silicate than slower growing diatoms (at low light).

**Photosynthetic efficiency \((F_V/F_M)\):**

As mentioned in chapter 2, the herbicide DCMU blocks the transfer of electrons from the acceptor side of PS2 to the electron carrier \( Q_A \). In other words, it blocks photochemical quenching of fluorescence. \( F_V/F_M \) denotes maximal, or theoretical, photochemical quenching of cells. Whereas interspecific differences in \( F_V/F_M \) between groups of phytoplankton are primarily due to different pigment composition, photosynthetic unit arrangement and mechanisms that alter \( F_0 \) (e.g. dark reduction of PS2) (Buchel and Wilhelm, 1993; Juneau (unpublished results)), intraspecific differences in \( F_V/F_M \) are mainly due to damage in PS2 centers.

\( F_V/F_M \) had been applied in the field to assess nutrient limitation of phytoplankton with mixed results. Parkhill et al. (2001) showed that under conditions of unbalanced growth, \( F_V/F_M \) provides a good indicator of nutrient stress, specifically nitrate stress. However, once
cells acclimated to nutrient stress (balanced growth), Fv/FM rose to control levels despite nutrient limitation. A similar trend was observed by Lippemeir et al. (1999) for silicate-limited *Thalassiosira weissflogii*. However, Fv/FM is an excellent indicator of Fe stress since it invariably makes cells more sensitive to photoinhibition (Kolber et al., 1994). In this study, Fv/FM (DCMU) values were reported, and PAM values were used mainly to compare both techniques to test whether the pulses of light employed in PAM studies were saturating.

For Fe-replete cells of *Pseudo-nitzschia grannii*, Fv/FM (DCMU) was ~0.6 (Fig. 3.8), which was within the range reported for diatoms (Buchel and Wilhem, 1993). Fv/FM (DCMU) was higher in Fe-replete cells compared to Fe-limited cells, which was in agreement with previously published results (Boyd et al., 2000). Fv/FM (DCMU) did not vary with light under Fe-replete conditions, which indicated that despite the damages incurred by the photosynthetic apparatus due to Fe limitation, the range of irradiances used in this experiment was not high enough to cause photoinhibition.

A comparison between Fv/FM obtained by the PAM fluorometer and by DCMU revealed a strong correlation between both ratios (r² = 0.67) (Fig. 3.9). This was very similar to what Parkhill et al. (2001) observed, and was a good indication that the pulses employed were saturating (Ting and Owens, 1993). Deviations from a 1:1 relationship between the ratios were partially due to optical differences between modulated and non-modulated fluorescence (Parkhill et al., 2001; Juneau, unpublished results).

The relationship between Fv/FM and chl cell⁻¹ was different for each Fe treatment (Fig. 3.10). Fe-limited cells fell within the linear range of the curve, where there was a strong relationship between chl and Fv/FM (r² = 0.71). Fe-replete cells fell on the plateau where increasing chlorophyll concentrations did not affect Fv/FM (r² = 0.85). One suggestion is that
photoinhibition might have been triggered only below a threshold of chlorophyll concentration of ~ 5 pg chl cell$^{-1}$. Above this threshold chlorophyll concentration, $F_V/F_M$ stabilized at a value of ~0.6. However, more research on the relationship between photoinhibition and chl cell$^{-1}$ is necessary before conclusive results can be drawn.

**PAM Fluorescence Results:**

**Quantum yield of PS2 ($\phi_{PS2}$):**

Under Fe-replete conditions, there was an inverse relationship between light and $\phi_{PS2}$ (Fig. 3.11A), a trend also observed by Flameling and Kromkamp (1998) for a variety of phytoplankton species. Many factors cause the reduction of $\phi_{PS2}$ under high light, such as cyclic and pseudocyclic electron transport processes that compete with linear electron transport (Ivanov et al., 2000), as well as respiratory mechanisms, such as mitochondrial respiration and photorespiration (Flamling and Kromkamp, 1998).

$\phi_{PS2}$ was significantly lower under Fe limitation than under Fe-replete conditions (Fig. 3.11A). This was most likely due to the sluggishness of electron transfer induced by damaged reaction centers and electron carriers in the photosynthetic unit. The adverse effects of Fe limitation on primary productivity and oxygen evolution has been well documented in the laboratory (Greene et al.; 1991). At OSP and the Southern Ocean, the rate of carbon fixation was lower for *in situ* phytoplankton species as opposed to those to which Fe was added during on-deck incubation experiments (Maldonando et al., 1999). It was interesting that $\phi_{PS2}$ does not vary with light under Fe limitation; this was most likely due to damage to components of the photosynthetic unit which render the cells photoinhibited even under low light intensities, or to increased levels of respiration under lower light caused by Fe limitation.
Though cells were more efficient under low light than high light, they were nonetheless growing faster under high light. This was most likely due to an increase of the rate of linear electron transport ($J_e$) with increasing irradiance (Figs. 3.11B, 3.12).

Photochemical quenching ($P_q$), redox poise ($1 - P_q$) and Non-photochemical quenching ($Q_n$):

Photochemical quenching ($P_q$, Table 3.1) is the measure of the fraction of light used in photochemistry (the fraction of oxidized $Q_A$). In other words, $P_q$ denotes the functionality of the PS2. Unlike $F_v/F_M$, which indicates damage of the photochemical system, or the fraction of damaged PS2's, $P_q$ indicates the efficiency of PS2 centers regardless of their number.

In *P. granii*, $P_q$ was not significantly affected by Fe limitation, but rather, it was only affected by light (Fig. 3.13A). This agrees with Belkhodja et al. (1998) who demonstrated that in sugar beets, Fe limitation affected photosynthesis by decreasing the number of active PS2 centers rather than altering their efficiency. It was interesting that the only significant differences between Fe treatments were in intermediate irradiances that correspond to the transition between light-limited growth and light-saturated growth. The most drastic differences in cellular chlorophyll and growth rates between Fe treatments were also observed in the middle range of the irradiance. This was the region of the $D$-I curve where cells were in transition between light-limited growth and light-saturated growth (Fig. 3.3A), and was probably where most of the cellular machinery is dedicated to increasing cellular resources and activating xanthophyll pigments needed for light-saturated growth (Olaziola and Yamamoto, 1994). The results of $P_q$ and $\theta_{PS2}$ showed that Fe and light affected the photosynthetic apparatus differently, and whereas Fe affected the quantum yield of
photosynthesis (by affecting the number of reaction centers), light affected photosynthetic efficiency. High photosynthetic efficiency and high cellular chlorophyll were most likely acclimations to maximize light absorption at low light.

The redox poise (or excitation pressure) of the cells, $1-P_q$, represents the fraction of closed $Q_A$. It is an indicator of accumulation of electrons on the acceptor side of PS2 that slows electron transfer (Maxwell et al., 1995a). $1-P_q$ acts like the clutch of the photosynthetic cell, and affects various physiological processes, such as modulating the production of m-RNA, light harvesting proteins and the activation of photoprotective mechanisms (the xanthophyll cycle) (Maxwell et al., 1995a; 1995b).

The redox poise, $1-P_q$, was high under Fe stress and under high light where cells were bombarded with photons faster than they can absorb them, causing electrons to accumulate on the acceptor side of PS2 (Fig. 3.13B). This was supported by examining the trend in $Q_n$ (Table 3.1; Fig. 3.13).

Non-photochemical quenching, $Q_n$, is defined as the measurement of the fraction of chlorophyll fluorescence quenched by non-photochemical, usually photoprotective means (Krause and Weis, 1991). Several components of $Q_n$ could be deciphered from chlorophyll fluorescence signals depending on the kinetics by which they relax (Maxwell and Johnson, 2000). Due to the limitation of the PAM system employed by this experiment, only the overall $Q_n$ was measured. It has been shown that the $Q_n$ correlated well with increasing the activity of the xanthophyll cycle in diatoms.

Under high light, Fe-replete cells of *P. granii* had a higher $Q_n$ than under low light. This was probably due to the activation of the xanthophyll cycle to protect the cells from photodamage. Under Fe limitation, non-photochemical quenching was higher under low light
than it was for Fe-replete cells due to the damage incurred on the photosynthetic unit that impaired light absorption and caused cells to be more susceptible to photoinhibitory stress even under low light intensities. Therefore, whereas high light compensated for growth rates under Fe limiting conditions, it was not an ideal situation for the cells that incur physiological costs in the form of enhanced non-photochemical activity. In addition, under both Fe treatments, growth rates were a function of the rate of electron transport (Fig. 3.12). The differences between the magnitude of growth rates between Fe treatments were most likely due to the activation of non-photochemical mechanisms (such as Qn) that compete with photosynthesis and growth under Fe limitation.

Conclusions

_Pseudo-nitzschia granii_ is a pennate diatom isolated from OSP, and Fe-limited station in the NE Subarctic Pacific. In this study, _P. granii_ was successfully grown in artificial seawater culture medium. The effect of light and Fe co-limitation on the physiology of _P. granii_ was tested with the following conclusions:

1. _P.granii_ was remarkably acclimated to Fe stress and to light stress.
2. Growth rates of _P. granii_ were light dependent for light levels between 20- 100 μmol photons m⁻² s⁻¹. Fe-stress was more evident in cells in transition between light-limited and light-saturated growth.
3. Fe-limited _P. granii_ grew slowly, was chlorotic and fluoresced at a much higher level than Fe-replete cells.
4. Fe-limited _P. granii_ showed signs of silicate limitation (low BSi cell⁻¹). Under Fe limitation, the steady-state BSi: N ratios were different than the uptake BSi:N ratios.
Under high light, *P. granii* was more silicified than under low light possibly due to increased photorespiratory activity at high light.

5. Photoinhibition might have been triggered by low cellular chlorophyll concentrations.

6. Fe limitation affected photosynthesis by decreasing the number of photosynthetic centers (low $F_v/F_M$) rather than altering their efficiency ($P_q$). Photochemical efficiency was modulated by light.

7. Though $\phi PS2$ was higher at low light, $J_e$ was still significantly higher under high light. Therefore cells still grew faster at high light despite their lower photosynthetic efficiency.
Chapter 4

Effect of Irradiance and Temperature on the Physiology of

*Pseudo-nitzschia granii*

Introduction

Despite circadian and seasonal fluctuations of oceanic and coastal surface temperatures, the effects of temperature on phytoplankton productivity have not been well studied. Temperature affects the activity of cellular enzymes, and by changing membrane fluidity, and it also affects the activity of membrane-bound proteins (see Falkowski and Raven, 1997 and Davison, 1991 for reviews). Temperature is thought to affect enzymatic activity linearly until an optimum is reached, and the degree of dependence on temperature, as derived from the Arrenius equation, is defined as $Q_{10}$ (Davison, 1991).

Thermoacclimation, the process by which organisms acclimate to temperature, is a complicated process to study for a myriad of reasons. Cellular enzymes often have temperature optima independent of those for growth rates (Gao et al., 2000; Davison, 1991). Thermoacclimation is influenced by growth irradiance, stage of acclimation (balanced vs. unbalanced growth) and by genetic constraints on metabolic activity (Thompson, 1999; Suzuki and Takahashi, 1995; Davison, 1991). Temperature-induced responses in marine phytoplankton are also taxa specific and sometimes, species specific depending on the geographic region from which they were isolated. It has been shown that Antarctic species of phytoplankton have lower temperature optima than equatorial species (Davison, 1991). Therefore, extrapolating thermal responses from one group of phytoplankton to another is misleading, and thermal responses should ideally be studied on a species-by-species basis.
In addition to the physiology, changes in temperature have been shown to influence the species composition of phytoplankton assemblages (Suzuki and Takahashi, 1995). For example, changes in oceanic, surface temperatures associated with El-Nino/La-Nina events are accompanied by changes in primary productivity in the NE Subarctic Pacific (Boyd and Harrison, 1999) and on the west coast of Vancouver Island (Harris, 2001). A single phytoplankton species faces a wide range of temperatures because of horizontal advection between different regions and because of seasonal and daily changes in temperature. Therefore, understanding how temperature affects phytoplankton physiology is essential for understanding fluctuations of primary production in the oceans.

Recent evidence suggests that photoacclimation and thermal acclimation are opposite faces of the same physiological coin (Maxwell et al., 1995a; 1995b). As mentioned in previous chapters, irradiance affects the concentrations of cellular pigments, photosynthetic efficiency and m-RNA production of various compounds. Temperature also affects photochemical efficiency by affecting electron transfer through photosystem 2 (PS2) (Raven, 1988) and photosynthetic cells grown under low temperature are often chlorotic (Geider, 1987). Maxwell and colleagues (Maxwell et al., 1995a; 1995b) showed that low temperature-acclimated, *Dunaliella salina* is biochemically identical to *D. salina* acclimated to high light. The same response is shown for *Chlorella* by Wilson and Huner (2000). These researchers argue that temperature and irradiance interact to co-modulate the redox pressure (poise) of the cell, and that cells grown under different temperatures, but the same irradiance, have different redox statuses and cannot be compared. Therefore, the effects of temperature or irradiance on physiology cannot be studied independently of each other. The redox pressure \((1-P_q)\) is the accumulation of electrons on the acceptor side of PS2, and can be easily...
measured using chlorophyll fluorescence (Chapter 3). Because studying the effects of
temperature and irradiance is complicated, only a few studies have been attempted.
Unfortunately, many of these studies have used irradiance and temperature ranges that are
ecologically irrelevant to the majority of phytoplankton species.

Because studies on species isolated from low temperature regions such as the NE
Subarctic Pacific are rare, this study attempts to elucidate the combined effects of
temperature and irradiance on the physiology of the oceanic diatom *Pseudo-nitzschia granii*
(Chapter 3). In this study, the physiology of *P. granii* was examined under five temperatures
(8, 10, 14, 17 and 20°C) and four irradiances (sub-saturating: 20, 50, 100 µmol photons
m⁻¹ s⁻¹ and saturating: 150 µmol photons m⁻¹ s⁻¹). Because PS2 is the most thermo-labile
component of photosynthesis (Davison, 1991), and the majority of chlorophyll fluorescence
originates from PS2 under normal physiological conditions (Falkowski and Raven, 1997),
chlorophyll fluorescence is an ideal tool to measure the response of photosynthetic efficiency
to changes in temperature. The following questions were addressed in this study: what is the
optimum growth temperature of *P. granii*? How do temperature and irradiance influence
growth rates of this species? How do temperature and irradiance affect pigment composition
and how do they interact to affect photosynthetic efficiency? Finally, does cold temperature
acclimation mimic high irradiance acclimation in *P. granii*?
Materials and Methods

Semi-continuous 30 ml batch cultures of *Pseudo-nitzschia granii* were grown in pFe 19.4 AQUIL medium (Chapter 3, and Appendix 1). The experiment was conducted in a graduated, aluminum temperature bath (8-24°C) following the design of Strezpek and Price (2001; Appendix 2). Continuous light was provided using 110 W Vita-lite cool lamps. Light intensities were attenuated using neutral density screening to achieve final growth irradiances (20, 50, 100 and 150 μmol photons m$^{-2}$ s$^{-1}$). Cultures were grown in triplicate and allowed to acclimate to respective treatments for at least 10 generations, or until growth rates stabilized. At mid-exponential growth, cellular numbers and chlorophyll were measured following the method discussed in Chapter 3. Photosynthetic efficiency was measured using a 101 Walz PAM fluorometer following Chapter 3 as well. $Q_{10}$ for growth rates, $Q_{10\mu}$, was calculated as

$$Q_{10\mu} = \frac{10}{(OPTI-8)} \times \ln\left(\frac{\mu_{OPTI}}{\mu_{8}}\right)$$

where OPTI is the optimum growth temperature for each treatment and $\mu_{OPTI}$ and $\mu_{8}$ are the specific growth rates at the optimum growth temperature and 8°C, respectively. Statistical analysis consisted of ANOVA testing followed by Students t-test following Zar (1984)($\alpha = 0.95$) (assuming data were normally distributed).
Results

Growth rate and photosynthetic pigments:

Growth rates increased with increasing growth irradiance (between 150 and 20 μmol photons m\(^{-2}\) s\(^{-1}\)) (Fig. 4.1A). Optimum growth temperature was 14°C, and Q10 increased linearly with decreasing light intensity (Fig. 4.1B). Optimum growth temperatures were independent of growth irradiance. Beyond 14°C, growth rates decreased with increasing growth temperature. There was no detectable growth at 20°C and 20 μmol photons m\(^{-2}\) s\(^{-1}\).

Chl cell\(^{-1}\) was high (P > 0.05) at low light compared to high light (Fig. 4.2). The maximum chl cell\(^{-1}\) was observed at 10°C. Cells grown at 8 and 20°C were chlorotic.

Chlorophyll fluorescence:

The theoretical yield of photosynthesis, \(F_V/F_M\), was slightly depressed at 8 and 20°C, especially at high light (Fig. 4.3). This was more pronounced at 8 than at 20°C. In the middle temperature range, \(F_V/F_M\) was not significantly different (P > 0.05) between different light and temperature treatments.

The quantum efficiency of photosynthesis, \(\phi_P\), was lower at 20°C than in the middle range of temperature and was significantly higher (P < 0.05) at low light compared to high light (Fig 4.4A).

The rate of linear electron transport, \(J_e\), increased with increasing irradiance. At high light, \(J_e\) reached a maximum at optimum growth temperature (14°C). However, at low light, \(J_e\) was unaffected by growth temperature (Fig. 4.4B).

At high light, photochemical efficiency, \(P_q\), was the highest at 8°C and it decreased with increasing temperature (Fig. 4.4A). At low light, \(P_q\) was unaffected by changes in
temperature. In general, \( P_q \), was significantly higher at low light compared to high light (\( P < 0.05 \)).

The redox pressure, \( 1-P_q \), had was inversely related to temperature and positively related to irradiance (Fig. 4.5B).

Non-photochemical quenching, \( Q_n \), was significantly affected by light only at low temperature (8 and 10°C) where \( Q_n \) was high at high light (Fig. 4.6). At high temperatures, \( Q_n \) was higher at high light compared to low light.
Figure 4.1: A) Growth rate ($\mu$) for *Pseudo-nitzschia granii* grown at 150, 100, 50 and 20 $\mu$mol photons m$^{-2}$ s$^{-1}$ vs. temperature. Error bars are standard error (n = 3) and are smaller than the symbol when invisible. B) $Q_{10\mu}$ for *P. granii*
Figure 4.2: Chl cell\(^{-1}\) for *P. granii* grown at 150, 100, 50 and 20 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\) vs. temperature. Error bars are standard error \((n = 3)\) and are smaller than the symbol when invisible.
Figure 4.3: $F_V/F_M$ for *P. grani* grown at 150, 100, 50 and 20 μmol photons m$^{-2}$ s$^{-1}$ vs. temperature. Error bars are standard error (n= 3) and are smaller than the symbol when invisible.
Figure 4.4: A) $\phi_{PS2}$, and B) $J_e$ of *P. granii* grown at 150, 100, 50 and 20 μmol photons m$^{-2}$ s$^{-1}$ vs. temperature. Error bars are standard error (n=3) and are smaller than the symbol when invisible.
Figure 4.5: A) \( P_q \) and b) \( 1-P_q \) of \( P. \) granii grown at 150, 100, 50 and 20 \( \mu \)mol photons \( \text{m}^{-2} \text{s}^{-1} \) vs. temperature. Error bars are standard error (\( n=3 \)) and are smaller than the symbol when invisible.
Figure 4.6: $Q_n$ for *P. granii* grown at 150, 100, 50 and 20 µmol photons m$^{-2}$ s$^{-1}$ vs. temperature. Error bars are standard error (n=3) and are smaller than the symbol when invisible.
Discussion

Temperature is one of the most important physical factors influencing primary production and community composition of phytoplankton in the oceans. Recent evidence suggests that thermoacclimation and photoacclimation are essentially the same processes in green algae (Wilson and Huner, 2000; Maxwell et al., 1995a; 1995b). However, no such evidence exists for temperate, oceanic phytoplankton. This study examined how light and temperature interact to influence growth rates, pigment composition and photosynthetic efficiency of the oceanic diatom *Pseudo-nitzschia granii* over an ecologically meaningful range of irradiances and temperature. The results of experiments, such as this one, are crucial for models of primary productivity in the ocean (Thompson et al., 1999).

**Growth rates and Cellular Chlorophyll:**

This study clearly demonstrated that the growth of *Pseudo-nitzschia granii* was modulated by light and temperature (Fig. 4.1A and 1B). As commonly reported in the literature (Kudo et al., 2000; Thompson, 1999), growth rates increase linearly with increasing growth temperature until an optimum is reached (~14°C) (Fig. 4.1A). Beyond this optimum, growth rates decreased. The optimum growth temperature of *P. granii* was similar to temperature optima reported for other temperate species (Suzuki and Takahashi, 1995). Light intensity not only influenced the magnitude of growth rates, but also the degree to which growth rate depended on temperature such that $Q_{10}$ decreased with decreasing irradiance (Fig. 4.1B). At high light, some of the cell’s energy was allocated towards alleviating light-induced stress, rather than responding to increased growth temperature (Anning et al., 2001), as demonstrated by a slightly elevated $Q_n$ at high light, especially at low temperature (Fig
4.6). Short-term responses of diatoms to low temperatures included increased concentrations of cellular carotenoids (Anning et al., 2001b). Savitch et al. (2001) showed that an increase in $Q_n$ at lower temperature was due to xanthophyll pigment activity and not to the Mehler reaction in *Arabidopsis thalina*. The increase in $Q_n$ at high light was probably due to increased protective activity of the xanthophyll pigments (Olaziola and Yamamoto, 1994).

The concentration of cellular chl was high at 10°C and low on either side of the temperature gradient. Though chl cell$^{-1}$ was generally high at low light (Chapter 3 - discussion), some bleaching was observed for cells grown at low light and at temperature > 10°C. There are conflicts in the literature regarding the response of chl cell$^{-1}$ to temperature; where some researchers reported a decrease of chl cell$^{-1}$ at low temperature (Strzepek and Price, 2001), others reported an increase (Thompson, 1999). Data from these experiments show that this conflict in the literature might, in fact, be due to the non-linear relationship between temperature and cellular chlorophyll concentration. The response of chl cell$^{-1}$ to temperature may also depend on the geographic range from which the species in study was isolated. It had been shown that the growth optima for species isolated from cold regions were well below those isolated from warmer regions (Suzuki and Takahashi, 1995). Though the low chl cell$^{-1}$ values obtained from *P. granii* grown at 8°C supported the popular hypothesis that low temperature acclimation mirrored high light acclimation, the overall response of chl cell$^{-1}$ to temperature indicated that this comparison might not be as simple as originally thought. An in-depth examination of the response of cellular chlorophyll concentrations to temperature was needed before comparing low temperature and high light acclimation strategies in marine phytoplankton.
Chlorophyll Fluorescence:

$\frac{F_V}{F_M}$ was slightly depressed at both ends of the temperature gradient (8 and at 20°C). This was most likely due to chlorosis-induced photoinhibition (Chapter 3). However, chl cell$^{-1}$ at 20°C was comparable to that at 16°C, which had a much higher $\frac{F_V}{F_M}$ value. The measured decrease in $\frac{F_V}{F_M}$ at 20°C was most likely the result of a combination of physiological processes induced by supra-optimal temperature. Strasser (1997) proposed that stress induced by supra-optimal growth temperature induced changes in the kinetics of the oxygen evolving complex (donor side) of PS2, thereby reducing $F_M$ (and $\frac{F_V}{F_M}$) in *Scenedesmus obliquus*. Unfortunately, donor side kinetics of *P. granii* were not examined in this study. In addition, the experiment performed by Strasser (1997) represented an effect of short-term temperature stress (~2 hours) at very high temperature (40°C; *S. obliquus* was grown at 30°C). Whether a similar response could be induced in diatoms is unknown.

$\frac{F_V}{F_M}$ decreased at low light at both 8 and 20°C. Between 8 and 20°C, there were no significant differences between various light and temperature treatments. However, $\frac{F_V}{F_M}$ was slightly higher at 10°C than at higher temperatures, consistent with what was reported in the literature about low temperature acclimation increasing $\frac{F_V}{F_M}$ (Davison, 1991). These observations further strengthened the argument proposed in the previous section regarding the care with which the results of experiments on temperature should be interpreted.

The relationship between $\phi PS2$, the quantum yield of photosynthesis, and temperature indicate that $\phi PS2$ was slightly depressed at 8°C and more severely depressed at 20°C. Savitch et al. (2001) showed that photosynthetic rates of low-temperature acclimated *A. thalina* were comparable to, if not slightly higher than high-temperature adapted plants. The same results were observed in marine phytoplankton (Davison, 1991). In low temperature,
short-term incubations seemed to dramatically decrease the quantum yield of photosynthesis in diatoms (Anning et al. 2001b). Thermal acclimation often modulated cellular concentrations of Rubisco and various Calvin cycle enzymes (Davison, 1991). Other results show that the relatively unchanged photosynthetic rates observed at low temperature were due to increased rates of electron transport (Davison, 1991) rather than elevated Rubisco activity. An examination of the effect of temperature on $J_e$, the linear rate of electron transport, revealed that $J_e$ was lower at 8 and 20°C compared to $J_e$ in the middle of the temperature spectrum (Fig 4.4B). This indicated that the observed decrease of $\phi_{PS2}$ at 8 and 20°C might be due to a decrease in $J_e$ (due to impaired light absorption created by low chlorophyll concentrations). Unlike, $J_e$, there was no striking temperature optimum for $\phi_{PS2}$. In general, $\phi_{PS2}$ was higher at low light compared to high light (see Chapter 3 for discussion).

At low light, photochemical efficiency, $P_0$, was slightly higher at 8°C and decreased with increasing temperature. At low light, $P_0$ was maximum at 14°C and decreased on either side of the temperature spectrum. This result disagreed with previously observed trends in low temperature acclimated Dunaliella salina (Maxwell et al., 1995b), which had higher $P_0$ at high light than D. salina acclimated to high temperatures. The exact mechanism by which temperature affects photochemical quenching is not understood.

Purely photochemical reactions are independent of enzymatic activity, and are theoretically independent of temperature. However, the photochemical reactions of photosynthesis take place along the thylakoid membrane, which is, like other phospholipid bilayers, vulnerable to temperature. At low temperature, often called the critical temperature, biological membranes lose their flexibility and become crystalline (Falkowski and Raven,
1997). With the loss of flexibility, the function of membrane proteins is also lost. In addition, the diffusion of electron carriers is temperature dependent (Maxwell et al., 1995b). In addition, phytoplankton cells from different taxa and different environments have been shown to have different responses to temperature. $P_q$ is linearly related to $\phi_{PS2}$ (Maxwell and Johnson, 2000). Increasing photochemical efficiency at low temperature provides cells with a physiological mechanism to maximize photosynthetic capacity at low temperature in order to keep electron rates at high enough levels capable of supporting growth yield and carbon fixation. This also explains why in diatoms in contrast to green algae (Maxwell et al. 1995b), the redox pressure on the acceptor side of PS2 is low at low temperature. This strategy is advantageous for phytoplankton native to the Northeast Pacific where the average temperature is between 8 and 9°C (Freeland et al., 1997). However, green algae, such as *D. salina*, have much higher growth temperature optima (30°C) and have no need for such a strategy. $P_q$ was also high at high light and low at low irradiance (see chapter 3 for discussion).

Are Thermoacclimation and Photoacclimation similar processes in *P. granii*?

By comparing, *P. granii* grown at 8°C and 20 μmol photons m$^{-2}$ s$^{-1}$ (8/20) to *P. granii* grown at 17°C and 150 μmol photons m$^{-2}$ s$^{-1}$ (17/150), it cannot be concluded that low temperature acclimation mimics high light acclimation in *P. granii*. In fact, $F_v/F_M$, $\phi_{PS2}$ and $P_q$ were all higher at 8/20 compared to 17/150. This is strikingly different than what is observed in green algae (Wilson and Huner, 2000; Maxwell et al., 1995b) where cells grown under 5/20 and 27/150 are biochemically indistinguishable. There are many reasons for the differences between the results of this study and those of Wilson and Huner (2000) and Maxwell et al. (1995b). Diatoms (chlorophyll c based) and green algae (chlorophyll b based)
are different physiologically and functionally so it is not surprising that they employ different thermoacclimatory strategies. In addition, most green algae have much higher temperature growth optima than temperate phytoplankton (~27°C compared to ~15°C) and live in environments that are on average much higher in temperature than the open ocean. Since temperatures fluctuate between 6 and 12°C at OSP, *P. granii* may benefit by employing a thermoacclimatory strategy that is better suited for cold environments rather than warm ones.
Conclusions:

- The growth optimum for *Pseudo-nitzschia granii* was ~14°C and was not affected by growth irradiance (Fig 4.1).

- $Q_{10}$ of *P. granii* depended on the photoacclimatory status of the cell and cells grown at low irradiance were more temperature-dependent (higher $Q_{10}$) than those grown under high light.

- Changes in the quantum yield of photosynthesis, $\phi_{PS2}$, might have been due to changes in the rate of electron transport, $J_e$, through the cells (Fig 4.4A and B). Changes in $J_e$ were most likely due to impaired light absorption due to low concentrations of cellular chlorophyll.

- Increased photochemical efficiency, $P_o$, at low temperature was, perhaps, a strategy to maintain high $J_e$, $\phi_{PS2}$ and growth rates.

- Finally, the results of this study provide preliminary evidence that whether Thermoacclimation mimics photoacclimation may depend on the physiology and the growth temperature optimum of the organism in question.
**Future Research**

This thesis provided basic information on the physiology of the oceanic diatom, *Pseudo-nitzschia granii*, isolated from OSP in the NE Subarctic Pacific. The effects of iron (Fe) and light, as well as the effects of light and temperature on the physiology of *P. granii* were investigated. This study was by no means comprehensive and there are many directions in which future research may proceed. Here are some questions that should be addressed in future studies:

1. How is the Fe quota of *P. granii* affected by Fe and light co-limitation?
2. How does the Fe quota of *P. granii* compare to those measured in other diatoms?
3. Is the physiological response of *P. granii* Fe and light co-limitation comparable to the response of coastal, pennate diatoms?
4. How does Fe and light co-limitation affect nutrient uptake in *P. granii*?
5. Why are Fe-limited *P. granii* cells thinly silicified?
6. What is the exact effect of Fe deficiency on nitrogen metabolism in *P. granii*?
7. What is the effect of Fe and light on the production of domoic acid in *P. granii*?
8. What is the effect of Fe and light on sexual reproduction in *P. granii*?
9. How does temperature affect Fe quota in *P. granii*?
10. How does $\phi_{PS2}$ compare to $^{14}$C-based quantum yield measurements in the laboratory?
11. How does $\phi_{PS2}$ relate to the activity of photorespiration in *P. granii*?


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KOLBER, Z. S. and others 1994. Iron limitation of phytoplankton photosynthesis in the


Olaizola, M., and H. Y. Yamamoto. 1994. Short-term response of the diadinoxanthin cycle and fluorescence yield to high irradiance in Chaetoceros muelleri


### Appendix 1
Media used in this study

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* Recipe from Price et. Al. 1988/1989
** Recipe for iron replete medium used in this thesis
*** Recipe for iron limiting medium used in this thesis
**** Trace contamination of Fe estimated at < 2 nM
*****Recipe from Harrison et al. (1980)
Appendix 2

Schematic of Temperature Block
Top View

Cold Water (4°C)

Temperature

8 10 12 14 16 18 24

Hot Water (30°C)

Culture Chambers
Appendix 2 - continued

Temperature block - side view