AN ABSTRACT OF THE DISSERTATION OF

Samuel R. Laney for the degree of Doctor of Philosophy in Oceanography presented on August 11, 2006.

Title: Seconds to Hour Scale Photosynthetic Responses in Marine Microalgae

Abstract approved:

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Our view of phytoplankton has historically revolved around their inability to control their location in space. The term *phytoplankton* itself underscores this particular difference between phytoplankton and their sessile terrestrial counterparts. Yet there are other differences between land plants and the phytoplankton that are perhaps equally important, beyond this sessile-planktonic dichotomy, to their growth, survival, and productivity. For example, phytoplankton are microbes and thus are short-lived, with generational scales on the order of days or less. An intriguing question to ask is how today's pelagic ecology would differ, had this temporal difference between plants and phytoplankton been initially emphasized, perhaps by naming these microbes *phytoephemera* instead? This dissertation addresses certain aspects of the ecology of phytoplankton that result from their having short generational scales. Because they are so short lived, phytoplankton need to adjust their photosynthetic physiology to cope with more rapid changes in irradiance than may matter to longer-lived plants. Photoacclimation on the hours-plus time scales has been studied extensively in the phytoplankton, because its temporal scales match those of vertical mixing processes in the ocean. Yet most phytoplankton exhibit faster photosynthetic responses as well, down to the time scales of seconds. These photosynthetic responses have received considerably less attention in phytoplankton ecology. This dissertation specifically examines these rapid, seconds-to-hour scale photosynthetic responses in phytoplankton. First, the physiological bases of rapid

photosynthetic regulation were examined using a numerical model that shows how specific physiological changes in phytoplankton photosystems either constrain or enhance light harvesting. This model is stochastic, and thus replicates certain nonlinear aspects of light harvesting better than equation-based analytical models. Also in this dissertation, a laboratory study is described that examined rapid photosynthetic regulation in three model phytoplankton. Results suggest that rapid photosynthetic regulation is not only constrained to higher eukaryotic phytoplankton, but also occurs in the two dominant marine photosynthetic prokaryotes, *Synechococcus* and *Prochlorococcus*. Finally, rapid photosynthetic responses were examined in field assemblages at Station ALOHA in the North Pacific. This ocean region experiences considerable cloud cover, which may result in a strong degree of rapid photosynthetic responses, even in near-surface assemblages.

Seconds to Hour Scale Photosynthetic Responses in Marine Microalgae

by
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A DISSERTATION
submitted to
Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented August 11, 2006
Commencement June 2007

Doctor of Philosophy dissertation of Samuel R. Laney presented on August 11, 2006

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Samuel R. Laney, Author

ACKNOWLEDGMENTS

> By now everyone knows that you can earn a lot of money and respect and perhaps even a Nobel Prize by becoming a scientist, so many will become scientists. (noted but obviously mistaken UC Berkeley philosopher of science Paul Feyerabend, in a 1974 address at Sussex University).

I'd like to express my thanks to:

- First, Ricardo Letelier and Mark Abbott, my doctoral co-advisors. Having both Mark and Ricardo together as advisors was especially valuable to me in my doctoral work. Mark was my Master's advisor, and he didn't have to accept back this prodigal graduate student. He introduced me to the works of Graham Harris. Most of my interest in ecology is a result of reading papers and books that Mark suggested, and from following his direction and advice. Ricardo is both a scientist and a scholar, something I admire and have benefited considerably from in my time at COAS. His "open-door" policy made it always easy for me to get advice I needed, be it about science, ethics, practical advice, and so on.
- Members of my doctoral committee: Pat Wheeler, Ron Zaneveld, and Tim Cowles from COAS, Tom Plant from Electrical Engineering and Computer Science in the College of Engineering, and Steve Giovannoni from Microbiology in the College of Science. All have been generous with their time, scientific advice and academic counsel. Tim has been especially generous in providing me with equipment and resources to do my research over the past several years. Tom has been a very supportive advisor for my Electrical Engineering minor, and also taught one of the best classes I've had the pleasure to take at OSU. Steve Giovannoni, in addition to serving as my Graduate School representative, also provided me with unique opportunities to become involved with research of his into phototrophy in rhodopsin-containing marine microbes.

- Colleagues and professors at COAS, but especially Scott Pegau, for having me along on GAK-GLOBEC in 2003 and for also providing me with much-appreciated equipment and instruments.
- Research staff in the Abbott, Letelier, Cowles, and Pegau lab groups, for considerable assistance over the past years, especially including Curt Vandetta, Amanda Ashe, and Russ Desiderio.
- Captain and crew of *RV Wecoma*, and the COAS martechs. I've gone on 3 cruises on *Wecoma* while at COAS. Getting research done on that ship has always been smooth and easy.
- Similarly, thanks to Dave Karl and the HOT program, for providing me opportunities to participate on several HOT cruises. Thanks in this respect especially to Lance Fujieki.
- Squirrel's Tavern, Bomb's Away Café, and American Dream Pizza, for not bothering me when I showed up to read papers and wanted to be left alone with beer and snacks.
- Young's Kitchen, for their unparalleled kimchee fried rice.
- The Usual Suspects and the Friday Bomb's Away crowd, for providing much-needed stress relief.
- Krista Longnecker, for all the help she's given me with the things I am particularly poor at, like making MSWord behave. Krista also introduced me to EndNote, and I can't convey just how much EndNote has helped me in writing this damned thing.

CONTRIBUTION OF AUTHORS

Contributions and advice by Ricardo M. Letelier and Mark R. Abbott were central to the preparation and intellectual development of Chapters 2 through 4. They will be co-authors when these chapters are submitted as manuscripts.

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1 Introduction

1.1 Overview

> It is at very small spatial scales that we have had problems. We have tended to see the world in terms of cubic metres and kilometres, convenient scales for us, but entire universes for organisms such as bacteria and phytoplankton. This has been particularly true in the study of marine phytoplankton where the size of oceans and the ships in use has led oceanographers to concentrate on scales of many kilometers and to neglect scales more relevant to the organisms. We have regarded phytoplankton as paradoxical because we have looked at the environment of the organisms at too large a scale. (Harris 1986, p. 1).

Phytoplankton is a general and imprecise term in ecology that refers to those aquatic, photoautotrophic microbes that are neither benthic nor epiphytic. Like other microbes, phytoplankton are very small and short-lived. Many phytoplankton are motile, and others can vary their buoyancy and thus exert some level of control as to where they reside vertically in the water column. The phytoplankton contain a considerable a range of cellular forms, including single cells, coenebia, small-number chains or colonies, and massive multicellular colonies of hundreds of cells. Some classes, notably the *Dinophyceae*, contain mixotrophic and predatory taxa, and numerous taxa have epiphytic or benthic cogeners (especially in the pennate *Bacillariophyceae*).

Such a broad range of physiological and ecological traits make it difficult to produce a precise definition of *phytoplankton*. However, their common feature – photosynthesis – is an integral aspect of pelagic marine ecosystems, as photosynthesis is the primary conduit through which metabolic energy enters the marine food web. As the dominant primary producers in pelagic ecosystems, phytoplankton are the main

prey for protists and small invertebrates. This trophic role of phytoplankton has long been recognized as an essential aspect of the productivity of marine fisheries. As a result, there has been considerable effort in fisheries oceanography to examine how variability in phytoplankton productivity affects higher trophic levels. The biogeochemical importance of primary production by phytoplankton is also becoming a very widely examined aspect, especially with respect to important bioelements such as carbon. Phytoplankton's role in the global carbon cycle has been a central issue in long-term, international biogeochemical research programs, including the Joint Global Ocean Flux Study (JOGFS, Steinberg et al. 2003).

As a field of study, phytoplankton ecology began as a distinct scientific discipline in the late 19th century, after technological advances in microscopy provided scientists with tools to observe these organisms directly. The term *phytoplankton* is thought to have been coined by Christian Gottfried Ehrenberg in 1897 (Mills 1989). It derives from Greek roots: φψτος (*phytos*) – pertaining to plants – and πλαγκτον (*plankton*) meaning a "wanderer" or a "drifter" in the sense first used by Hensen ten years earlier (Hensen 1887). As with many scientific disciplines, the research trajectory that phytoplankton ecology has taken over the past century has been biased to some extent by the anthropocentric perspective of us human observers. The opening quote to this dissertation, from Harris's own introduction to his (1986) text, describes one particular bias associated with spatial scales. Harris argues that because we are so much larger than phytoplankton, we may have not yet observed some fundamentally important aspects of their ecology that would become apparent, if only we could better observe phytoplankton on the more relevant, much smaller spatial scales of the individual microbe. The danger of not appreciating phytoplankton ecology at these most relevant spatial scales is that these unobserved facets of phytoplankton ecology may perhaps be at odds with some of our current paradigms, and may be misguiding our current research.

Harris's criticism is not unique to phytoplankton ecology, and many important advances have come about in other subdisciplines of microbial ecology, when these small spatial scales are examined in detail. Small-scale spatial heterogeneity in

environmental conditions is an important factor affecting assemblage structure at hydrothermal vent communities (Kormas et al. 2006), in cyanobacterial mats (Ward et al. 1998), and in seawater at the millimeter scale (Long and Azam 2001). Just as how light microscopy led to the emergence of plankton ecology over a century ago, these recent observations directly result from new technologies that allow researchers to examine marine phytoplankton assemblages at smaller spatial scales. Flow cytometry (Li and Wood 1988), laser sheet spatial imaging (Franks and Jaffe 2001), and *in situ* systems to measure phytoplankton assemblages on the microscale (Cowles et al. 1998) provide three examples where novel instruments or sampling techniques have resulted in observations that call into question some previously held concepts regarding phytoplankton dynamics in the surface ocean.

Yet there is a temporal analog to the spatial bias in phytoplankton ecology that Harris claims, which has received less attention over the past century. It may also be the case that we may have failed to identify important aspects of phytoplankton ecology by not examining their behaviors and interactions on the appropriately short time scales. The generational scale of phytoplankton, G – following the nomenclature of Fogg and Thake (1987) – is typically between 0.5 and several days. Such short generational scales makes it very easy for us to examine ecological processes in populations of phytoplankton, such as competition, exclusion, and evolutionary adaptation. In fact, this particular aspect of phytoplankton was identified by Harris as the main reason these microbes are ideal model organisms for monitoring the outcomes of ecological aspects, as a means to test theory (Harris 1986, p. 14). However, this advantage inherently comes with a drawback: in organisms having such short *G*s, it is much less easy to observe the interactions and physiological responses of the individuals in these populations that lead to competition and exclusion outcomes.

The days-to-years time scales that are more relevant to us human observers, and to the higher trophic levels that depend on phytoplankton productivity, occur on scales of many phytoplankton generations. Thus, much of the past research in phytoplankton ecology concerns changes in the structure and function of

phytoplankton assemblages over many *G*. It is easy to forget that these outcomes and these assemblage dynamics simply reflect a large numbers of interactions between a single phytoplankter and its environment, a predator, or a competitor. Theoretical models for describing changes in phytoplankton growth over multi-*G* scales involve only idealized, abstract parameterizations of these interactions, such as the population growth rate *R* and the environmental carrying capacity *k*. The underlying interactions between physical and biological properties in an ecosystem that give rise to these observed kinetics, i.e., the dynamics of these assemblages (sensu Jumars 1993), occur more rapidly, on the shorter, sub-*G* time scale. It has long been recognized that these sub-*G* interactions fundamentally determine the overall growth and productivity of a phytoplankton assemblage (Abbott et al. 1982; Marra and Heinemann 1982). However, observing the interactions on sub-*G* scales that lead to such ecological outcomes in natural assemblages has been challenging experimentally. Laboratory studies can only provide a limited view of the interactions that are most relevant to phytoplankton assemblages in the actual surface ocean.

A historical bias against the relevant temporal scales of individual phytoplankton can be inferred from the considerable emphasis that has been placed on more spatially related processes. A broad review of the major research themes in this field during the past century will reveal a focus on the spatial, e.g., patchiness and the effects of vertical displacement on access to resources. If in fact there is a temporal analog to Harris' perceived spatial bias, it may have in part resulted from our overwhelming focus on such spatial topics. There are considerable temporally driven differences between phytoplankton in planktonic systems and sessile plants in terrestrial systems. The environmental perturbations that are important to the former may not be so to the latter. An interesting thought experiment is to consider what trajectory phytoplankton ecology would have taken over the past century, had Ehrenberg considered first the short generational scales of these photosynthetic microbes and named them *phytoephemera* instead of *phytoplankton*.

This dissertation addresses a small facet of the potential biases in phytoplankton ecology that result from a failure to appreciate their responses and

interactions at short, sub-*G* scales. Specifically, this dissertation examines the role of seconds-to-hour responses in photosynthetic physiology and light harvesting. In general, the availability of phytoplankton's primary energetic resource – ambient sunlight – varies considerably more over sub-*G* time scales than do the solutes and ions that phytoplankton require as nutrients. A considerable fraction of the overall biomass and metabolism of phytoplankton is directed toward assimilating this light energy. The photosynthetic light reactions responsible for this assimilation must therefore accommodate these sub-*G* changes in light availability and quality. The so-called "plasticity" of phytoplankton photosynthetic structure and function is a reflection of the ecological requirement to acclimate to strong resource perturbation. This plasticity has been well examined on certain temporal scales, in natural assemblages and in cultures. On other time scales, photosynthetic plasticity has received comparatively very little attention. Photosynthetic responses on the sub-hourly time scale, occurring within seconds to many minutes, are among the least well examined. This gap in our examination of photosynthetic response scales in phytoplankton suggests that we may be missing certain important aspects of their ecology.

Historically, rapid, sub-hourly photosynthetic responses in phytoplankton have been difficult to identify and quantify. Establishing their physiological bases has been technologically challenging, since these responses do not involve changes in rates of biosynthesis or degradation. They are invisible to most biochemical assays, and their influence is generally apparent only in the rates of production of photosynthetic waste products, i.e., oxygen and chlorophyll fluorescence emission. These rapid responses are most readily examined by dynamically stimulating the light harvesting apparatus of phytoplankton, and observing the resulting change in oxygen and fluorescence. Such techniques have been central for examining photosynthetic responses on the sub-hourly scale (Falkowski and Raven 1997). This dissertation describes research in which fluorescence techniques in particular have been used to examine the photosynthetic responses of marine phytoplankton on the sub-hourly time scale. This background will include some physiological background pertaining to specific modes

of these rapid responses, some ecological ramifications of rapid photosynthetic responses in phytoplankton, and some discussion of the sources of rapid fluctuations in the marine light field that may serve to stimulate these similarly rapid, directional photosynthetic responses.

1.2 Photosynthetic responses available to an individual phytoplankter: regulation and photoacclimation

The first step in photosynthesis involves absorbing photon energy from the ambient light field and utilizing this energy to generate products that will be metabolically useful in biosynthesis. Like their counterparts in Plantae, phytoplankton employ a chlorophyll-based light harvesting apparatus to mediate this energetic absorption and conversion. Photon-absorbing structures called photosystems and a series of cytochrome intermediates work together to generate a supply of reductant in the form of NADPH, and chemical energy in the form of ATP, using absorbed photon energy. The generation of these two products is commonly referred to as the light-dependent, or "light" reactions in photosynthesis. These products are subsequently used in the light-independent ("dark") reactions for carbon assimilation, as well as to support other metabolic processes such as active nutrient uptake (Button 1985; Raven 1999), cellular motility (van den Hoek et al. 1995), and buoyancy regulation (Villareal and Carpenter 2003). The strong interest in phytoplankton productivity vis-à-vis carbon cycling over the past few decades has resulted in comparatively less attention to these other metabolic requirements for light reaction products, although these processes are nonetheless critical to the growth and survival of many phytoplankton species.

A review of the photosynthetic literature reveals little standardization in the terminology used to discuss photosynthetic responses in phytoplankton. Observed changes in empirical photosynthetic parameters are often interchangeably referred to as *photoacclimation* or *photoadaptation*, although acclimation and adaptation

represent distinctly different phenomena in ecology (Ricklefs 1990; Roughgarden 1998). In this dissertation, the term *photoadaptation* will be reserved specifically to describe irreversible changes in the genotype that occur over evolutionary time scales, orders of magnitude greater than *G*. In contrast, *photoacclimation* will refer to the those responses available to the individual that involve changes in its macromolecular photosynthetic structure. Thus, an individual phytoplankter never photoadapts, although the suite of photosynthetic responses available to that individual reflect photoadaptation. The alteration to pigment quotas or stoichiometry, or in other components of the light or dark reactions, occur with time scales of transcription and biosynthesis of several hours or more. There are other changes in photosynthetic structure or function that an individual phytoplankter can effect, which do not require alteration of photosynthetic architecture or macromolecular composition. These are referred to as *regulation*, and since they require no transcriptional changes, they can occur much faster, down to the time scales of seconds or less. These definitions of photoadaptation, photoacclimation, and regulation are consistent with those presented by MacIntrye et al. (2002) and Raven and Geider (2003), in recent reviews.

Photoacclimation in the phytoplankton has been studied extensively, and the current level of understanding is well detailed in the two aforementioned reviews. The physiological bases of photoacclimation are generally well understood, although they are better understood in eukaryotes than in the prokaryotes. Current research into photoacclimation focuses to a large degree on understanding the physiological signals that trigger these responses, and how photoacclimation is controlled at the transcriptional level (e.g., Paul and Foyer 2001). Since these responses involve transcriptional regulation, genomics and proteomics have become key tools for identifying the upregulation and downregulation of the photosynthetic components that are modified during photoacclimation, such as pigments. The feedback control involved in photoregulation involves aspects of the light and dark reactions both (Paul and Foyer 2001). However, genomics and proteomics are not appropriate tools for examining rapid, regulatory responses in the light reactions, because there is no gene

or protein associated with these regulatory responses. These responses only involve adjustments to how an essentially fixed photosynthetic architecture behaves dynamically, although the presence or absence of a particular gene can indicate the potential for certain of these responses. In a sense, these rapid adjustments occur "automatically" when a specific photosynthetic architecture experiences a transient change in energetic stimulation.

Several different types of photosynthetic regulation have been identified in phytoplankton, the earliest of which was identified independently in 1969 by two different laboratories: in *Chlorella* by Bonaventura and Myers (1969) and in *Porphyridium* by Murata (1969). These so-called "state transitions" involve the movement of light harvesting antennae within the thylakoid membrane, between Photosystem II (PSII) and Photosystem I (PSI). This movement redistributes absorbed light energy between the two photosystems, which is generally thought of as a means to optimize electron transport by balancing photochemical activity in PSI and PSII (Allen 2003). This mechanism has been subsequently found in other algal eukaryotes, and appears to occur in phytoplankton with the time scales of several minutes. State transitions are defined phenomenologically, not physiologically, and thus several different regulatory responses fall under this category (Lunde et al. 2003; Williams and Allen 1987). State transitions that are observed in cyanobacteria involve an undetermined physiological mechanism that is still a matter of debate. There is evidence for direct energy spillover between PSII and PSI (Koblížek et al. 1998), but migration of the light harvesting phycobilisome between PSI and PSII on the thylakoid surface also has strong experimental support (Campbell et al. 1998). Since the light harvesting antennae in cyanobacteria reside on the thylakoid, and not embedded in it as in eukaryotes, migration-meditated state transitions in these prokarytes can be very fast, on the order of tens to hundreds of milliseconds (Mullineaux et al. 1997; Sarcina et al. 2001). In both prokaryotes and eukaryotes, state transitions appear to be triggered by the same physiological signal: the redox state of the electron carriers between PSII and PSI (Mullineaux and Emlyn-Jones 2005).

A second physiological mechanism for rapid photosynthetic regulation involves special carotenoid pigments whose optical properties can be modified enzymatically. This mechanism, referred to as the xanthophyll cycle, can occur in eukaryotes that employ violaxanthin or diadinoxanthin as photoaccessory pigments (Blakenship 2002). Epoxidation of these pigments converts them to violatoxanthin and diatoxanthin respectively, which behave photoprotectively by thermal dissipation of any absorbed photon energy that they receive. Thus, expenditure of a small amount of ATP to effect this epoxidation can considerably affect the bio-optical and photosynthetic properties of a phytoplankton. Neither Prochlorophytes nor cyanobacteria appears to have an analog to the xanthophyll cycle (Siefermann-Harms 1985), although both are known to contain at least one important xanthophyll, zeaxanthin (van den Hoek et al. 1995). The xanthophyll cycle appears to be triggered by the translumenal gradient in pH (Oliazola et al. 1994; Oliazola and Yamamoto 1994), and occurs with time scales on the order of several minutes (Demmig-Adams and Adams 1992; Lavaud et al. 2004; Yamamoto and Nakayama 1963). This same translumenal pH signal also appears to trigger a third mechanism for photosynthetic regulation in phytoplankton, changes in the aggregation and conformation of PSII light-harvesting complexes (Horton et al. 1991; Wentworth et al. 2000).

Both photoacclimation and photosynthetic regulation act to mitigate the physiological stress that results from a change in the intensity or spectral content of the ambient light field. There are other changes in photosynthetic physiology that phytoplankton exhibit when experiencing changes in light, but which are not considered to reflect photoacclimation or regulation. For example, changes in photosynthetic physiology due to photodamage only represent the unavoidable, essentially toxic effect of light at very high irradiances (Lavaud et al. 2004). Similarly, the so-called “flashing light effect” (Phillips and Myers 1954) is also not considered to be regulatory. This effect describes the apparent enhancement of photosynthesis and photosynthetic rates, in plants and algae that are stimulated with very rapid, multi-Hz flashes of light. It is a widely used in algal mass cultivation to increase yield (Degen et

al. 2001; Eriksen et al. 1996). This phenomenon is an essential aspect of the photosynthetic ecology of shade plants in terrestrial systems (Pearcy 1990; Pearcy et al. 1994), yet this phenomenon does not involve adjustment to photosynthetic physiology on the time scales of the fluctuations in light that characterize this response. Unlike state transitions or the xanthophyll cycle, the flashing light effect does not represent a means to optimize photosynthesis, continually and directionally. It appears to play a role in enhancing phytoplankton growth in natural assemblages under certain conditions (Quéguiner and Legendre 1986; Walsh and Legendre 1983), but it does not appear to be in response to individual transients or fluctuations in irradiance

1.3 Two apparently different, but inherently related, roles for rapid photosynthetic regulation in marine phytoplankton

1.3.1 Responses to similarly rapid perturbations in irradiance

In the surface ocean, an individual phytoplankter experiences considerable variability in both the intensity and spectral content of ambient irradiance over a wide range of time scales (Harding et al. 1987). Much of this irradiance variability results from their being vertically displaced in the water column by hydrodynamic processes. Since irradiance displays a near-exponential decay with depth, vertical displacement through this gradient materially changes the ambient light field that an individual phytoplankter experiences. There are numerous mechanisms in the ocean that cause this displacement, including mesoscale eddies, internal tides, storms and hurricanes, and inertial oscillations. How vertical displacement affects photosynthesis and growth in marine phytoplankton has been widely examined both from an experimental and theoretical perspective (Cullen and Lewis 1988; Falkowski and Wirick 1981; Huisman et al. 1999; Marra 1978a; Marra 1978b; Schubert et al. 1995). Given the characteristic, ≥ hour time scales of vertical displacement (Denman and Gargett 1983), changes in

photosynthetic properties on these times scales largely represent photoacclimation, not regulation.

However, there are other environmental processes, independent of vertical displacement, that introduce variability in the marine light field which are often overlooked. These displacement-independent processes include intermittent cloud cover, foam and bubbles at the air-sea interface, and focusing by surface waves, which can introduce fluctuations in irradiance that can occur with time scales down to seconds or minutes (Dera and Gordon 1968; Stramska and Dickey 1992). Of the three, the effect of bubbles or foam on photosynthesis is the least well examined, having been considered primarily from an optical, radiative transfer perspective only (Mobley 1994). Surface wave focusing has been examined in more detail, but also primarily from an optical or bio-optical perspective (e.g., Dera and Gordon 1968; Stramska and Dickey 1998; Stramski and Dera 1988). Short period waves have received the most attention, as they occur on the same time scale of the flashing light effect. These waves are estimated to modulate underwater irradiances by 3- to 5-fold, primarily at depths very close to the ocean surface (Stramski 1986). Recent modeling efforts suggest that longer-period waves, with their longer focal distances, may modulate irradiances much deeper in the water column (Zaneveld et al. 2001). Intermittent cloud cover, the third displacement-independent process, can introduce irradiance fluctuations over a very wide range of time scales, from seconds to days. Unlike the wave focusing effect, cloud-induced irradiance fluctuations are experienced throughout the entire water column (Stramska and Dickey 1998).

In a more general sense, the distinction between photoacclimation and regulation is a physiological one. Such a physiological distinction is somewhat irrelevant when considering the ecological role that each serves in photosynthetic metabolism. Both provide a mechanism for enhancing light harvesting under suboptimal irradiances, and for constraining light harvesting under supraoptimal intensities. Viewed this way, the only material difference between the two is their characteristic time scales. Thus, regulation is nothing more than an additional strategy for photosynthetic optimization in phytoplankton, that responds on time scales much

faster than can be accomplished over the time scales of transcription through photoacclimation. Yet despite the fact that virtually all phytoplankton taxa examined to date do exhibit fast regulatory photosynthetic responses on the rapid time scales of cloud cover, surface waves, and interface processes, the extent to which photosynthetic regulation is used to optimize photosynthesis on the seconds-to-hour scale has not been well examined. Research into photosynthetic responses in marine assemblages has focused almost exclusively on photoacclimation and on responses having time scales of biosynthesis and degradation (Flöder et al. 2002; Gallegos et al. 1980; Gallegos and Platt 1982; Lewis et al. 1984a; Lewis et al. 1984b; Litchman 2003; Litchman and Klausmeier 2001; Marra 1978b).

On theoretical grounds, it has been argued that rapid photosynthetic regulation is essentially negligible with respect to phytoplankton production, because photoacclimation at slower, hours-plus scales is the factor that is rate limiting to growth (Lewis et al. 1984b). A current text (Mann and Lazier 1996) uses this Lewis et al. study as experimental proof that rapid regulatory responses have only a minor role in phytoplankton photosynthesis and productivity. However, a central assumption in this argument is that vertical displacement mechanisms are the dominant source of the variability in irradiance that phytoplankton experience. Lewis et al. examined a heavily mixed coastal region, where survival depends on the ability to photoacclimate. The largest changes in irradiance experienced by phytoplankton in that region resulted from vertical displacement. Thus, it is a scale-matching argument that suggests that the slower, hours-plus perturbations due to mixing are what most limit photosynthesis and growth.

In this dissertation, open ocean field data are examined to raise important questions about the presumed negligibility of rapid photosynthetic responses in marine assemblages. A multiyear record of irradiance at 25 m depth, collected by the HALE ALOHA mooring (Hawaii Air-sea Logging Experiment, A Long-term Oligotrophic Habitat Assessment) between 1997 and 2000 (Letelier et al. 2000), suggests that intermittent cloud cover may have a role equal to, or perhaps greater than, vertical displacement, in determining the light fluctuations experienced by phytoplankton. In

this time series, intermittent cloud cover introduced considerable perturbations in irradiance on the time scales on the order of minutes. The vertical velocities that would be required for vertical displacement processes to equal these cloud-driven fluctuations are only rarely seen in this region. Thus, phytoplankton at Station ALOHA experience a variable light field that is driven more by ambient cloud cover than by vertical displacement mechanisms such as internal waves or inertial oscillations. In such an environment, the interpretation of Lewis et al. (1984b) no longer holds. In a tidally mixed environment, there is a clear-cut separation between the environmental perturbations that would stimulate photoacclimation, and those that would require regulation. In the open ocean at Station ALOHA, there is no such separation. Fluctuations in irradiance occur continuously across time scales between several minutes and several hours. It is not possible to claim that rapid photosynthetic regulation is negligible in the open ocean, because the environmental forcing on those sub-hourly scales may be stronger.

1.3.2 Rapid responses in the absence of rapid light perturbations

The preceding section summarizes a widely held view of the ecological role that photosynthetic regulation plays in optimizing photosynthesis in response to rapid perturbations in irradiance. Yet rapid photosynthetic responses can be observed on the seconds to minutes scale in phytoplankton growing in light fields which have no rapid fluctuations in irradiance (Bruyant et al. 2005; Laney et al. 2005). Scale-matching arguments cannot explain the need for rapid responses under light conditions that have no similarly scaled perturbations. Instead, rapid regulation under such conditions represents a threshold behavior in the energetic dynamics of the light harvesting apparatus, as it shifts suddenly in a matter of seconds to minutes from one photosynthetic "state" to another.

Such sudden shifts in the state of the light harvesting apparatus reflect the basic nonlinear nature of the light reactions. Such threshold behavior is a hallmark of

nonlinear systems, along with hysteresis and feedback (Strogatz 1994). Nonlinear phenomena have been long recognized in photosynthetic function, but historically these phenomena have been identified and discussed independently, not as a family of related responses of a single nonlinear system. The canonical photosynthesis-irradiance curve, in which changes in irradiance do not always result in changes in photosynthesis, reflects only the most rudimentary nonlinear aspects of light harvesting. Given that even simple nonlinear systems exhibit complex nonlinear dynamics (May 1976), feedback, hysteresis, and threshold behaviors in the light reactions should not be surprising. Such dynamics have been shown to have material ramifications to photosynthesis and growth in phytoplankton, and are fundamental in determining the outcome of ecological interactions under both equilibrium and nonequilibrium conditions (Levins 1979; Pascual-Dunlap 1995).

If we are to use terms in phytoplankton ecology such as "photosynthetic machinery", or "photosynthetic apparatus", then it makes sense to examine light harvesting and utilization using techniques developed for analyzing mechanical systems. Throughout this dissertation, the photosynthetic light reactions will be discussed in terms of a nonlinear, dynamical system. Many sophisticated techniques have been developed in engineering and the physical sciences for analyzing nonlinear systems dynamics, and these techniques are slowly migrating to the biological sciences and phytoplankton ecology. This slow migration is similar to how spectral analysis techniques, developed and well established in engineering, remain to be widely adopted in mainstream ecology despite their considerable usefulness (Legendre and Legendre 1988). The few recent studies that have utilized these nonlinear analysis techniques reveal interesting nonlinear behavior in the light harvesting dynamics of certain phytoplankton taxa (Nedbal and Brezina 2002; Nedbal et al. 2003). These behaviors may have important ramifications to how we interpret how phytoplankton respond photosynthetically under nonequilibrium conditions. Future research using these analytical techniques will be central to developing robust dynamical models that describe light harvesting and utilization in continually varying light fields.

The benefit of taking a systems-oriented perspective to light harvesting and utilization in phytoplankton is that it synthesizes some of the different nonlinear aspects of photosynthesis under a single, unifying conceptual framework. From a systems perspective, the two roles of photosynthetic regulation described above are not inherently different, only different manifestations of how a single photosynthetic system responds to particular form of energetic perturbation. A systems-oriented perspective also allows us to progress beyond the pervasive but flawed concept that phytoplankton "integrate" short-term environmental variability in irradiance, or that they acclimate to some "mean" or "average" light conditions (Demers and Legendre 1981; Dusenberry et al. 1999). Such an interpretation implies that phytoplankton are insensitive to fluctuations in irradiance on short time scales, despite the fact that widespread existence of directional, sub-hourly photosynthetic responses among the phytoplankton demonstrates a clear relationship between rapid environmental stimuli and rapid physiological responses. Using a systems-oriented approach, the "filtering" effect of photosynthetic physiology on environmental perturbations (sensu Harris 1986) can be quantified using tools and concepts developed in computer sciences and signal processing (Mitra 2001). There is considerable overlap between the nonlinear numerical systems being investigated in these fields, and the nonlinear photosynthetic systems that are central to phytoplankton ecology.

1.4 The current relevance of rapid photosynthetic responses in oceanography and phytoplankton ecology

Rapid regulation of light harvesting and photochemistry is an essential part of the photosynthetic metabolism. It has long been recognized as a primary factor that determines the outcome of higher-order processes in phytoplankton assemblages such as competition and exclusion (Abbott et al. 1982; Marra and Heinemann 1982). Plants and algae do not compete for light directly, as they do for space or nutrients, and light

is relatively abundant in the marine euphotic zone. Instead, light "competition" is indirect, where differences in the uptake and utilization of light energy leads to a differential ability to uptake nutrients through active pathways. Thus, understanding the role of photosynthetic regulation in how phytoplankton utilize their ambient energetic resource is an essential part of understanding a mechanism that drives change phytoplankton assemblages over multigenerational scales. This alone provides a scientific basis for examining rapid regulatory responses in marine phytoplankton assemblages.

A renewed interest in rapid photosynthetic regulation is timely for several other reasons. A recent paper by Strzepek and Harrison (2004) suggested that a differential ability for rapid photosynthetic regulation was a reason why both neritic and oceanic cogeners can be found in *Thalassiosira*. These authors hypothesized that iron is a necessary nutrient for effecting rapid photosynthetic responses, and that the availability of iron in coastal waters allows neritic *Thalassiosira* to survive in the highly dynamic coastal light environment, where irradiance fluctuations due to vertical displacements are considerable and rapid. These authors suggested that such rapid responses are largely unnecessary in the open ocean, because the open ocean is far more quiescent in terms of irradiance fluctuations. Thus, since oceanic *Thallasiosira* experience comparatively less irradiance perturbation, the lack of iron in oligotrophic regions does not hamper growth of oceanic *Thalassiosira* cogeners.

As mentioned earlier in this Introduction, the canonical view of the open ocean as quiescent may be appropriate in terms of vertical displacements, but it is not appropriate in terms of irradiance fluctuations. Intermittent cloud cover over the open ocean can introduce strong perturbations in irradiance throughout the entire water column, on time scales where rapid photosynthetic regulation may very well be important. Strzepek and Harrison's (2004) assertion may still be correct in the sense that the relative lack of iron does restrict rapid photosynthetic responses in oceanic *Thalassiosira* cogeners. However, an absence of rapid photosynthetic responses in these oceanic cogeners cannot be considered to be an evolutionary result of surviving in an environment with low short-term irradiance variability. The canonical view, that

the open ocean is quiescent in terms of irradiance perturbations can be found in many other recent studies (e.g., Havelková-Doušová et al. 2004; Peers and Price 2006).

Another topic of renewed interest in photosynthetic regulation in oceanography involves data streams that are being collected by long-term ocean observational platforms. The NSF-sponsored Hawaii Ocean Time-series (HOT) at Station ALOHA off Hawaii and the Bermuda Atlantic Time Series (BATS) at Ocean Station S off Bermuda have greatly expanded our understanding of both the kinetics and dynamics of phytoplankton assemblages in the surface ocean (Karl and Lukas 1996; Karl and Michaels 1996). Observations at HOT, BATS, and other sites have documented extensive variability in biomass, production, and taxonomic composition of phytoplankton assemblages on seasonal, annual, and decadal scales (Karl et al. 2003; Letelier et al. 1996; Letelier et al. 2004; Winn et al. 1995). Yet these time series also indicate considerable variability in photoautotrophic processes and variables on shorter time scales, both between consecutive months and when comparing seasonal patterns between years. The approximately monthly intervals between sampling at both HOT and BATS make it difficult to determine if observed month to month changes in autotrophic biomass and production reflect the actual assemblage kinetics, or instead represent artifact kinetics generated by aliasing due to inadequate sampling of unmeasured dynamics acting at shorter time scales.

Several ocean observing systems have added or will be adding instruments to measure optical properties of phytoplankton on these time scales, in the Gulf of Maine (Bogden and Richert 2003), off New Jersey (Schofield et al. 2002), and off Martha's Vineyard (Austin et al. 2000). These systems will be able to observe how phytoplankton assemblages respond to physical forcing with sub-monthly time scales, including inertial oscillations, internal tides, Rossby waves, storms, hurricanes, mesoscale eddies, and cloud cover. In many cases, optical or physiological data will be well sampled on sub-diurnal scales. To interpret these data robustly, a greater understanding is required of how photosynthetic regulation on the sub-diurnal scale affects these optical and photosynthetic properties. This same requirement applies to

the ocean drifters, gliders, autonomous vehicles, moorings, and even satellites, that now carry sensors that continuously sample not only proxies for phytoplankton biomass, but proxies for photosynthetic characteristics and physiology.

There has also been interest in recent years to place an ocean color sensor on the NOAA geosynchronous orbiting environmental sensor (GOES) weather satellites (Davis 2005). Such *in situ* and remote sensing platforms can and will provide phytoplankton ecologists with much more detailed information about the dynamics of phytoplankton photosynthesis over sub-generational time scales of minutes to hours. It will allow us to build climatologies of diurnal behaviors in these properties, from very long term averages of diurnal-scale measurements. However, interpreting these highly resolved photosynthetic data will be difficult without a greater understanding of how rapid photosynthetic regulation and acclimation work together to determine the light harvesting state of the phytoplankton, at any particular instant.

1.5 Research objectives and summary of results

A general question was posed early on in this Introduction: has a lack of attention to the appropriate microbial scale of phytoplankton kept us from understanding important aspects of their ecology? In the remaining chapters of this dissertation, I will examine the photosynthetic responses in phytoplankton on the seconds to sub-hourly scale, and discuss how this less-well explored time scale may be physiologically and ecologically relevant. I approach this from three different perspectives, including a theoretical study of light harvesting by phytoplankton, a laboratory study that examines rapid photosynthetic responses in cultures, and a field study that investigates similar responses in natural assemblages.

Different modes of photosynthetic regulation can occur simultaneously in phytoplankton, and often do. The overall effect of these concurrent physiological changes is difficult to predict, however, because the photosynthetic light reactions are highly nonlinear, and the behavior of such systems is generally difficult to model

exactly. Nonlinear systems that are modeled using equations often involve considerable approximation of certain interactions that may not accurately represent their dynamics or responses. The first major research section of this dissertation, Chapter 2, describes a stochastic approach based on Monte Carlo principles for more accurately simulating the nonlinear effects of specific physiological changes to Photosystem II structure and function. These simulations provide a means to describe the relationship between irradiance and photosynthesis for a particular state of photosynthetic regulation, defined in terms of specific properties of Photosystem II. Since several of these properties can be directly measured using variable fluorescence techniques, this new approach can be used to predict how regulatory changes in the photosynthetic properties of actual phytoplankton assemblages affect how they harvest and utilize ambient light. The basis of these simulations is described in detail, as are the results of several modeling exercises used to examine the behavior of this stochastic model when provided with synthetic and actual time series of PSII physiology.

In the second major research section, Chapter 3, results are presented from a laboratory study designed to examine how specific phytoplankton taxa respond to rapid step transients in irradiance. Cultures of the neritic marine diatom *Thalassiosira weissflogii* were grown under semi-sinusoidal, diurnal light histories over a range of nutrient availabilities. During the light period, these cultures were exposed to rapid decreases in irradiance of 5 to 10 minute duration. These cultures were continuously monitored using a single turnover, variable fluorescence technique, to identify how specific physiological aspects of PSII varied in response to these perturbations. The stochastic model of Chapter 2 was then used to predict how the rapid changes observed in these cultures affected their light harvesting rates and efficiencies during these irradiance transients, and to compute the energetic gains that these rapid responses conferred. Two prokaryotic marine phytoplankton, *Synechococcus* and *Prochlorococcus*, were also examined. Both prokaryotes exhibited photosynthetic changes on the rapid scales of regulation, albeit of a different character than *T. weissflogii*. Such taxon-specific differences in rapid photosynthetic responses to light

perturbations shed light on the particular strategies of these microbes for coping with different scales of variability in light availability.

The third major research chapter in this dissertation examined rapid photosynthetic responses in phytoplankton grown in natural light environments. Chapter 4 examines these responses in surface assemblages in the North Pacific subtropical gyre, at Station ALOHA, 100 nm north of Oahu. These responses were examined using the variable fluorescence method described above, as well as with a radiometric method to detect the natural, sun-stimulated fluorescence emitted by these assemblages.

A fifth chapter of this dissertation describes an ancillary study in which a method was developed to improve the retrieval of PSII properties using a commercially available fast repetition rate (FRR) fluorometer. This instrument is widely used in oceanographic field studies to measure many of the physiological properties of PSII that control the rate and efficiency of light harvesting. However, these instruments have significant hardware biases that introduce errors and artifacts into these physiological measurements. These errors are particularly important when using these instruments in the near-surface, oligotrophic ocean, where phytoplankton abundances are small, representative taxa have weak fluorescence yields, and high irradiances often lead to considerable fluorescence quenching.

Several additional issues related to rapid photosynthetic regulation are presented in the summary, Chapter 6. Included are discussions of some of the more important ramifications that this doctoral research has for phytoplankton ecology, both in theory and in practice. Specific attention is directed toward how rapid photosynthetic responses on the seconds to hour scale can be exploited to understand better some higher level ecological processes in the ocean. These are discussed in terms of expected advances in satellite remote sensing and in *in situ* autonomous observations.

Table 1-1. Symbols and abbreviations used throughout this dissertation.

Symbol / Term	Description	Units
JGOFS	Joint Global Ocean Flux Study	n/a
G	Generational scale of an organism	Time
NADPH	Nicotinamide adenine dinucleotide phosphate	n/a
ATP	Adenosine triphosphate	n/a
PSI, PSII	Photosystem I, Photosystem II	n/a
LHCII, RCII	Light-harvesting complex in PSII, reaction center in PSII	
E or *I*	irradiance	
PAR, E_{PAR}	Photosynthetically active radiance	μmol quanta m^{-2} s^{-1}
P, P_{max}	Photosynthetic rate (maximum rate)	Product $time^{-1}$
α	Light-limited constant for *P*	*P* $time^{-1}$ $irradiance^{-1}$
ϕ	General notation for quantum yield	unitless
ϕ^f, ϕ^p	Quantum yields of fluorescence and photosynthesis	unitless
σ_{PSII}, σ_{PS2}	Functional cross section of PSII	$Å^2$ $photon^{-1}$ $PSII^{-1}$
n, n_{PSII}	Number of PSII in a cell, sample	
P, P_f, P^{RCII}, P_e	Light-driven electron flow rates	Electrons $time^{-1}$
σ	Generalized functional cross section	Area $photon^{-1}$
τ	Time constant for electron flow	time
τ_{PSII}	Time constant specific to PSII	time
p	Index of energetic connectivity between PSII reaction centers	unitless

Table 1-1 (Continued)

Symbol / Term	Description	Units
F	Apparent fluorescence	Instrument units
F_o, F_m	Initial and maximal F	Instrument units
F_v	Variable fluorescence: F_m-F_o	Instrument units
F_v/F_m, $\Delta\Phi_{max}$	Normalized variable fluorescence	unitless
Φ^F, Φ^P	Apparent yields of fluorescence, photosynthesis	unitless
L	A length index for PSII simulations	length
E_K	Photosynthetic saturation irradiance	Units of E
FRR, FRRF	Fast-repetition rate (fluorometry)	
EX[], EM[]	FRRF excitation and emission	Instrument units
KPF, KPF98	The Kolber et al. (1998) model of variable fluorescence kinetics	
X^2	"chi-squared" quality of fit metric	unitless
I*	The critical light intensity of Huisman and Weissing (1994)	Units of I
Lu_{670}, Lu_{683}	Radiances at 670, 683 nm	$\mu W\ cm^{-2}\ sr^{-1}$
FLH	Fluorescence line height	$\mu W\ cm^{-2}\ sr^{-1}$

1.6 Literature cited

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2 Using stochastic simulations to assess how specific physiological changes in Photosystem II affect rates of primary photochemistry in phytoplankton

2.1 Abstract

A numerical simulation was developed to examine how specific changes in the structure and function of Photosystem II (PSII) affect light harvesting and photochemistry in phytoplankton. The energetic fate of photons interacting with idealized PSII populations were modeled dynamically using a Monte Carlo approach. By randomly directing photons through these populations, the yields of light-driven electron flow and PSII fluorescence can be determined if each simulated PSII is assigned certain properties: a photochemical yield, a mean functional cross section, potential energetic connectivity with other PSII, and a turnover time-scale of electron acceptors. The simplest form of this simulation represents the stochastic analog of analytical equations that have been used to predict light-driven electron flow rates from measurements of irradiance and PSII parameters. Stochastic models can replicate nonlinear dynamics within PSII populations that equation-based models cannot. Light-driven electron flow rates, simulated over a large physiological parameter space, were used as a look-up table to predict the time series of photochemistry in a phytoplankton culture, from measured PSII physiology and irradiance alone. The predicted relationships between photochemistry and irradiance reflected photosynthetic saturation at moderate irradiances and photoinhibition at supraoptimal irradiances. For a given combination of PSII physiological properties, simulating the light-driven electron flow over a range of irradiances generates the photosynthesis-irradiance relationship for that specific physiological state. In these simulations this relationship was best described by a rectangular hyperbola. This simulation also reflected transient kinetics in fluorescence that would be expected given current theory of variable fluorescence in PSII.

2.2 Introduction

2.2.1 Background

Phytoplankton are the primary conduit through which biologically utilizable energy enters pelagic marine ecosystems. The process by which ambient photons are absorbed and converted into metabolically useful energy is referred to as the photosynthetic light reactions. In these reactions, membrane-bound photosystems I and II (PSI and PSII) catalyze the conversion of photon energy into excited state energy. Subsequent biophysical and chemical reactions use this energy to provide a source of electrons for the regeneration of reductant (NADPH) and to support a transmembrane proton gradient for the phosphorylation of ADP into ATP. The NADPH and ATP generated support photoautotrophic metabolic.

Many phytoplankton taxa exhibit considerable physiological plasticity in photosystem structure and function, presumably to optimize light harvesting and to minimize light damage in the highly variable marine light environment. Physiological responses to perturbations in irradiance occur in individual phytoplankton across the entire temporal range of their generation scales, from seconds to days (Harding et al. 1987). Of the two photosystems, the physiological dynamics of PSII have been examined in more detail, because the activity of PSII can be readily examined by its *in vivo* fluorescence yield and oxygen evolving properties. Photochemistry in PSI has no oxygen signature and its *in vivo* fluorescence yield is much weaker.

A powerful tool for measuring specific physiological properties of PSII *in vivo* is single turnover variable fluorescence analysis. These methods rely on stimulating a population of PSII using a brief, 100 μs time scale flashes of saturating light (e.g., Johnson 2004; Koblížek et al. 2001; Kolber and Falkowski 1992; Olson et al. 1996). This time scale of saturating PSII with energy is much shorter than the time scale over which electron acceptors can drain this energy from PSII, i.e., a single turnover of acceptors. A model of photosystem energetics can be used to interpret the kinetics of

fluorescence yield that occur during and after this flash, in terms of specific photophysiological properties of PSII and its downstream electron acceptors. The physiological properties of PSII that are measured using such methods have found use as proxies for assessing nutrient stress in phytoplankton (e.g., Behrenfeld and Kolber 1999; Kolber et al. 1988; Vassiliev et al. 1995), although there is some debate about the types and scales of ecological inferences that can be drawn (e.g., Parkhill et al. 2001).

Additionally, photophysiological properties of PSII are often used along with irradiance measurements as inputs to deterministic models, which compute rates of primary photochemistry and light driven electron flow from irradiance and photosynthetic physiological properties alone (Table 2-1). Generally speaking, light harvesting and primary photochemistry are strongly nonlinear processes and are therefore difficult to model. The canonical *P-E* relationship clearly illustrates this inherent nonlinearity by saturating at high irradiances. Other feedbacks, thresholds, and lagged behaviors that occur throughout the photosynthetic light reactions suggest a broad role of nonlinear dynamics in light harvesting and utilization. Nonlinearity in the relationship between incident irradiance and photosynthetic electron flow arises from the inherent photosynthetic architecture shared by phytoplankton and all photoautotrophs: ambient photons are absorbed by discrete photosystems, finite in number, of finite effective optical cross section, interacting with a similarly finite pool of electron acceptors that can transport energy away from PSII on a finite time scale. Such a discrete and limited photosynthetic architecture has important ramifications for how a population of PSII collectively absorbs and processes a flux of incident photons.

Table 2-1. Seven different analytical models for computing light-driven electron flow from PSII photosynthetic parameters and irradiance. Notation and equation structure is identical to that presented in the original publication.

Model equation	**Reference**
$P = n \cdot \sigma_{PSII} \cdot \phi \cdot I$	Sukenik et al. 1987 (Eq. 1)
$P = I \cdot \sigma_{PSII} \cdot \Phi_q \cdot \Phi_t \cdot \Delta\phi_{sat}$	Kolber and Falkowski 1992 (Eq. 1)
$P_f = \left[\frac{\Delta\phi_m}{0.65}\right] \cdot q_p \cdot E \cdot \sigma_{PSII}$	Falkowski and Kolber 1993 (Eq. 5) Kolber and Falkowski 1993 (Eq. 12)
$P_{O_2}^B(E) = \sigma_{PS2} \cdot \Phi_{RC} \cdot q_P(E) \cdot \phi_e(E) \cdot f \cdot n_{PS2} \cdot E$	Falkowski and Kolber 1995 (Eq. 4)
$P = I \cdot \sigma_{PSII} \cdot n_{PSII} \cdot qP \cdot \left[\frac{\phi_{sat}}{1.7}\right]$	Falkowski and Kolber 1995 (Eq. 9)
$P_f = E \cdot \sigma_{PSII} \cdot \frac{\Delta F'}{F_v'}$	Gorbunov et al. 2000
$P_f = E \cdot \sigma_{PSII}' \cdot \frac{\Delta F'}{F_v'}$	Gorbunov et al. 2001
$P^{RCII}(E) = E \cdot \sigma_{PSII} \cdot qP(E) \cdot \phi_e(E) \cdot f$	Suggett et al. 2001

Light harvesting and the light-driven flow of photosynthetic electrons in populations of photosystem subunits can be described using analytical models derived from Poisson statistics, or "target theory" (sensu Dubinsky et al. 1986). If each photosystem in a population is assigned a mean functional cross section to absorb a single photon (σ, units of $Å^2$ $photon^{-1}$), as well as a turnover time representing the rate

at which acceptors relieve these photosystems of generated electrons (τ, units of ms), an exponential relationship between irradiance E and photosynthetic electron flow P_e can be derived.

$$P_e(E) = P_{\max} \cdot (1 - e^{-E \cdot \sigma \cdot \tau}) \qquad \text{Eq. 2-1}$$

The relationship between irradiance and photochemical electron flow P_e that is predicted by a Poisson-based model of electron flow well reflects the saturating behavior of commonly used functional *P-E* equations like Eq. 2-2 (Figure 2-1).

$$P_e(E) = P_{\max} \cdot \left(1 - e^{-\frac{E}{E_K}}\right) = P_{\max} \cdot \left(1 - e^{-\frac{\alpha E}{P_{\max}}}\right) \qquad \text{Eq. 2-2}$$

The threshold between light-limited and light-saturated photosynthesis in the target theory model is established jointly by the product of σ and τ, with the former representing a control on energy flux into PSII reaction centers, and the latter representing a drain of this energy out of the PSII population. Variable fluorescence techniques can be used to measure both of these parameters directly *in vivo* (Kolber et al. 1998; Mauzerall 1972). With appropriate conversion factors it can be shown that the inverse product of these parameters, $(\sigma \cdot \tau)^{-1}$, is a physiologically derived analog to the empirical light saturation parameter E_K (Falkowski 1992) that is computed in the exponential *P-E* model presented by Webb et al. (1974), as shown by analogy in Eq. 2-2.

Although Eq. 2-1 and Eq. 2-2 both express similar functional forms as exponentials, a P_e-*E* relationship derived using Poisson statistics differs importantly from the empirical functional *P-E* form because both σ and τ represent specific physiological properties. In contrast, empirical parameters such as E_K and α reflect a convolution of numerous physiological mechanisms that have no specific physiological interpretation, and thus are solely mathematical conveniences (Abbott

1993). Changes in measured P_e-E relationships, such as would be observed from oxygen evolution experiments, can be interpreted using the Poisson framework of Eq. 2-1 in terms of specific physiological changes in PSII populations, whose effect on light harvesting and photochemistry are explicit, and which can be independently and experimentally measured using several techniques.

Similarly, models of light-driven electron flow that are based on specific physiological aspects of light harvesting and photochemistry, e.g. equations in Table 2-1, also represent a more causal and mechanistic description of the relationship between irradiance and electron flows. Providing that they accurately reflect the dynamics of light harvesting and photochemistry among PSII populations, equations in Table 2-1 – coupled with an understanding of how environmental properties affect PSII photophysiology – in theory present a more deterministic explanation of how photosynthesis in natural phytoplankton varies under differing environmental conditions. Such determinism represents a considerable improvement on the empirical functional P-E relationships that appear in current models (e.g., Flynn 2001; Thornley 1998).

2.2.2 Assessing the dynamics of light harvesting and electron flow in PSII populations

Whether the equations in Table 2-1 are or are not in fact dynamically accurate is an important caveat. A robust model of any dynamical system includes not only all of the dominant parameters that control system behavior, but also properly integrates these parameters. Thus, any representation of light harvesting and photochemistry by PSII must include not only the major physiological properties that control these processes, but must also reflect accurately the dynamics of PSII energetics, i.e., how photon energy is absorbed by and migrates among individual PSII.

It is unclear how best to improve equations for P_e (e.g. Table 2-1), or to validate which PSII properties have the most effect on light harvesting and how they

should be integrated dynamically. Primary photochemistry in PSII and variable fluorescence are both mediated by same identical physiological structures, and it would seem that the best equations for P_e that include all measurable physiological properties of PSII should more accurately reflect the energetic dynamics of light-driven electron flow in PSII populations. Yet none of the analytical equations in Table 2-1 include all the PSII physiological parameters that can be recovered using single turnover variable fluorescence methods. The control on P_e by τ is never explicitly incorporated, nor is any potential enhancement in P_e that may result from connectivity p among PSII reaction centers (Joliot and Joliot 1964). One argument for omitting parameters like p from these P_e equations is that p affects fluorescence yield kinetics in phytoplankton only negligibly, by only a few percent (Kolber et al. 1998), and therefore should have a similarly negligible effect on P_e. However, in nonlinear systems this is a dangerous assumption, and changes in PSII properties that only minimally affect $F(t)$ may in fact considerably affect P_e. Thus, analytical equations in Table 2-1 are not necessarily the best basis for improving physiological models for P_e. Even very simple nonlinear systems do not have an exact analytical expression (May 1976; Strogatz 1994), which makes it impossible to describe some nonlinear dynamics in light harvesting and utilization using equations.

An alternative approach for examining certain types of nonlinear systems is to use stochastic techniques that model the structure and function of dynamical systems without restrictions and approximations that would be required by employing equations (Kantz and Schreiber 1997). We applied one such stochastic technique, a Monte Carlo method, to examine the light driven electron flow P_e that would result from a flux of photons of irradiance E that dynamically interact with a population of n PSII. The simulation is based on the same conceptual principles that underlie the equations in Table 2-1: a population of n PSII having physiological properties F_v/F_m, σ, p, and τ, which can be measured using techniques such as single turnover variable fluorescence analysis. This simulation allowed us to examine how individual and concurrent changes in specific PSII physiological properties affect P_e in idealized populations of photosystems. A fundamental advantage of this approach is that

stochastic simulations can be easily configured to replicate light harvesting dynamics in phytoplankton such as pigment packaging and variability in PSII spatial density, which are not easily represented with equation-based models. A second advantage of this stochastic simulation is that it is very easy to examine its response to step impulses. This is relevant with respect to PSII energetics because the dynamic response of absorbed photons that are fluoresced in response to a step increase in irradiance is equivalent to the variable fluorescence transient that can be measured by single turnover techniques, such as fast repetition rate fluorometry.

2.3 Methods

2.3.1 Structure of the simulation

The simulation tracks the fates of individual photons as they interact with a population of *n* idealized PSIIs, randomly distributed within a two-dimensional area with sides of length *L* (Figure 2-2). Individual PSII whose centers are initially located within one radius of the edge of the model space are "nudged" back into the model space before running the simulation, so that no PSII has any light harvesting area outside of the model space. Each PSII is assigned a functional cross section σ_{PSII}, a time constant τ corresponding to the average PSII electron throughput rate, and a probability *p* of transferring absorbed photon energy to another PSII if the absorbing PSII is already busy processing a prior photon. These three physiological properties of PSII are identical to those measured in actual phytoplankton using variable fluorescence techniques such as FRR fluorometry or similar methods. A fraction of these *n* PSIIs can be individually designated as photochemically nonfunctional, to simulate varying degrees of reaction center damage to a PSII that affect its photochemical yield. A fourth variable fluorescence parameter, $F_v/F_m = (F_m - F_o) \cdot F_m^{-1} \equiv \Delta\Phi_{max}$, is used to represent this behavior (Kolber et al. 1998).

The number of photons that are directed through L^2 for a given simulation is calculated from the desired irradiance E. These photons are sequentially directed through L^2 randomly in space. Their energetic fate is determined by the decision tree shown in Figure 2-3. Photons that pass through L^2 and that intersect the area representing the functional cross section of any PSII are considered to be absorbed by that PSII. Photons that do not intersect a PSII pass through the model space and thus cannot contribute to P_e. Whether or not an absorbed photon contributes to P_e depends on the photochemical state of the PSII that absorbed it. If that PSII is defined to be photochemically incompetent, simulating reaction center damage for example, that absorbed photon is considered to be dissipated thermally. If absorbed by a competent PSII that has not recently absorbed a prior photon, i.e., having an "open" reaction center, this photon contributes to P_e and photochemically closes the reaction center in that PSII. That reaction center stays closed for a photocycle lasting 3τ, approximately 99% of the time it takes for an electron acceptor to relieve that reaction center of that photon energy and reopen it. Additional photons that intersect a closed PSII during its 3τ closure period will be reemitted as fluorescence, unless this photon energy is redirected by energetic connectivity between reaction centers to another randomly chosen PSII. The strength of this connectivity is set by the value assigned to p, with units of a probability. A stochastic approach is particularly advantageous with respect to modeling the influence of connectivity on P_e because such models make it easy to constrain connectivity within predefined PSII 'domains' of small numbers of 2 to 5 PSII (Bernhardt and Trissl 1999). Such restrictions are often difficult to represent using analytical equation-based models, although some aspects of limited energetic connectivity can be modeled using such an approach (Trissl 2003).

The simulation continuously monitors the entire PSII population to determine how long each individual PSII remains closed following a photochemical event. To ensure an accurate representation of the dynamics of these PSII populations and to minimize statistical noise in the output, each simulation was terminated after a period of 200τ. The steady-state rate of electron flow through the PSII population P_e was calculated by summing all photon events leading to reaction center closure, for each

simulated physiological state and irradiance. The apparent yield Φ^P of this electron flow was computed by dividing P_e by the number of photons that were directed through L^2 during that 200τ period. Steady-state yields of fluorescence emission F were similarly calculated (Φ^F) by dividing the total number of fluoresced photons within 200τ by the total number of photons directed through L^2. The yield of thermal dissipation is fixed at zero in these simulations, but Φ^F and Φ^P will not sum to unity because these are defined as apparent yields, i.e., normalized to the total number of photons directed through the model space, not to the number of photons absorbed by PSII. For a given combination of PSII physiological parameters, the values of P_e that are predicted over a given range of irradiances indicate the P_e-E relationship corresponding to that PSII physiological state.

The physiological model of single turnover PSII variable fluorescence (Kolber et al. 1998) includes no description of nonphotochemical mechanisms that dissipate absorbed photon energy and thus quench fluorescence, such as thermal dissipation pathways or chemical interactions that quench fluorescence. For simplicity and for consistency, the yields of such loss processes are fixed at zero for these simulations, although this restriction can be readily relaxed as desired. Additional terms appear in some of the specific P_e models listed in Table 2-1, e.g., the efficiency of transfer of absorbed energy within a PSII to its reaction center, or the quantum yield of charge separation. However, these are typically defined to be unity and are included in those equations for heuristic purposes only, not as actual variables that take on different values. Finally, the simulation described here can be run in a trace mode, where the dynamic response of a population of PSII to an impulse of light can be predicted. This provides a means to simulate transients in electron flow or in variable fluorescence, such as those that are induced using single turnover variable fluorescence techniques.

The length dimension L is chosen so that physiologically reasonable values of simulated σ_{PSII}, τ, p, and F_v/F_m will lead to saturation of electron flow at roughly the same irradiance intensities that are observed in cultures and in natural assemblages of phytoplankton. For example, the parameter values in Table 2-2 lead to a simulated E_K

occurring at exactly 100 μmol quanta m^{-2} s^{-1}. Such length scaling of this model preserves the energetic dynamics of the PSII population, provided that the scaling does not introduce significant self-shading of PSII within the population, as would occur by compressing a large number of PSII into a comparatively small L^2.

Table 2-2. The range of photophysiological PSII properties and irradiance intensities over which P_e was computed in these simulations.

Parameter	Simulated values	Units
n	250	dimensionless
L	$0.5 \cdot 10^{-7}$ & $1 \cdot 10^{-7}$	m
E	0.1, 0.2, … 50, 60, … 200, 250 … 500	μmol quanta m^{-2} s^{-1}
σ_{PSII}	200, 300, … 1200	$Å^2$ $quanta^{-1}$ $PSII^{-1}$
p	0, 0.05, … 0.5	dimensionless
τ	1, 2, … 10	ms
F_v/F_m	0.05, 0.1, … 0.65	dimensionless

Finally, because the simulation is stochastic, there is a possibility that using an inadequate random number generator will not provide a truly random distribution, if the number of random numbers required is larger than the numerical period of the generator. This simulation was written in C and used the "ran1" algorithm presented in Press et al. (1992) to generate random numbers. The period of this algorithm is greater than 10^8, which is much larger than the 6 million photons that are required to be randomly directed through L^2 when simulating the highest irradiance intensity examined in this study (500 μmol quanta m^{-2} s^{-1}).

2.3.2 Simulations to assess dependence of electron flow rate on irradiance

Simulations following the decision tree in Figure 2-3 were performed for every permutation of irradiance E and the PSII physiological properties F_v/F_m, σ_{PSII}, τ, and p listed in Table 2-2. The ranges of the physiological properties of PSII are representative of published values found in the literature. The simulation irradiances were spaced unevenly to provide fine resolution at low irradiance intensities, then progressively coarser resolution up to the highest intensity that was simulated: 0.1 μmol quanta m^{-2} s^{-1} from 0.1 to 50, 10 μmol quanta m^{-2} s^{-1} from 50 to 200, and 50 μmol quanta m^{-2} s^{-1} from 200 to 500.

Photosystem-photon interactions were examined over this entire irradiance and photophysiological model space, in two sets of simulations to compare how the spatial density of PSII in L^2 affects light harvesting dynamics. These two simulations differed only in the length scale L, which was 100 nm in the former PSII population, considered to be "sparse", and 50 nm in the latter population, representing a factor of 4 decrease in L^2 (Figure 2-4) and thus a more "dense" population.

2.3.3 Application: merging PSII physiology and irradiance measurements with simulation predictions

The estimates of P_e that were generated over the five dimensional ranges of E, F_v/F_m, σ_{PSII}, τ, and p were used as a look-up table to compute a time series of P_e for an actual phytoplankton culture, directly from actual measurements of irradiance and PSII physiology. This look-up table approach saves considerable computational time as the Monte Carlo simulations need not be performed for every combination of actual data, only over a model space of physiology and irradiance that is finely enough resolved to capture relevant variability in PSII physiology.

The test data chosen for this application come from the "constant dilution rate" model diatom culture presented in Laney et al. (2005). In brief, a culture of *Thalassiosira weissflogii* was maintained in a chemostat at very low growth rates (dilution rate of 0.11 d^{-1}) and provided sinusoidal daily irradiances with midday maxima of ≈ 50 μmol quanta m^{-2} s^{-1}. After approximately 5 volume turnovers under these low irradiance conditions, the midday maximum was increased to ≈ 400 μmol quanta m^{-2} s^{-1}. Samples of this culture were continuously measured using fast repetition rate fluorometry to provide the fluorescence transients *F(t)* that are needed to estimate F_v/F_m, σ_{PSII}, τ, and p on ≈ 1 min intervals. Details of culture maintenance and single-turnover variable fluorescence data collection can be found in Laney et al. (2005).

For the current study, we reprocessed the original fluorescence transients *F(t)* measured by Laney et al. (2005), using a more advanced version of the *F(t)* analytical software. This analytical software (v6, S.R.L) improves upon the instrument characterization used by Laney et al. (2005). The photochemical yield F_v/F_m was computed from F_o and F_m as $F_v/F_m = (F_m - F_o) \cdot F_m^{-1}$. We use these reprocessed estimates of F_v/F_m, σ_{PSII}, τ, and p, and the concurrently measured irradiance data, from the twenty days that bracket this step increase in irradiance, and find the simulated P_e that most closely correspond to that PSII physiological state and irradiance, using a nearest neighbor search algorithm.

2.4 Results

2.4.1 Reconstructing photosynthesis-irradiance relationships from simulation output

The simplest PSII-photon interactions to consider are those in which there is no energetic connectivity between PSII reaction centers, i.e., a PSII population where $p = 0$. In such a model system, individual PSII operate wholly independently in their absorption and processing of photon energy. The relationship between P_e and E that the simulation predicts for such a PSII population closely reflects the canonical "saturating" behavior that is expected in a photosynthesis-irradiance relationship. The magnitude and irradiance-dependence of P_e change as expected for specific changes in F_v/F_m, σ_{PSII}, and τ when there is no connectivity between PSII reaction centers: decreases in F_v/F_m led to decreases in P_e, a twofold decrease in σ_{PSII} resulted to irradiance-saturated P_e at higher irradiances, as did a fivefold decrease in τ (Figure 2-5a). At very low irradiances with minimal self-shading, the P_e that were predicted asymptotically, but not linearly, approached $P_e = 0$ for $E = 0$. However, it is clear from the simulation output that even at these very low irradiances, $\frac{dP_e}{dE}$ is not constant and the relationship P_e and E is not linear (Figure 2-6).

Several functional forms of the *P*-*E* relationship, including Jassby and Platt (1976), Bannister (1974), Platt et al. (1980), and Thornley (1998), were fitted to an example P_e-E relationship that was generated by this simulation to see which functional form best fit the simulated output. The example P_e-E was chosen to exhibit clearly saturating dynamics, to provide the functional *P*-*E* equations an appropriate dynamic range of behavior to which to fit. Of the different functional forms, the non-rectangular model, a rectangular hyperbolic models, and the Bannister (1974) model fit best and virtually identically (Figure 2-5b). The rectangular hyperbola provided an equally good fit using one less degree of freedom than the other two models. The shape parameter predicted by the non-rectangular hyperbolic model (ξ) was 0.0626, which is essentially identical to the implicit shape parameter of 0 of a rectangular hyperbola (Thornley 1998). Thus the non-rectangular hyperbolic fit to these P_e data can not be considered to represent a truly distinct functional form. The shape parameter in the three-parameter model of Bannister (1974) is differently defined from that in Thornley (1998) and fitted these data at almost exactly 1.00. This similarly

reduces this three-parameter model to a simple rectangular hyperbola. An exponential functional form, e.g., one using an empirical saturation parameter E_K such as Eq. 2 and mathematically equivalent to the Poisson-derived model of Eq. 1, did not fit these simulated P_e-E relationships as well as the rectangular hyperbola.

Reconstructing variable fluorescence transients from simulation output – As is shown by the decision tree in Figure 2-3, this simulation tracks not only the photons that undergo primary photochemistry, but also those that are fluoresced from an irradiated PSII population. Thus, this simulation can also be used to examine fluorescence yield transients *F(t)* in these idealized PSII populations, like those that would be induced by single turnover variable fluorescence techniques. This ability provides a means to examine the impulse response of these modeled PSII populations and to check the energetic dynamics of these populations against the physiological model of variable fluorescence presented by Kolber et al. (1998). This provides an independent means to validate the dynamical behavior of the photon-PSII interactions in these simulations, to ensure that the dynamics of these PSII populations are properly incorporated.

Fluorescence transient kinetics were tracked in an example simulation for a PSII population having the physiological state F_v/F_m = 0.65, σ_{PSII} = 1200.0, τ = 10000, p = 0.50, n_{PSII} = 500, and L = 1.00e-07, for the first 100 ms following a step impulse in irradiance of 10,000 μmol quanta m^{-2} s^{-1}. This impulse replicates the saturating flashes of excitation irradiance that are used in single turnover variable fluorescence techniques, such as fast repetition rate fluorometry. A larger number of PSII were required in this simulation than in the steady-state runs to generate the P_e matrices as a function of PSII physiological state, in order to minimize the stochastic noise in the simulated *F(t)* transients. The variable fluorescence kinetics of the resulting $F(t)$ compare well to the saturating curves predicted that the Kolber et al. physiological model over ms time scales, showing rapid saturation of *F(t)* and eventual slow decay due to the action of electron acceptors up to ≈ 600 μs (Figure 2-7a). The overall behavior of *F(t)* over this period is polyphasic; a rise is evident at ≈ 650 μs which also

appears in replicate simulations (data not shown), suggesting that this feature is not a stochastic artifact. Over shorter time scales of 100 μs, the simulated fluorescence transient kinetics well replicate the shape of the Kolber et al. physiological model of variable fluorescence (Figure 2-7b).

Enhancement of electron flow rates due to connectivity among PSII – When energetic connectivity between reaction centers was allowed in these simulations, i.e., $p \neq 0$, the electron flow rates predicted by these simulations were increased. This enhancement was most evident at low irradiances, below that at which P_e "saturates", but since this simulation reflects a dynamic and nonlinear system, the eventual maximal electron flows that were computed at "saturation" were also enhanced slightly compared to equivalent PSII states where $p = 0$ (Figure 2-8a). The term "saturate" is not completely accurate and is only used here for the sake of historical consistency; in fact when the P_e-E relationship reaches a steady state at high irradiances, a small fraction of PSII remain photosynthetically open at any given instant in time. For the example shown ($F_v/F_m = 0.6$, $\sigma_{PSII} = 1200$, $\tau = 5000$, in a "sparse" PSII population), connectivity of $p = 0.5$ resulted in an enhancement of P_e of up to 25% at low irradiances above that predicted when $p = 0$, and an ≈ 2% enhancement at saturated electron flow (Figure 2-8b).

The enhancement of P_e across the entire 5-dimensional field of PSII physiological state and irradiance was examined by plotting histograms of P_e enhancement due to connectivity, for both sparse and dense PSII populations. For these plots, only simulations corresponding to the range of irradiances 10 to 200 μmol quanta m^{-2} s^{-1}, evenly spaced in 10 μmol quanta m^{-2} s^{-1} intervals, were considered, to minimize bias in these histograms that may result from also including the very fine or very coarse irradiance intervals in Table 2-2 for the low and very high irradiance levels, respectively. The resulting histograms show different distributions of enhancement, depending on the level of connectivity as established by p and the degree to which the simulated irradiance differs from that of electron flow saturation.

Of the 28,600 P_e-E curves that were generated between 10 and 200 μmol quanta m^{-2} s^{-1} in the "sparse" PSII population simulation, the central tendency of enhancement by p was less than 10% with p = 0.25 (Figure 2-9a). Doubling p to 0.5 increased the central tendency of this enhancement only somewhat. In the simulation run that examined a densely packed PSII population (L = 50 nm), in general the central tendencies of these two distributions were increased somewhat but not materially so (Figure 2-9b). By computing the photochemical E_K for each simulation from the simulated σ_{PSII} (in $Å^2$ $photon^{-1}$) and τ (in ms):

$$E_K = \frac{6.022 \cdot 10^{-23} \cdot 10^6}{(\sigma_{PSII} \cdot 10^{-20}) \cdot \tau \cdot 10^{-3}} \qquad \text{Eq. 3}$$

it becomes possible to estimate where along the P_e-E curve, at any PSII physiological state, the threshold occurs between light-limited and light-saturated electron flow rates. The constants in Eq. 3 convert the $E_K = (\sigma \cdot \tau)^{-1}$ of (Falkowski 1992) into units of irradiance in order to compare these E_K directly with the E of these simulations. For the 1300 simulations in the sparsely packed PSII population where p = 0.25, enhancement in P_e due to p was typically 9% for irradiances that were about half of the expected photochemical E_K of that physiological state. Increasing p to 0.5 increases the magnitude of this enhancement approximately twofold to 18% (Figure 2-9c). At higher irradiances relative to E_K, simulation results for p = 0.25 and 0.5 show that the magnitude of enhancement decreases as simulated E approaches and exceeds the E_K predicted from photochemical principles (Figure 2-9e,g). However, the enhancement never drops to zero, even at E = 1.5 E_K. For the equivalent set of simulations where the PSII population was more densely packed, enhancement of P_e becomes an even stronger function of p, with up to 40% increases in P_e as a result of connectivity between PSII (Figure 2-9d,f,h).

2.4.2 Estimating electron flow of actual cultures from PSII physiology and irradiance

The reprocessed variable fluorescence parameters from Laney et al. (2005) are shown in Figure 2-10. Clear differences in photosynthetic parameters are evident following the shift to higher irradiances on day 258. Midday quenching of fluorescence becomes evident in both F_o and F_m, and is reflected in F_v/F_m. The P_e-E relationships that were predicted from these measured time series of irradiance and PSII physiological data, by incorporating the simulation output, in general behave as saturating functions of irradiance. For steady-state growth under nitrate limiting conditions and low irradiance, P_e shows no saturation from the beginning of the light period to the highest irradiances at midday (Figure 2-11a). These trends appear generally linear with irradiance, despite considerable variability in the individual PSII physiological parameters during this time (Figure 2-10).

Immediately after the shift to high irradiance conditions, these P_e-E curves begin to show saturating behavior with irradiance but not necessarily photoinhibition in P_e at the highest irradiances, except perhaps somewhat at the very highest irradiances many days after the increase in irradiance (Figure 2-11c). These predicted P_e were approximately 5-10% smaller when computed with p set to 0 (asterisks), with this effect located primarily around E_K. Since σ_{PSII} is the only input parameter from Figure 2-10 that relies on a calibration, performed at the factory by the manufacturer, we also computed these P_e-E curves assuming a twofold error in this calibration. Assuming such an error changes the predicted P_e-E relationships considerably by shifting the saturation irradiance lower into irradiance space (Figure 2-11, plus symbols). There were no material differences evident between predictions of P_e based on either the "sparse" and "dense" simulation output, given the particular values of L chosen to represent those cases.

2.5 Discussion

2.5.1 Modeling PSII energetic dynamics in phytoplankton: ecological relevance

Capture of photons by membrane-bound pigments, and their conversion by photosynthetic reaction centers into metabolically useful energy, is the first step in photosynthesis. From the perspective of cellular energetics, understanding the efficiency of photon energy capture and utilization is important because it establishes the upper limit of energy available to the cell for metabolism. Processes such as carbon assimilation, nutrient uptake, and motility all require supplies of the two direct products of the photosynthetic light reactions: ATP and NAPDH. A central goal of marine phytoplankton ecology is to understand how environmental and physiological processes constrain photosynthesis in oceanic assemblages, and the rate-limiting step in the generation of these internal cellular resources occurs in these initial steps of the process, when PSI and PSII absorb photon energy from the environment and transform it into a light-driven electron flow.

Because generation of ATP and NAPDH are mediated by specific photosynthetic structures on the thylakoid membranes, primarily the photosystems and cytochromes, the energetic dynamics of this collective system can be modeled from first principles. This modeling will be robust only if the interactions with these structures and the ambient light field and with each other can be adequately described. However, it is difficult to measure the important physiological properties associated with all the primary structures of the photosynthetic light reactions. Activity of PSI and the cytochromes intermediates does not produce easily measured fluorescence and oxygen byproducts that PSII generates, and consequently the physiological and dynamical aspects of these structures are less well understood. Even though the physiological dynamics of PSII provide only a partial picture of the whole-system energetics of the photosynthetic light reactions, this partial picture is nonetheless

valuable because it indicates when and how certain photosynthetic adjustments are made. Such adjustments either 1) indicate a specific photosynthetic response to a change in environmental conditions, or 2) reflect a general metabolic cycle on diurnal or generational scales. Metabolic cycles that affect photosynthetic structure and function also indicate photosynthetic responses to environmental change when day-to-day differences in diurnal patterns in PSII behavior can be associated with specific environmental stressors Laney et al. (2005).

Using a numerical approach to simulate the energetic dynamics of PSII provides insight into the specific physiological adjustments of PSII structure and function that affect the supply of metabolic energy. The simulation described in this study was initially developed as a heuristic tool, and the stochastic model is well suited for examining the complex, nonlinear system of light harvesting by PSII. Because it is based on target theory of photon-photosystem interactions, this particular stochastic model provides a physiological basis for determining the relative importance of specific photon-photosystem interactions and the key photosynthetic adjustments that affect the overall capacity and efficiency of energy capture (Falkowski 1984). Relevant physiological properties of PSII that can be examined include heterogeneity within PSII populations, energetic sharing between PSII and PSI, different models of electron acceptor processes, and different photosystem organizations that affect reaction center connectivity. The degree to which physiological variability of these forms affects photosynthesis in marine phytoplankton is poorly understood, because the effect of this variability on light harvesting is difficult to assess using currently available measurement techniques. In contrast our simulation approach provides a framework for quantifying how photosynthetic rates are constrained by specific physiological states of PSII. Thus, simulations provide a means to identify which facets of PSII physiology should be examined in more detail in order to improve the use of PSII physiological data to assess photosynthetic variability in marine phytoplankton.

The ranges of σ_{PSII}, τ, p and F_v/F_m that were used in these simulations were experimentally measured values reported in the literature, and thus can be considered

representative for modeling purposes. Saturation of photosynthetic electron flow rates occurred at low irradiances (E < 200 μmol photon m^{-2} s^{-1}), comparable to the order of magnitude of E_K found in the literature. Consequently, it was not necessary to perform simulations at very high irradiances to examine the range of P_e-E behaviors that can be expected in the PSII populations of most taxa under typical environmental conditions. An upper limit of E = 500 μmol photon m^{-2} s^{-1} in the simulation provided a comprehensive range of P_e-E behaviors for the range of PSII physiological states measured.

There are aspects of the physiological data in Figure 2-10 that are causes for concern, however. The values computed for σ_{PSII} are low compared to what has been presented in the literature. A estimated value for the low range of σ_{PSII} would probably be around 500 $Å^2$ $photon^{-1}$ $RCII^{-1}$ (Falkowski and Raven 1997), about twice that measured in these cultures. Of all the physiological parameters measured using the FRRF approach, σ_{PSII} is the only one that relies directly on an instrument calibration. How the manufacturer conducts this calibration has not been made publicly available and no estimate of the error in this calibration is provided. An error in this excitation intensity calibration will scale directly into computed values of σ_{PSII}, and therefore into the photochemical E_K we compute (Figure 2-10). Thus, if our measured σ_{PSII} is too low by at least a factor of two, the E_K will be too high by the same factor. Thus, assuming an error in the manufacturer's calibration would explain the presumably unrealistic values of σ_{PSII} and E_K that we observed. Assuming this error would also result in P_e-E relationships that demonstrate earlier saturation at lower irradiances, and photoinhibition at the highest midday irradiances.

2.5.2 Energetic dynamics within PSII populations that enhance electron flow

The results of this study indicate that at least two physiological aspects of a PSII population – spatial density and the degree of energetic sharing – can enhance

light-driven electron flow considerably, depending on its physiological state. Such interactions would not be predicted by the simple electron flow equations in Table 2-1, which express systems in which photosystems act independently in the harvesting and processing of light energy. A high spatial density of PSII introduces self-shading among the population, an optical phenomenon which has been broadly referred to as "pigment packaging". Pigment packaging has been examined previously but primarily from a purely optical perspective, for the purposes of refining estimates of chlorophyll-specific absorption (a^* of Bannister 1974 and Dubinsky and Berman 1984, or k_c of Morel and Bricaud 1981 and Kiefer and Mitchell 1983).

The energetic ramifications of pigment packaging are less well explored. Variability in photosystem location within a cell and spatial density can be expected to affect light harvesting and overall cellular photosynthetic yield considerably (Fisher et al. 1998). In an experimental analysis, Berner et al. (1989) concluded that photoadaptation observed in *Dunaliella tertiolecta* represented both changes in pigmentation and changes in how these pigments were packaged, in roughly equal proportions. If pigment packaging occupies as important a role in photoadaptation in natural assemblages as Berner et al. (1989) suggests, these simulations then indicate that even moderately packed PSII populations may benefit considerably from energetic sharing among PSII. Published measurements of environmental variability in connectivity (p) in natural phytoplankton assemblages are rare. Laboratory studies suggest that the degree of energetic connectivity among PSII reaction centers ranges from 0.2 to 0.6 in most algae (Falkowski and Raven 1997). Our own measurements, in cultures and in natural field assemblages indicate a comparable range of p (Laney 2003), that in some instances exhibits a strong diurnal pattern (this study).

An important caveat for using the Kolber et al. (1998) physiological model of PSII variable fluorescence to estimate p from single turnover variable fluorescence transients is that the two PSII parameters σ_{PSII} and p are not numerically independent in that model (see Laney, this dissertation, Chapter 5). Thus, it is possible that numerical tools used to fit this physiological model to measured variable fluorescence transients do not estimate robustly the typically weak influence of p on $F(t)$ (Laney

2003). In order to understand better any natural variability in p in phytoplankton assemblages, and the role this variability plays in enhancing P_e in PSII populations that exhibit some degree of packaging, improved experimental tools for measuring p need to be developed.

It is questionable to examine how self-shading of PSII or PSII connectivity affect light harvesting using analytical models of PSII energetics (e.g. Table 2-1). For those analytical models that are derived from Poisson statistics, using them to examine self-shading would violate a fundamental requirement that all PSII targets have an equal probability of interacting with the photon flux. As shown in this study, replicating a PSII population with material self-shading is trivial using a simple stochastic approach. Given the degree to which these equations still contain empirical parameters, it is doubtful whether expansions of the analytical equations in Table 2-1 would be able to replicate the energetic dynamics that occur within this complex, nonlinear system. The parameters used to parameterize the quantum yield of photochemistry (e.g., Φ_q, q_p, $q_p(E)$, qP) and the light-dependent terms used to represent reaction center closure (e.g., ϕ_{sat}, $\Delta F'/F_v'$) encapsulate the behavior of physiological processes. Although equations can be written that encapsulate the overall effect of such physiological factors in empirical parameters like q_p and ϕ_{sat}, defining such closure terms does not improve our fundamental understanding of the energetic dynamics that govern photosynthetic light uptake, similarly to the way in which defining an E_K in a P-E functional form does not either (Abbott 1993).

2.5.3 Functional forms and physiological principles

These simulations establish the energetic dynamics between E and P_e from first principles, through specific PSII physiological properties and fixed interactions between PSII. The P_e-E relationships predicted by these simulations for a given physiological state of PSII derive from physiological first principles and incorporate more measurable physiological parameters than any other functional form for P_e-E has

to date. Presumably, this complete dynamic model provides a more accurate description of the actual P_e-E relationship in PSII, than do the empirical forms from measured proxies such as rates of oxygen evolution or radiocarbon uptake. However, the observed relationship between simulated E and P_e behave similarly to what is expected from experimentation and observation (Figure 2-11). Yet it should not be automatically expected that the canonical functional forms of P-E relationships found in the literature should well fit the simulated P_e-E relationships we observe here. Although it is tempting to assign functional P-E curves to these simulation results, subtle differences exist between the simulated P_e-E curves and functional P-E relationships that appear in the literature, which can affect the interpretation of P_e-E relationships generated by these simulations.

Different functional forms of the P-E relationship reflect specific inherent principles using a mathematical representation. The empirical parameters in these equations often reflect the combined influence of numerous physiological factors and are solely mathematical conveniences. As has been discussed (Abbott 1993), this ambiguity complicates how changes in the shape of photosynthesis-irradiance relationships should be interpreted. Of the various functional P-E forms examined, a rectangular hyperbola (Maskell 1928; Thornley 1998) provided the best fit to the P_e-E relationships generated by these simulations, with the fewest parameters. A rectangular hyperbola expresses the principle of diminishing returns. The analogy with respect to photosynthetic light harvesting is that as photon flux increases for a fixed number of PSII with set physiological state, light-driven electron flow rates will decrease.

The failure of other functional forms of P-E to fit the simulated P_e-E relationships suggests that any principle they may represent does not well reflect the energetic dynamics of PSII reaction centers. The simple exponential of Webb et al. (1974) and the hyperbolic tangent of (Jassby and Platt 1976) can both be derived from identical first principles, by assuming that change in P as a function of E follows a specific infinite power series, the Maclaurin series.

$$\frac{dP}{dE} = a_0 + a_1 P + a_2 P^2 + a_3 P^3 + a_4 P^4 + a_5 P^5 + \ldots$$

Eq. 4a

The two solutions differ in the order to which Eq. 4a is approximated. The exponential *P-E* relationship of Webb et al. (1974) derives from taking a linear approximation of Eq. 4a,

$$\frac{dP}{dE} = a_0 + a_1 P \qquad \text{Eq. 4b}$$

whereas to derive the hyperbolic tangent *P-E* relationship of (Jassby and Platt 1976) derives from taking a quadratic approximation.

$$\frac{dP}{dE} = a_0 + a_1 P + a_2 P^2 \qquad \text{Eq. 4c}$$

Solving these differential equations requires information on boundary conditions, one for each unknown coefficient. Chalker (1980) derives the Webb et al. (1974) exponential form $P = P_{\max} e^{-(E \backslash E_K)}$ from Eq. 4b, solving for a_0 and a_1 by assuming that $P(E=0)$ is 0 and that $P(E=\infty)$ is some value P_{max}. The derivation by Chalker, and restated by Platt and Sathyendranath (2000), is needlessly complex because such an exponential form is the implicit solution of any separable ordinary differential equation such as Eq. 4b. The numerous changes of variables from a_0 and a_1 into various combinations of α, P_{max}, and I_K described by Platt and Sathyendranath (2000) are not required to integrate Eq. 4b and may possibly introduce tautologies in their derivation. The added assumption of Platt and Sathyendranath (2000) that the initial slope of the *P-E* relationship be nonzero simply avoids the trivial solution of such differential equations, that P_{max} is 0 everywhere, but has no inherent physiological meaning.

Integrating Eq. 4b to arrive at the hyperbolic tangent *P*-*E* functional form $P = P_{\max} \tanh(E / E_K)$ is more challenging mathematically. Given now three coefficients in Eq. 4c instead of the two in Eq. 4b, it would appear that a third boundary condition is required. However, a hyperbolic tangent is also an implicit solution to quadratic approximations of infinite power series, provided that the first order coefficient a_1 is zero. It is unclear what the additional assumption is that allows Platt and Sathyendranath (2000) to conclude that $a_1 = 0$; again, changes of variables and redefinitions such as $\alpha = P_m/I_K$ may introduce tautologies in their derivation.

Solving power series approximations in exponential $y = ae^{bx}$ and hyperbolic tangent $y = a\tanh(bx)$ forms is well studied in introductory calculus and physics, as such equations describe terminal velocity solutions for falling bodies. It is questionable, however, if either of these functional forms reflect any underlying inherent principle, other than the basic mathematical fact that any analytical curve can be represented using a power series of infinite order. The only assumptions needed to arrive at these functional forms is for a curve to exist that has nonzero initial slope and that asymptotically approaches some maximal *P*. Little insight into the physiological implications for photon-photosystem interactions can be obtained from this, especially regarding the kinetics of *P* below P_{max} which are of immediate interest here.

Expecting a functional form for *P*-*E* to apply to these simulated P_e-*E* relationships may lead to one final issue regarding how the output of these simulations is interpreted. These simulated P_e-*E* relationships do not exhibit any "photoinhibition", as would be expected from the complete version Platt et al.'s (1980) model that parameterizes this behavior using a term β. This issue is easily resolved by realizing that these simulated P_e-*E* curves represent an instantaneous "snapshot" of the electron flow that can be expected for a given physiological state, at a particular irradiance. In the multidimensional P_e matrix, these curves are only the projections of P_e onto a range of *E*, for a fixed combination of PSII physiological parameters. The shape of *P*-*E* curves that are measured in nature and in cultures show a decrease in P_e with *E* at higher irradiances because these measurements unavoidably convolve time-

dependence and physiological changes in the PSII population. This behavior is evident when we use actual time series of irradiance and PSII physiology to drive our P_e predictions (Figure 2-11). Such curves represent photosynthetic states that are not fixed but that vary, i.e., the structure and function of PSII populations at high irradiances differs from those at lower irradiances. At a fixed physiological state of PSII there are only two possibilities: either there is additional capacity in the population for additional light to be harvested, or the population has no excess capacity. There is no mechanism for a local maximum in electron flow at intermediate irradiances for a fixed photosynthetic state.

2.5.4 Future directions

Using analytical equations to model how the physiological state of PSII affects P_e is computationally easier than using stochastic models. However, estimates of P_e derived using analytical equations will not be robust if they fail to include an accurate physiological description of the energetic dynamics of light harvesting and electron flow. With refinement, our stochastic simulations may provide a practicable alternative to the analytical models in Table 2-1, by incorporating a larger number of PSII physiological parameters and dynamics into computations of photosynthetic electron flow. The potentially strong enhancement of P_e identified here indicates that both PSII packaging and energetic connectivity among reaction centers should receive more attention, in photosynthetic electron flow models. Since the analytical equations in Table 2-1 do not contain either of these properties, such a finding would not have resulted from use of these models to examine PSII electron flow dynamics.

The potential role of other aspects of light harvesting physiology in phytoplankton, such as photosystem stoichiometry and organization (Kim et al. 1993) and mobility (Mullineaux et al. 1997; Sarcina et al. 2001), mechanisms of photoprotection (e.g., Lavaud et al. 2004), energy transfer among PSII (Ley and Mauzerall 1986), and spectral variability in light utilization (Falkowski and LaRoche

1991), present a wide range of applications for simple dynamic simulations. Being able to use the output of these simulations as look-up tables to predict P_e from a specific photosynthetic state and irradiance also has widespread application in examining how phytoplankton in dynamic light environments respond photosynthetically to perturbations in the ambient light field. In natural assemblages it is now possible to measure several key physiological aspects of PSII *in situ*, using variable fluorescence techniques. However, these measurements do not provide any information about how physiological responses to irradiance perturbation actually mediate photodamage under high light transients, or enhance light harvesting during low light transients. The integrated effect over time of nonlinear dynamical photosynthetic light reactions, cannot be examined using linear or simple nonlinear analytical equations (Levins 1979). Stochastic simulations, although computationally cumbersome, have no such limitation.

One aspect of PSII structure that was not examined in this study, but which is readily explored using this stochastic framework, are the energetic ramifications of different models of PSII organization. Since the discovery of energetic connectivity among PSII (Joliot and Joliot 1964; 2003) there have been efforts to identify how this connectivity actually occurs within PSII populations. Limited "domains" of connectivity have been hypothesized, of size 2 to 5 PSII, as well as widespread connectivity throughout the entire PSII population, i.e., the "lake" model of PSII organization. The issue of how exactly this energetic sharing occurs within PSII populations is difficult to validate experimentally (e.g., Bernhardt and Trissl 1999), yet it may have considerable implications in how phytoplankton utilize a combination of physiological responses in PSII to optimize light harvesting. Stochastic simulations provide a means to identify and quantify these energetic ramifications in a manner that heavily formalized analytical treatments (e.g., Trissl 2003) have not to date been capable of replicating.

Computationally, the Monte Carlo implementation used in this study is crude and inefficient compared to other more refined techniques commonly used to simulate dynamics of such systems. There is considerable overlap between the approach being

taken in this study to model photon-photosystem interactions, and other fields such as medical radiography where the underlying dynamic systems are similar (e.g., Andreo 1991). For this exploratory study, it was decided to focus on the physiological structure first and the computational refinement second, in order to provide maximum flexibility in replicating known structural and functional aspects of light harvesting in phytoplankton. The Monte Carlo approach lends itself well to parallel structured computing, and future versions of this simulation may use such refined methods to reduce the computational effort required to predict electron flow rates from irradiance and PSII photophysiology over finer physiological grids.

Acknowledgements We would like to thank Curt Vandetta for providing valuable system administrative support on the computer systems that were used in this study. Comments and criticisms by P. Wheeler, T. J. Cowles, J. R. V. Zaneveld, and T. Plant helped improve this manuscript considerably. This research was supported in part by the National Aeronautics and Space Administration (NAS5-31360 to M. R. A., and NNG04HZ35C to R. M. L. and M. R. A.).

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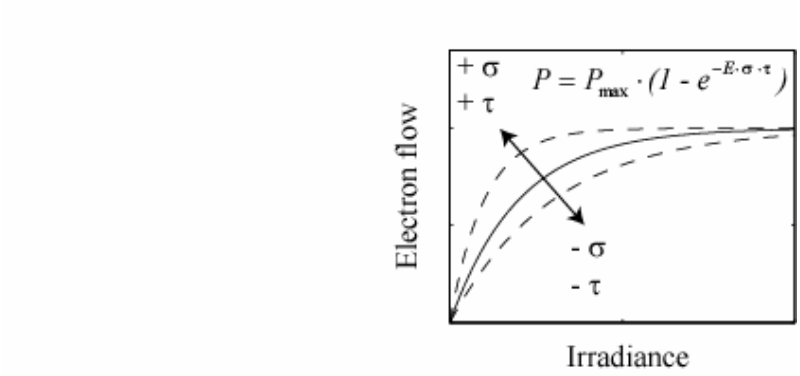

Figure 2-1. An example of how relationships between irradiance intensity and light-driven electron flow can be derived from first principles using cumulative one-hit Poisson models (Eq. 1). The effect of changes in σ_{PSII} or τ by 50% on *P* are indicated by dashed lines.

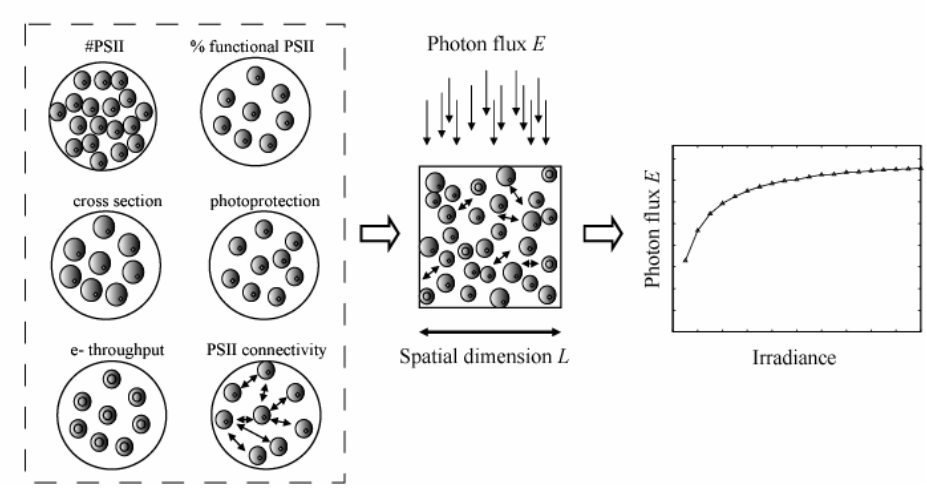

Figure 2-2. Basic diagram of the structure of the simulations. Physiological parameters are associated with *n* individual PSII in a model space L^2. The light-driven electron flow from these PSII populations can be computed over a range of simulated irradiances, to generate representative *P-E* curves for any particular photosynthetic state.

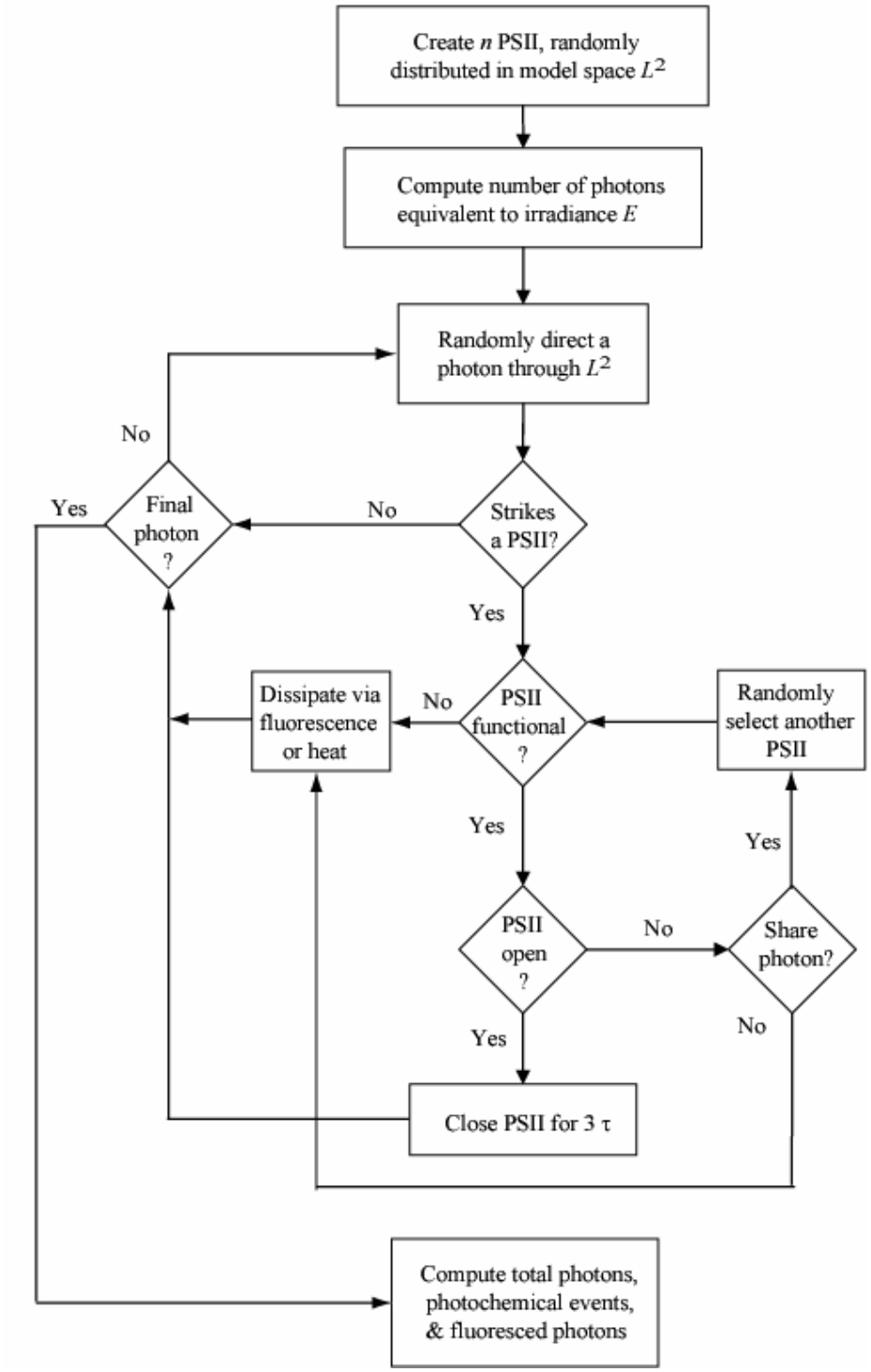

Figure 2-3. A flowchart of the stochastic simulation.

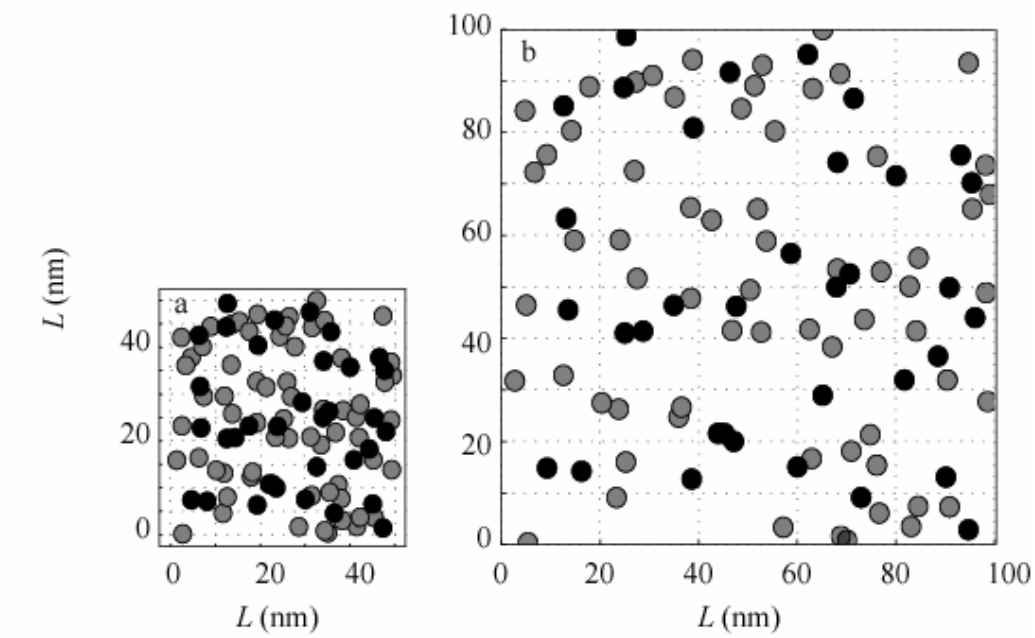

Figure 2-4. Visual representations of the model space used to generate the a) "enhanced shading" and b) "minimal shading" PSII populations. The length scale *L* in the former is one-half of that in the latter, therefore increasing self-shading among the photosystem population fourfold.

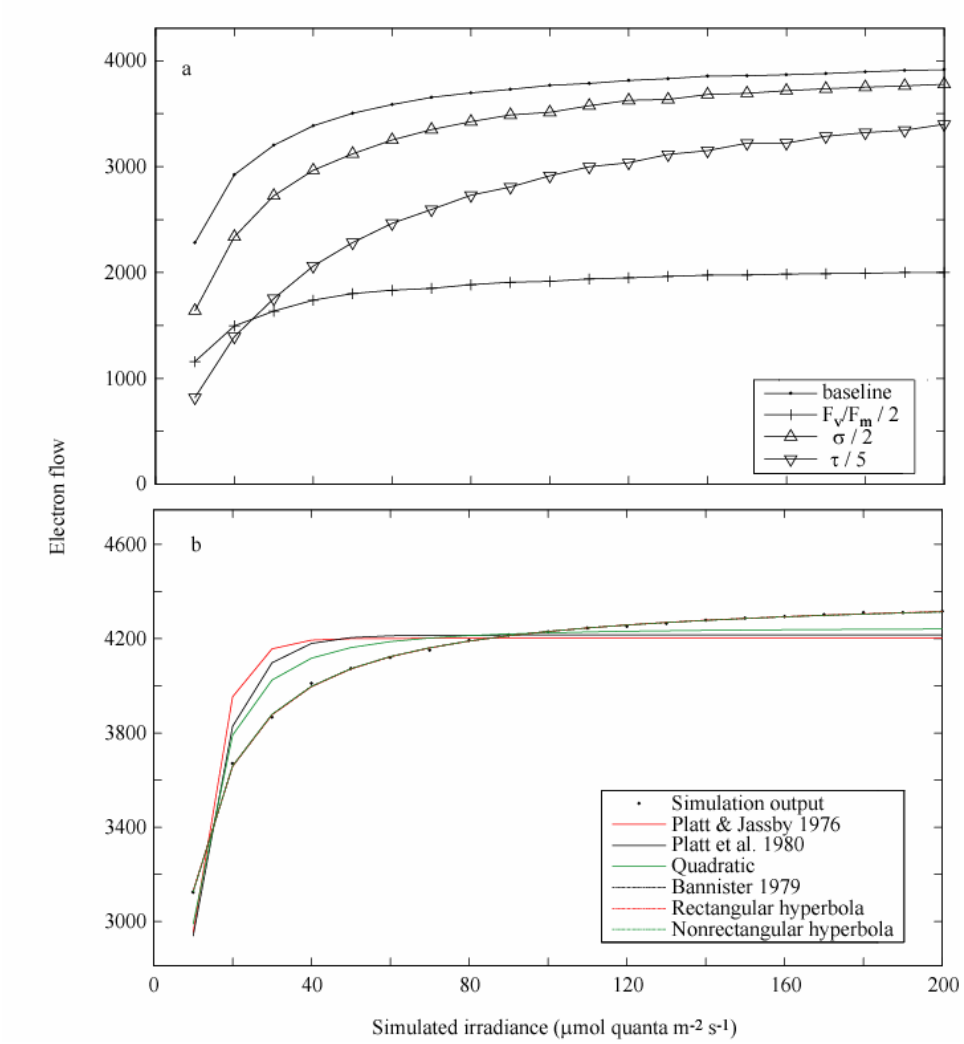

Figure 2-5. a) Example photosynthesis-irradiance relationships that were predicted by the simulation for various physiological states as set by particular combinations of F_v/F_m, σ_{PSII}, p, and τ, and b) examples of fits to a simulated P_e-E relationships, using other P-E models from the literature.

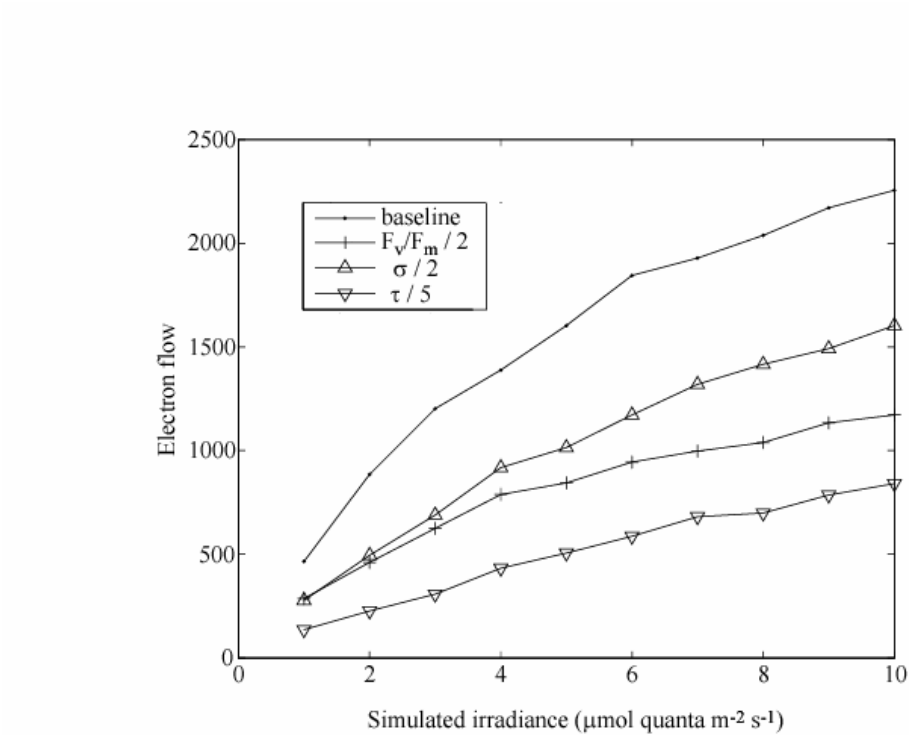

Figure 2-6. The behavior of the P_e-E relationship at very low simulated irradiances, as irradiance asymptotically approaches zero.

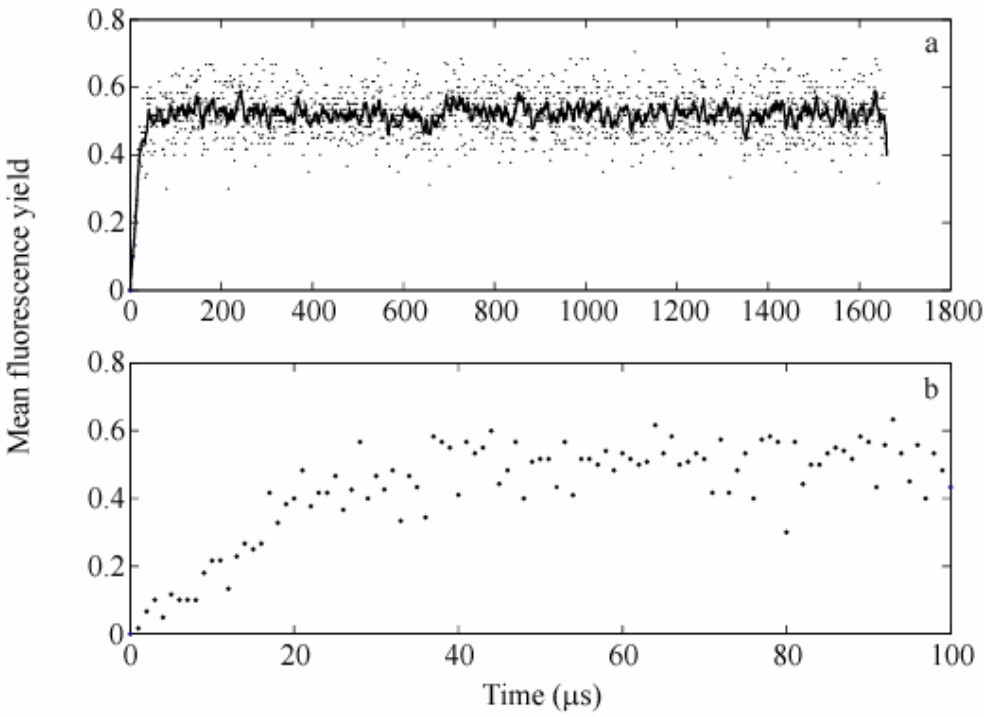

Figure 2-7. The impulse response of this model to a step increase in irradiance from 0 to 10,000 μmol quanta m^{-2} s^{-1}, in terms of the apparent fluorescence yield, for a) a period of time on the order of single electron acceptor turnover (≈ 2 ms), and b) on time scales much shorter than those of single turnovers (≈ 100 μs). The variable fluorescence kinetics of this response exhibit the behavior predicted by the Kolber et al. (1998) physiological model of variable fluorescence.

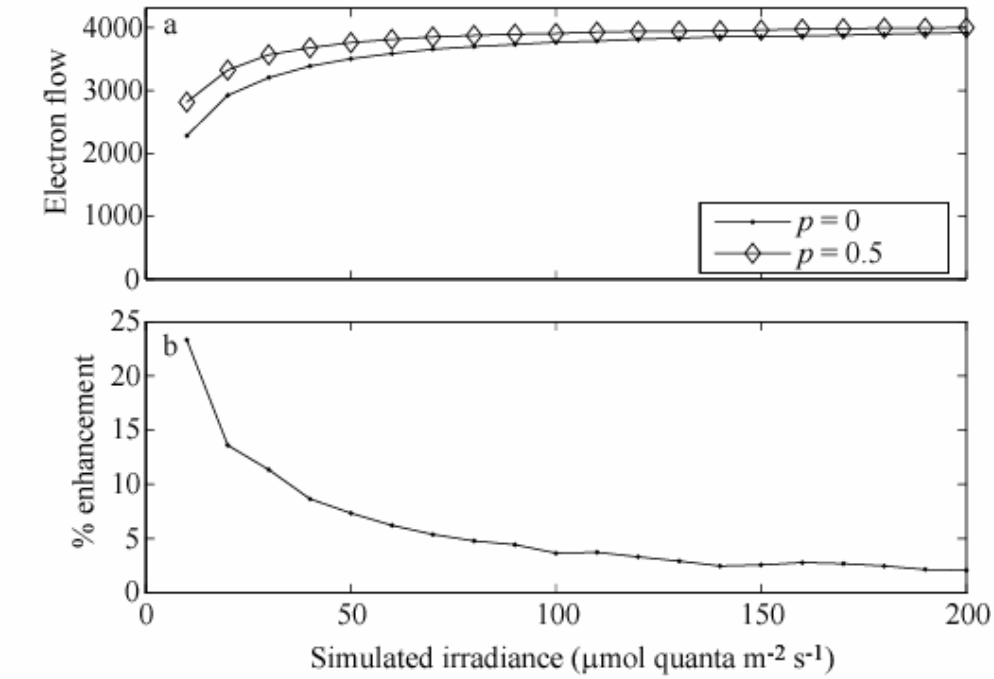

Figure 2-8. An example of enhancement in P_e due to energetic connectivity between PSII, in terms of a) electron flow with $p = 0$ and $p = 0.5$, and b) the percent enhancement in P_e that this increase in p confers.

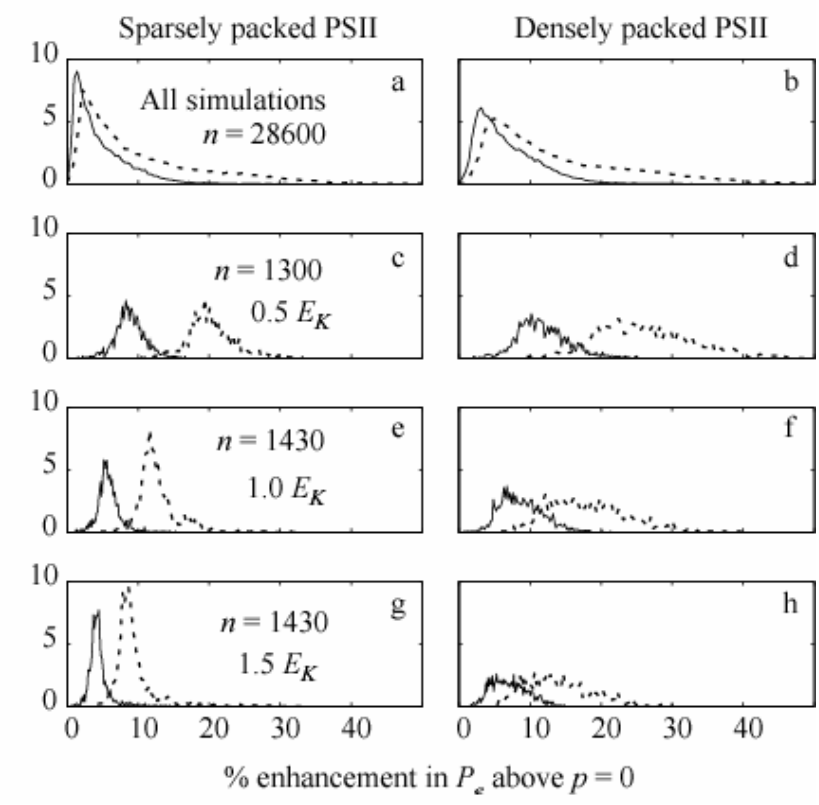

Figure 2-9. Histograms showing the percent enhancement of P_e due to energetic connectivity among PSII reaction centers (p), comparing model spaces which are sparsely populated with PSII (left column) and those that are densely populated (right column), for $p = 0.25$ (solid lines) and $p = 0.5$ (dotted lines). Histograms in the top row show the total results of all simulations. The bottom three rows show subsets of the data in the first row, for irradiances at 0.5 E_K (row 2), 1.0 E_K (row 3), and 1.5 E_K (row 4), with E_K being computed as discussed in the text.

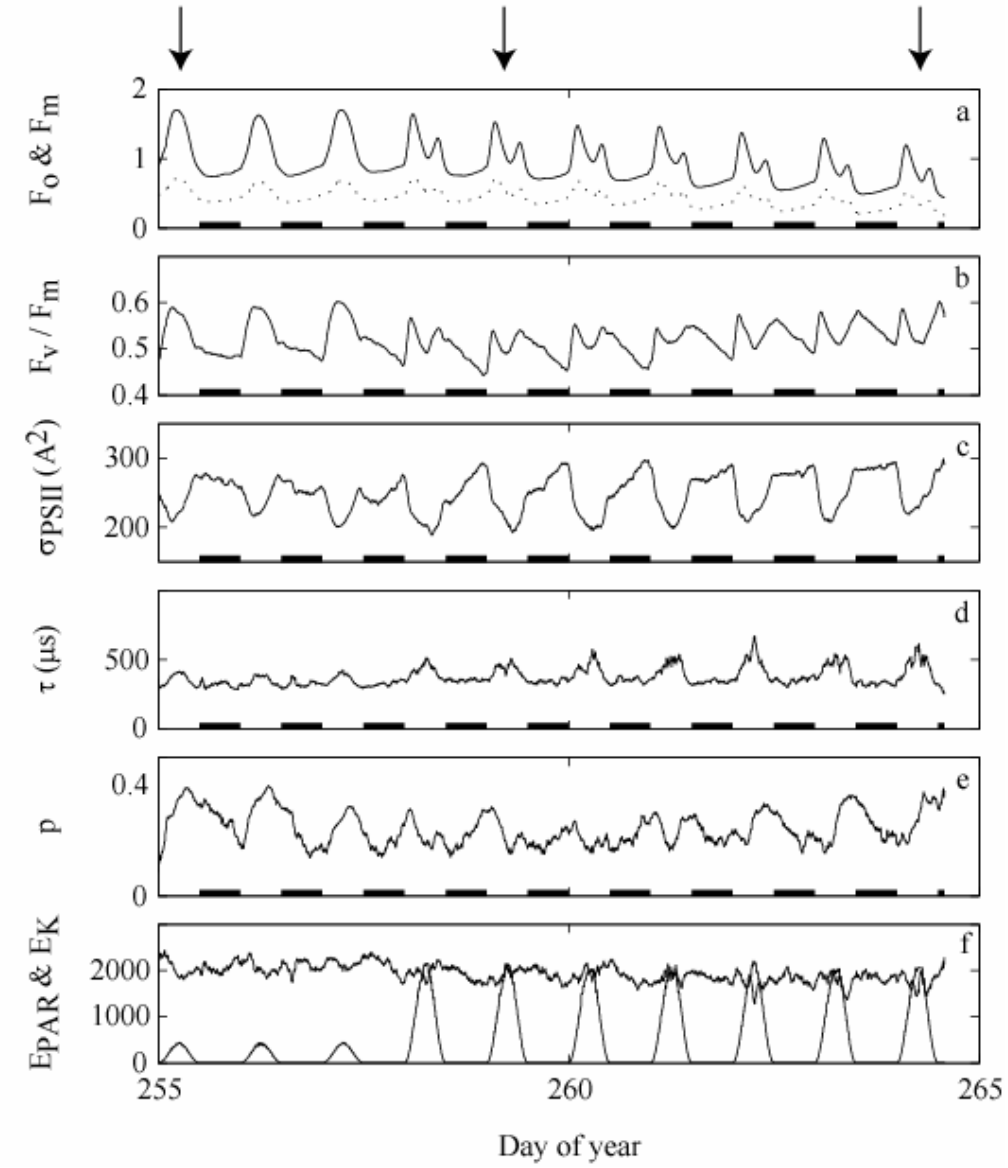

Figure 2-10. The time series of a-e) physiological properties of PSII, and f) irradiance, that were used as the physiological input to predictions of P_e using a look-up table approach. Arrows indicate specific days referenced in the text.

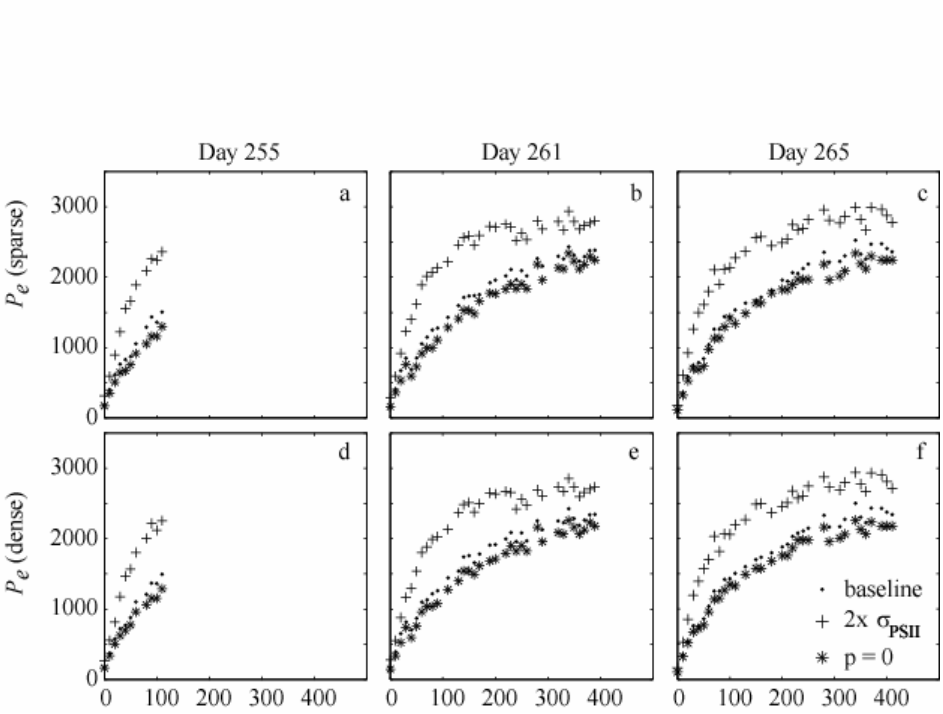

Figure 2-11. Examples of the P_e-E relationships that were generated by using the output of the "sparse" (top row) and "dense" (bottom row) simulations to predict photosynthetic electron flow rates from the measured irradiances and PSII physiological parameters, for the three days indicated in Figure 2-10. Panels show how this P_e-E relationship differs when the culture is acclimated to low light, low nitrate conditions (left column), transiently acclimating to high irradiances (center column), and well-acclimated to high irradiances (right column). Different traces in each panel indicate the P_e-E relationship for the physiological parameters as measured (dot symbols), for a PSII population with $p = 0$ but all other PSII parameters incorporated (asterisk symbols), and how a twofold error in instrument calibration of excitation would affect P_e (plus symbols).

3 Rapid changes in photosystem II properties in Thalassiosira weissflogii (Bacillariophyceae) in response to minutes-scale perturbations in irradiance

3.1 Abstract

Phytoplankton metabolism is driven by the energy of photons, absorbed from the ambient light field by Photosystems I and II. The structure and function of these photosystems constrain the rates and efficiency of primary photochemistry. Physiological adjustments that affect how these photosystems absorb and process photon energy are important for maintaining optimal photosynthetic rates in dynamic light environments. Although the marine light field exhibits considerable variability on the seconds to minutes time scales, physiological responses to such short fluctuations in irradiance are poorly understood, as are their impact on light harvesting. Using a single turnover variable fluorescence method, physiological responses in Photosystem II to minutes-scale transients in irradiance were examined in laboratory cultures of the marine diatom *Thalassiosira weissflogii*. The time scales and magnitudes of specific changes in PSII properties were quantified during and immediately following these transients, over varying growth conditions. A physiological model of Photosystem II was used to estimate how these observed minutes-scale photosynthetic responses either enhanced or constrained light-driven electron flow rates. During rapid growth conditions, minutes-scale responses to decreases in irradiance were predicted to enhance light-driven electron flows by over 20 percent. In contrast, identical decreases in irradiance during a period of nutrient starvation were predicted to reduce light harvesting by a similar percent. These laboratory observations are consistent with and can be interpreted using theoretical arguments that explain optimization of light harvesting in terms of the relationship between the supply of photon energy to Photosystem II and its utilization by the plastoquinone pool.

3.2 Introduction

Phytoplankton assemblages living in the surface ocean experience considerable variability in the ambient irradiance, which serves as their primary energetic resource. This variability reflects the combined effect of numerous planetary, atmospheric, and hydrodynamic processes. The solar cycle sets the fundamental scale of variability in the marine light field, and many aspects of algal metabolism are synchronized to these diel changes in irradiance (Doty and Oguri 1957; Suzuki and Johnson 2001). Physical processes that vertically displace phytoplankton in the water column further modulate the irradiance that phytoplankton experience. In the ocean such vertical displacement processes include wind-driven mixing, inertial oscillations, internal tides, and storm events. These processes have characteristic time scales that are estimated to range from 0.5 to hundreds of hours (Denman and Gargett 1983; Lewis et al. 1984b). How phytoplankton respond to these hours-plus scale perturbations in irradiance has received considerable attention in marine ecology. Such displacements have been shown to affect the growth and survival of marine phytoplankton materially, in a wide range of ocean environments (e.g., Cullen and Lewis 1988; Falkowski and Wirick 1981; Huisman et al. 1999; Marra 1978b; Schubert et al. 1995).

It is arguable that this longstanding focus on vertical displacements in the ocean may have created a paradigm in marine ecology where irradiance fluctuations caused by these displacements are considered to have the greatest effect on phytoplankton photosynthesis and growth. Indeed, by simply designating these microbes as *phytoplankton*, we emphasize their inability or only weak ability to maintain an optimal position in the water column. Reference texts contain considerable discussion of how light fluctuations on these time scales affect marine phytoplankton, either at the individual or assemblage level (Fogg and Thake 1987; Harris 1986; Mann and Lazier 1996). Yet it is important to remember that there are other sources of irradiance variability in the marine environment that can introduce equal if not greater perturbations in the irradiance experienced by phytoplankton. Two

examples are intermittent cloud cover and focusing of light by surface waves. Fluctuations in irradiance resulting from these two mechanisms differ considerably from those of vertical displacements. Cloud cover and wave focusing introduce variability on the time scale of seconds to minutes, well below the scale of fluctuations resulting from most vertical displacements.

Compared to the considerable attention that such rapid irradiance fluctuations has received in terrestrial plant ecology (Chazdon 1988; Pearcy et al. 1994), their effect on the ecology of marine phytoplankton is less well examined. A literature review indicates that there is a gap in prior research at these time scales (Table 3-1). There has been considerable historic focus on vertical displacement processes with time scales of hours, and on physical processes that introduce perturbations on the sub-second scale, i.e., the "flicker effect". It may be that intermittent cloud cover introduces considerable variability in irradiance on exactly those time scales. Irradiance data collected by the Hawaii Ocean Time-series (HOT) program at a mooring at Station ALOHA north of Oahu (22° 45'N, 158° W) show that seconds to minutes irradiance fluctuations are common at this ocean site (Figure 3-1). Since similar patterns are observed in surface irradiance time series, collected by ships on the monthly HOT survey cruises, this variability is presumably cloud-driven.

A simple computation demonstrates that the magnitude of irradiance perturbation resulting from this cloud cover is much greater than can be generated by vertical displacement on these sub-hourly time scales The water column at this oligotrophic site is very clear optically, with attenuation coefficients on average ≈ 0.04 m^{-1} (Letelier et al. 2004). In such clear water, sustained vertical displacements on the order of 0.1 m s^{-1} would be required for hydrodynamic processes to produce the same magnitude perturbations in irradiance as are generated by cloud cover (Figure 3-2). This degree of hydrodynamic forcing is not observed at Station ALOHA, and therefore intermittent cloud cover, not vertical processes, drive the fluctuations in irradiance.

Table 3-1. An overview of studies published in oceanographic and related journals that examine the impact of fluctuating light on phytoplankton photosynthesis, physiology, growth, or ecology.

< 1 min	**1 min – 1 h**	**> 1 h**	**Species**	**Study**
		1, 3, 6, & 12 d	Natural assemblage	(Flöder et al. 2002)
		8 or 24 h	2 cyanobacteria	(Litchman 2003)
		≈ 10 h	*T. pseudonana*	(Cullen and Lewis 1988)
		≈ 6 – 24 h	*Prochlorococcus sp.*	(Dusenberry 1995)
		1, 8, or 24 h	various	(Litchman 2000)
		1 or 8 h	various	(Litchman 1998)
			Modeling study	(Litchman and Klausmeier 2001)
		1.25 - 2.5 h	*Scenedesmus protuberans*	(Flameling and Kromkamp 1997)
		2 h	*Dunaliella tertiolecta*	(Havelková-Doušová et al. 2004)
		≈ 3 h	*Lauderia borealis*	(Marra 1978a)
		5 h	Modeling study	(Falkowski and Wirick 1981)
		2-6 h	Tidal estuary	(Frechétte and Legendre 1982)
		≈ 1 h	Natural assemblage	(Gallegos and Platt 1982)

Table 3–1 (Continued)

< 1 min	**1 min – 1 h**	**> 1 h**	**Species**	**Study**
	12 min		Natural assemblage	(Gocke and Lenz 2004)
1.25 s			Sea ice algae	(Legendre et al. 1986)
10 – 20 s (est.)			Natural assemblage	(Schubert et al. 1995)
1 s		4 h	*Chlorella sp.* & *Scenedesmus sp.*	(Grobbelaar 1991)
3 s		4 h	*Chlorella pyrenoidosa*	(Grobbelaar et al. 1992)
0.1 – 10 s			*Dunaliella tertiolecta*	(Quéguiner and Legendre 1986)
200 μs – 20 s			*Scenedesmus obliquus*	(Grobbelaar et al. 1996)
0.001 s			*Scenedesmus bicellularis*	(Mouget et al. 1995)
5 – 20 ms			*Dunaliella tertiolecta*	(Stramski et al. 1993)
0.4 s			Natural assemblage	(Walsh and Legendre 1983)
	Environmental		Natural assemblage	(Abbott et al. 1982)
	Environmental		3 diatoms	(Marra and Heinemann 1982)

The power spectrum of this high frequency variability is very different than what would be expected for irradiance fluctuations resulting from vertical displacements. The magnitude of these cloud-driven fluctuations remains roughly constant at time scales below ≈ 6 hours (Figure 3-3), rather than the overall negative trend that would be expected for vertical processes, e.g., Kolmogorov scaling of –5/3 for small scale turbulent motions. This spectral shape has some relevant implications for phytoplankton. If vertical displacements are considered the dominant source of irradiance fluctuations in these waters, phytoplankton photosynthesis and growth should be less affected by vertical displacements with short periods, because the irradiance fluctuations resulting from these small displacements would be correspondingly weak. Thus, a focus on irradiance fluctuations driven by vertical displacements would provide a reason to ignore photosynthetic responses at short, sub-hourly time scales. However, our field data refute this idea, and suggest that subhourly fluctuations in irradiance due to intermittent cloud cover may not be as readily ignored, as has been historically assumed, in its effect on growth and success in marine phytoplankton assemblages.

The responses of phytoplankton to short term variability in light can fundamentally affect the structure and evolution of marine assemblages (Marra and Heinemann 1982). Yet rapid modes of photoacclimation have received little attention in phytoplankton ecology (e.g., Bidigare et al. 1992; Harris 1978; Karl et al. 2002; Marra 1980). A recent review of photoacclimation in microalgae made no mention of responses on time scales shorter than those of biosynthesis (MacIntyre et al. 2002). Standard reference texts provide little or no discussion of the ecological role that rapid photoacclimation may play (Falkowski and Raven 1997; Fogg and Thake 1987; Harris 1986; Mann and Lazier 1996).

Virtually all phytoplankton taxa examined to date exhibit rapid physiological responses to short-term changes in irradiance. These responses appear to be localized primarily in the photosynthetic light reactions, at the photosystem level, triggered by unbalances in the photochemical activity of photosystem II (PSII) and photosystem I (PSI). Translumenal gradients in pH, established by excess photochemistry in PSII,

induce enzymatic modification of xanthophyll pigments in both chromophytes and chlorophytes (Oliazola et al. 1994; Oliazola and Yamamoto 1994). These modifications can either decrease or increase the supply of light energy to photosynthetic reaction centers (Demmig-Adams and Adams 1992; Lavaud et al. 2004; Yamamoto and Nakayama 1963). The same translumenal pH signal also appears to trigger changes in the aggregation and conformation of PSII light-harvesting complexes (Horton et al. 1991; Wentworth et al. 2000), which also affects their light harvesting properties. Neither prochlorophytes nor cyanobacteria appears to have a xanthophyll cycle analog (Siefermann-Harms 1985). However, mechanisms for energetic spillover between PSII and PSI have been observed in cyanobacteria (Koblížek et al. 1998), as have rapid migrations of phycobilisomes across the thylakoid membrane on the 100 ms time scale (Mullineaux et al. 1997; Sarcina et al. 2001). These mechanisms in the prokaryotes may provide a means to optimize photochemical activity at the photosystem level, on time scales shorter than those of biosynthesis.

The central goal of this study was to examine how a model diatom, *Thalassiosira weissflogii*, responds to perturbations in irradiance on the seconds to minutes time scales, and to predict how these responses may affect light harvesting and rates of photochemistry. Since rapid photoacclimation appears to be localized in the photosynthetic light reactions, we used a variable fluorescence technique to monitor how specific properties of PSII varied, during and after idealized, 10 min step transients in irradiance. These step transients in irradiance were designed to mimic the rapid and strong changes in irradiance that are characteristic of intermittent cloud cover. Photochemistry in PSII is central to the photosynthetic light reactions, and the variable fluorescence of PSII is a very sensitive indicator of its activity and energetic state (Krause and Weiss 1991). The fast repetition rate (FRR) method we used in this study allows several independent PSII properties to be estimated from variable fluorescence kinetics with sampling resolution of seconds (Kolber et al. 1998). The overall effect of the physiological responses that occurred during and after these transients was predicted using a physiological model of light harvesting in PSII.

3.3 Methods

The neritic marine diatom *Thalassiosira weissflogii* (Bacillariophyceae, strain UBC636) was grown in f/2 medium (Anderson 2005) in clear, 1 L polycarbonate bottles (Figure 3-4). Growth irradiance was provided by two, twin compact-fluorescent tubes (Philips PL-L 40W, 3150 lumens). Combinations of white plastic plate diffusers and window screen were used to attenuate the maximum lamp output to an irradiance (E) of ≈ 150 μmol quanta m^{-2} s^{-1}. The diffusers presumably minimize microscale spatial patchiness in irradiance that the screening introduced. The cultures were continuously bubbled with 0.2 μm filtered air. Temperature was maintained at 24 ± 1 °C using a water bath, to minimize thermal fluctuations caused by heat generated by the growth lamps.

The intensity of these bulbs was controlled by computer to generate semi-sinusoidal daily irradiance histories with a 14:10 h light-dark cycle. These cultures were allowed to acclimate in the incubators to this 14:10 diurnal pattern and to the spectral output of these lamps, in which a large fraction of their output occurred in narrow bandwidths (Figure 3-5a). After this initial acclimation period, the diel light history was changed to one having the same maximum midday intensity, but which also included hourly, 10 min long decreases in E to a low level of ≈ 20 μmol quanta m^{-2} s^{-1} (Figure 3-5b).

Using a peristaltic pump, a small volume of each culture was continuously circulated through a 1 cm pathlength flow-through fluorescence cell (131-OS, Hellma, Forest Hills NY) inserted into the "dark chamber" of a fast repetition rate fluorometer (FRRF, Fasttracka, Chelsea Instruments Ltd., West Moseley, UK). The FRRF in this study was placed within 15 cm of the culture so that only ≈ 10 s elapsed between removal from ambient light conditions and measurement of chlorophyll variable fluorescence transients inside the instrument's dark chamber. One hundred excitation

flashlets were delivered to the sample within ≈ 450 μs, to determine its variable fluorescence saturation kinetics. Twenty subsequent flashlets were delivered over an ≈ 7.25 ms period to probe its variable fluorescence relaxation kinetics. In this way, fluorescence transients were measured continuously in these cultures over the course of several days. Sixteen individual excitation and emission flashlet sequences were averaged internally by the instrument, resulting in an effective sampling rate of 1 acquisition every 16 s. Since this interval is short compared to the relaxation scale of nonphotochemical quenching (mins and longer), but long compared to the relaxation of photochemical quenching (ms to seconds), our assumption is that any fluorescence quenching evident in the measured variable fluorescence kinetics primarily reflects nonphotochemical processes. This approach is similar to that used by Laney et al. (2005) to continuously measuring variable fluorescence transients in *T. weissflogii*. These cultures were diluted periodically with fresh media, daily or once every several days, as needed to maintain culture biomass within a dynamic range optimal for these variable fluorescence measurements.

Specific physiological properties of PSII were estimated from the flashlet sequences using variable fluorescence transient analysis software written by one of the authors (v6, S. R. L.). Biases in the emission flashlets, due to optical scatter in the sample cell and to hardware artifacts in detector electronics, were characterized and corrected for using an impulse response method (Chapter 5). This method improves upon the empirical method described by Laney (2003). The analysis software numerically fits the physiological model of variable fluorescence kinetics of Kolber et al. (1998), hereafter KPF98, to these corrected emission/excitation ratios. The KPF98 physiological model describes changes in fluorescence yield over the single-turnover time scale in terms of an initial minimal level (F_o), a final, saturated maximal level (F_m), a functional cross section of PSII (σ_{PSII}), and a probability of exciton sharing between PSII reaction centers (p). From the initial and maximal fluorescence yields, a normalized variable fluorescence (F_v/F_m) can be computed to serve as a proxy for the quantum yield of photochemistry in PSII (Genty et al. 1989). The KPF98 model also allows for subsequent decreases in variable fluorescence over the ms time scale,

reflecting reoxidation kinetics on the acceptor side of PSII. With the FRRF used in this study, it is difficult to resolve these kinetics in terms of anything other than a single generalized time constant, which we will refer to as τ_{PSII}.

The 2π irradiance sensor that is integrated with this commercial FRRF was used to monitor the changes in irradiance. This sensor was too large to place in the polycarbonate bottles directly. Therefore, these 2π data were calibrated against a smaller 4π sensor that was periodically placed inside the 1 L culture flasks (QSL-2101, Biospherical Instruments Inc., San Diego CA), to convert the irradiance data from the 2π sensor into a more representative scalar irradiance.

3.4 Results

3.4.1 Diurnal and short term trends in PSII properties

Diurnal trends in F_o, F_m, F_v/F_m, σ_{PSII}, p, and τ_{PSII} are shown in Figure 3-6, for a 5 day period where the nutrient supply to a rapidly growing culture was stopped (columns I-V), and for a representative day of rapid culture growth during a period of repeated daily dilution (column VI). Grayed areas indicate periods where the lamp controller had an error (CE), or periods where the FRRF detector was saturated ("det. sat." where F_m levels approached 2). In rapidly growing cells (column I & VI), F_o and F_m both show increases during most of the light period. Decreases in the late afternoon most likely reflect fluorescence quenching and not changes in culture biomass, as shown in Laney et al. (2005). Changes in F_o and F_m are not coupled throughout the entire day; F_v/F_m rises quickly in the morning and gradually decreases over the course of the day, falling rapidly in the late afternoon. Gradual decreases in σ_{PSII} during the daytime are mirrored by gradual increases in τ_{PSII}. Diurnal changes in p largely follow those in F_v/F_m, most likely due to a theoretical constraint on p that restricts p to values less than or equal to F_v/F_m (Lavergne and Trissl 1995). Without this constraint, fitted

values of p would probably be greater and fits of the KPF98 physiological model would improve statistically.

Dilutions with fresh media were stopped on day 2, in order to examine how these diurnal patterns during rapid growth differ as nutrients become less available. The increasing diurnal trends in F_o and F_m evolved into a sinusoidal pattern that closely corresponded to the time rate of change in irradiance. A similar diurnal pattern became apparent in F_v/F_m over these 4 days. The diurnal trend in σ_{PSII} over this period also evolved into one that had distinct forenoon-afternoon differences: σ_{PSII} was lower in forenoon than in the afternoon. Within each day during this 5 day period, τ_{PSII} showed an increase in the early morning, but remained constant throughout the remainder of the day. Over these 5 days the mean diurnal value of τ_{PSII} increased steadily. Diurnal patterns in p again closely followed those in F_v/F_m, again presumably due to the fitting constraints.

How these PSII properties responded transiently to the brief, 10-minute, hourly reductions in irradiance also differed materially across these 5 days, presumably also as a function of nutrient availability. During rapid growth (column I & VI), and during most of the period with no dilution (columns II-IV), transient decreases in irradiance coincided with rapid decreases of F_o and F_m. An exception to this general pattern was observed well after dilution ceased (column V), when these transient decreases in irradiance coincided with increases in F_o and F_m, in the afternoon. In general, transient responses observed in F_m were of greater magnitude than those seen in F_o. The kinetics of these transients in F_o and F_m differed as well, as shown by the behavior of F_v/F_m during and after the brief perturbations in irradiance (Figure 3-7). Throughout the entire experiment, transient decreases in irradiance were always associated with increases in σ_{PSII} and with decreases in p and τ_{PSII}. A quality of fit metric, which was computed for each individual variable fluorescence measurement, did not exhibit any trends over time coincident with the irradiance transients to indicate that the transient behavior in these properties reflected any bias or artifact in the fitting of the KPF98 physiological model.

The contrast in these transient responses in PSII also differs across the spectrum of growth conditions in terms of their kinetics. The time course of these transient kinetics over a diurnal period (e.g., Figure 3-7) can be better examined as a composite (Figure 3-8). With rapidly growing cells, transient responses in F_o, F_m, and F_v/F_m were generally characterized with two time constants: a rapid decrease on the order of 2 min, and a subsequent longer-scale decrease over the remaining 8 min. This second, longer-scale decrease was sometimes absent in F_o, i.e., F_o displayed only the initial rapid decrease then stayed constant during the remainder of the irradiance perturbation. Similarly, two time constants were often observed following the perturbation, when the irradiance intensities were restored to their pre-transient levels. These responses in F_o and F_m were not tightly coupled, as F_v/F_m also generally exhibited two components in both the onset and recovery from these 10 min light perturbations. The fluorescence yields also exhibited very rapid, sub-minute peaks during rapid growth, immediately after each step decrease or increase in irradiance. These peaks were least apparent in F_o.

Under presumably nutrient-deplete conditions and slower growth (Figure 3-8, bottom panels), the very short, sub-minute "peaks" in F_o, F_m, and F_v/F_m were generally absent. The apparent decrease in these parameters with decreases in irradiance, and the apparent increase in them in the afternoon, was shown to be only relative. The baseline level to which each of these three parameters restores during these transients remains constant over the course of the day, e.g., a baseline of ≈ 0.7 for F_o and 0.95 for F_m. This suggests that the apparent afternoon increases in F_o, F_m, and F_v/F_m are artifactual, reflecting instead the decrease in these PSII parameters that occurs during higher-irradiance conditions during most of the afternoon.

A higher level of fitting noise in the σ_{PSII} and τ_{PSII} data makes it more difficult to resolve the transient responses in these PSII parameters during and immediately following these 10-min perturbations in irradiance. When distinct transient responses in σ_{PSII} are observed, they generally appeared as increases. When transient responses occurred in τ_{PSII}, they appeared as decreases. There are slight indications in the τ_{PSII} transients that suggest small sub-minute peaks immediately following the step

decreases and subsequent step increases in irradiance, but this is difficult to prove with such noisy data.

3.4.2 Effect of rapid responses on light-driven electron flow

The numerical model described in Chapter 2 (this dissertation) was used to predict how these simultaneous transient responses in F_v/F_m, σ_{PSII}, p, and τ_{PSII} together affect light-driven electron flow rates, during and immediately after these irradiance perturbations. Electron flows were first computed using as input data the actual measured time series of irradiance and PSII physiological properties. Electron flows were then recomputed with the same time series of irradiance, but with the PSII parameters fixed over the simulated irradiance transient, to the values they had immediately preceding the transient. These two electron flow rate time series were used to compute the percent enhancement in electron flow due to the actual PSII responses.

For the nutrient-replete, rapidly growing *T. weissflogii* culture with the PSII transients shown in column VI, Figure 3-6 and in Figure 3-8, top panels, the physiological model predicted that these coincident changes in PSII properties led to an up-regulation of light harvesting and electron throughput on these 10 min time scales (Figure 3-9, top panels). The magnitude of these enhancements in electron flow rates ranged between +20 and +40%. This up-regulation was reversed rapidly within ≈ 5-10 minutes following restoration to the prior high light levels. When used in an identical way to predict the transient changes in electron flow rates for nutrient-deplete, slowly growing culture (column V, Figure 3-6 and in Figure 3-8, bottom panels), the same model predicted that light harvesting during these transients was in fact down-regulated by ≈ -30% (Figure 3-9, top panels). This behavior was evident throughout the day, but most apparent in the mid-afternoon.

3.5 Discussion

3.5.1 Ecological relevance

3.5.1.1 Light as a resource

Understanding exactly how rapid photoacclimation affects photosynthesis in marine phytoplankton is part of a much larger issue regarding resource utilization theory. Developing and testing theories that describe how phytoplankton utilize light, their primary energetic resource, has been very difficult compared to developing and testing those that describe how they utilize nutrients, their primary material resources (Grover 1997). In a review of such theory, Tilman (1990) noted that

> "…light competition is conceptually more complex than nutrient competition. We do not yet have either rigorous theoretical predictions or experimental results that indicate that a single number, analogous to *R**, can predict the outcome of competition for light. A fuller understanding of light competition remains a major challenge."

Huisman and Weissing's (1994) analysis of such a single number, the critical light intensity *I**, provides a good case study for why such direct metrics may be less applicable for light, despite their proven utility with respect to nutrients (Tilman 1977).

A basic problem with adapting existing nutrient-based resource utilization theory to light is that irradiance, as a resource, has very different characteristics in the marine environment than the solutes or ions that are nutrients for phytoplankton. First, the dynamic range over which irradiance fluctuates in the ocean far exceeds that of nutrients. Concentrations of macronutrients in the ocean often are low enough to be suboptimal, but they rarely occur at concentrations high enough to inhibit growth. In contrast, it is common that irradiance varies from suboptimal to supraoptimal, almost continually in some environments. Second, the availability of light and nutrients differ

materially in their time scales with which they fluctuate. Strong transient increases in nutrients are generally a result of large-scale mixing processes, whose effects persist for a large fraction of the generational scale of an individual cell. Strong transient decreases in nutrient availability often result from their utilization among the assemblage, which also persists for at least significant portions of the generational scale. Higher frequency variability in the availability of nutrients, such as through "micropatches" on the spatial scale of a single mm and less, is thought to be negligible in the marine environment, having been largely discounted using scaling arguments based on turbulent dissipation (Currie 1984). The Station ALOHA irradiance time series that we examined in this study illustrates both of these features of variability in the marine light field: its large dynamic range and its considerable power at higher frequencies.

If the rapid responses that we observed in these cultures affect how these cultures utilized light energy, this photoacclimation may significantly affect higher-level ecological processes in the phytoplankton. This ability may have considerable consequences for growth and success in marine phytoplankton, given that the ambient light field is highly dynamic on these short time scales and that phytoplankton have very short generational periods (e.g., Ley 1980; Marra and Heinemann 1982). How these interactions occur in actual phytoplankton assemblages, however, has been very difficult to assess in these dynamic environments. It is presumed that rapid photoacclimation provides a means to either enhance or to constrain light harvesting on the time scale of minutes or less, if irradiance conditions suddenly deviate from optimal. Developing a quantitative framework that describe this qualitative behavior is not straightforward. Levins (1979) proposed a simple explanation for coexistence under non-equilibrium conditions by allowing for taxonomic differences in the time constants with which an organism can utilize a resource. If an ability to acclimate provides a metabolic enhancement, phytoplankton with faster photoacclimation scales could presumably enjoy a competitive advantage in dynamic environments (e.g., Abbott 1993; Allen 1998; Harris 1978; 1980; 1986). In theory, this quantitative

framework should also work if failure to acclimate incurs a metabolic penalty, e.g., if photoacclimation provides protection against photodamage.

These acclimation time constants have been difficult to measure and their effect on photosynthesis and growth have been difficult to assess, much in the same way that niches – a similarly useful ecological concept – have also proved difficult to measure directly (Pielou 1972). Variable fluorescence techniques provide a means to measure the time scales of specific changes in PSII. These time scales indicate the rate at which these properties are adjusted in response to light perturbations, and how these responses vary under different growth conditions (e.g., Figure 3-8). Using our physiological model of light-driven electron transport, these seconds to minutes scale changes in PSII had a substantial effect on the predicted rates of light-driven electron flow in the *T. weissflogii* clone we examined (e.g., Figure 3-9). Preliminary assessments of *Synechococcus* sp. (CCMP2370) and *Prochlorococcus marinus* (CCMP1983) cultures, using an identical approach, suggest that the effect of rapid photoacclimation in these prokaryotes may be quite different (Figure 3-10). It is unclear whether these preliminary assessments can be interpreted in the same was as the results we observed with the diatom. *Synechococcus* and *Prochlorococcus* have fundamentally different photosynthetic architectures than the diatom, which may make this variable fluorescence method, and the structure of our electron flow model, less appropriate for these prokaryotes. However, these taxa dominate much of the oligotrophic ocean, where cloud cover should be a major source of perturbations in irradiance. From an ecological perspective, therefore, rapid photoacclimation in these prokaryotes merit further examination.

3.5.1.2 Relevant sources and scales of variability in the marine light field

The radiometry data from Station ALOHA indicate that intermittent cloud cover can be a principal source of irradiance perturbations on time scales ranging from minutes to a few hours. In this study we have focused on the physiological changes in

PSII that can occur on similar time scales. However, it is important to note that other processes in the marine environment also introduce fluctuations in the marine light field, on as short or shorter time scales. Lensing of sunlight by short-period surface waves modulates the local light field by around 3- to 5-fold, at periods less than 1 s (Dera and Gordon 1968; Stramska and Dickey 1998; Stramski and Dera 1988). Because the focal lengths of these waves are on the order of meters, their effects on phytoplankton have commonly been thought to be restricted to the near surface ocean. Recent models, though, indicate that the effect of longer period waves may be deeper (Zaneveld et al. 2001). Foam and the entrainment of bubbles at the air-sea interface also affect how light from the sun enters the ocean, but these processes have been examined primarily from a radiative transfer perspective (Mobley 1994).

These very rapid, sub-second irradiance fluctuations have received some attention in phytoplankton ecology (Table 3-1), primarily because of evidence that such high frequency fluctuations can enhance photosynthesis in some phytoplankton (Kok 1953; Laws et al. 1983). This so-called "flashing light effect" has not been well studied in a marine context, compared to the considerable attention it has received in the terrestrial plant literature (e.g., Pearcy 1990). This enhancement is thought to occur when photoprotective mechanisms do not recognize these very rapid fluctuations as supraoptimal. Consequently, certain phytoplankton appear to utilize the energy contained in very short impulses of light without the energetic penalty that results from photoprotection. This phenomenon does not represent a directional response to changes in the availability of light and thus does not reflect any continual adjustment of E_K to optimize photosynthesis in response to these very rapid light fluctuations. Instead, enhancement due to this effect appears to vary over long time scales, reflecting an adaptation to mean conditions of the ambient light field (e.g., Legendre et al. 1986). Although the flashing light effect may be an important aspect of photosynthetic dynamics in light fields that fluctuate rapidly, how it affects photosynthesis and growth is considerably different from the directional responses in PSII that we examined here.

The characteristic time scales of the flashing light effect (sub-seconds) and of vertical displacements (hours-plus) bracket the comparatively understudied time scales of changes in PSII that were the focus of this study (Table 3-1). Two common misperceptions may be responsible for this lack of attention to these scales of photoacclimation. First, rapid photoacclimation could be considered unimportant if vertical displacements processes are presumed to be the primary source of fluctuations in irradiance that phytoplankton in the ocean experience. Since displacements are generally small on such rapid scales, the fluctuations in irradiance they would create would also be small, and so rapid acclimation to such minor perturbations thus would be expected to result in only minimal photosynthetic gains (sensu Strzepek and Harrison 2004). Our analysis of irradiance data from Station ALOHA does not support this argument and indicates instead that irradiance perturbations resulting from cloud cover in this region are considerable, roughly equivalent to what phytoplankton experience at this location when displaced ≈ 50 m on near-inertial oscillations (Karl et al. 2002; Letelier et al. 2004). If phytoplankton have evolved rapid modes of photoacclimation to cope with short-term changes in irradiance, these modes presumably have evolved in the context of strong, broadband perturbations due to clouds, and not to vertical displacement.

Second, rapid photoacclimation might also be considered unimportant if the physiological changes they incur are presumed to only modestly affect light harvesting. For example, state transitions have been estimated to alter absorption by only 10-20% (Allen 1992). Such changes are much smaller than can be realized by altering cell pigment quotas and photosystem stoichiometries over biosynthesis time scales (Falkowski and Owen 1980; Prézelin 1981). However, it is important not to confuse small changes with unimportant ones, especially when considering a dynamically forced, highly nonlinear system such as the light reactions. Such responses in light harvesting may be small when compared absolutely to longer-scale changes, but in relative terms over short periods, the cumulative effect of ≈ 20% enhancements are probably not negligible (e.g., Figure 3-9). The model of light-driven electron flow used here provides a theoretical basis for predicting the effect of these

rapid PSII responses in natural light fields having broadband but strong variability in irradiance (e.g., Figure 3-1). However, understanding how these responses actually affect cell metabolism requires that this model be validated, using more sophisticated physiological techniques.

3.5.2 Physiological interpretation of rapid responses in PSII

The dynamic responses of phytoplankton to energetic perturbation are poorly understood. Yet such a dynamical understanding is essential for predicting how a physiologically "plastic" system such as the light reactions behaves in a variable environment. There are a wide range of directional responses that can occur in PSII to affect light-driven electron flows, and many require no fundamental changes to photosynthetic structure (Table 3-2).

Table 3-2. A classification of some photosynthetic responses, based on whether these responses reflect changes in the fundamental structure of the light reactions, or more affect only their function independent of fundamental structure

Structural responses (on time scales of biosynthesis)	**Functional responses (below time scales of biosynthesis)**
Pigments: content & distribution	State of interconvertible pigments
PSI & PSII: number and stoichiometry	Aggregation of LHC with PS
Quota of electron acceptors & carriers	Mobility of phycobilisomes
Repair rates of photodamage	Energy redistribution among PSII & PSI
Thylakoid structure and position	Thylakoid structure and position (?)
Locational change (swimming, buoyancy)	(?)

Laboratory studies can be used to quantify the magnitude and energetic impact of rapid photoacclimation, under controlled environmental conditions. Variable fluorescence analysis techniques, like the FRR fluorescence method used here, are well suited for measuring how rapid photosynthetic responses in PSII affect light harvesting and primary photochemistry.

However, specific responses in individual PSII parameters are of less ecological interest than how these responses in concert affect the dynamics of the light reactions overall. Here, we used a numerical model to synthesize these various changes in PSII physiology into a prediction for their combined effect on light-driven electron flow (Chapter 2). This model is consistent with the physiological description of PSII presented in Kolber et al. (1998), and it expresses the same physiological assumptions that are used here to recover these PSII properties from variable fluorescence transients. Because it is a stochastic physiological model, it can replicate complex and nonlinear effects that changes in PSII physiology have on light harvesting and rates of electron flow. Changes in specific PSII properties that only weakly affect the fluorescence yield of phytoplankton, e.g., reaction center connectivity *p*, may not have such an negligible influence on light harvesting, and vice-versa. As a result, developing such a model requires not only identifying the PSII properties that exert the most control on photosynthetic electron flow, but also properly integrating these properties in a dynamically accurate manner. The importance of both individual components and their interrelatedness is a basic feature of nonlinear systems. When interpreting how changes in PSII physiology affect light harvesting, it is important to focus on the behavior of the system as a whole, and not on the kinetics of any particular PSII property *per se*.

3.5.2.1 How rapid changes in σ_{PSII} and τ_{PSII} affect light harvesting and E_K

In its most simple form, the numerical model we used to predict how rapid PSII responses affect light-driven electron flow reflects the interplay between two

processes: the delivery of excitation energy to reaction centers in PSII, and the utilization of this energy by downstream electron acceptors (Dubinsky et al. 1986). The rate of which absorbed photon energy is supplied to individual PSII reaction centers can be expressed with units of $RCII^{-1}$ s^{-1} as the product of irradiance and σ_{PSII}. At the reaction centers, this light energy is used to remove electrons from water and thus generate a light-driven flow of electrons. A pool of plastoquinone (PQ) molecules shuttle these electrons away from PSII, by individually diffusing back and forth to cytochrome. The inverse of the relaxation time constant τ_{PSII} measured in this study has units of s^{-1} and serves as a proxy for the average rate with which the PQ pool as a whole moves electrons away from PSII (Kolber et al. 1998). Therefore, in this physiological model there are only two photosynthetic states for any particular instantaneous combination of σ_{PSII}, and τ_{PSII}: the PQ pool either has additional capacity to accommodate a greater supply of electrons if irradiance suddenly increases, or it does not. Thus, all other factors being equal, there is a limit where additional increases in σ_{PSII} will result in a supply of electrons to PQ that exceeds its capacity to shuttle this energy away from PSII. This limit can be expressed quantitatively using the inverse product of σ_{PSII} and τ_{PSII}, i.e., an expression for the empirical light saturation parameter E_K, derived from first physiological principles (Falkowski 1992).

The lack of attention to a photochemically derived E_K may partly reflect a difficulty in recovering σ_{PSII} and τ_{PSII} with variable fluorometers (Laney 2003). Yet in this conceptual framework, changes in σ_{PSII} and τ_{PSII} are what express the fundamental changes in PSII that affect rates of light-driven electron flow. For *T. weissflogii* cultures, we make the simplifying assumption that neither the number of PQ nor PSII varies materially during the 10 min irradiance transients, as this time scale is far shorter than that of biosynthesis or repair. If the number of PQ is fixed, a sudden decrease in irradiance would immediately decrease the rate of energetic supply to PSII, which in turn would lead to an immediate increase in the rate of electron removal from PSII and appear therefore as a decrease in τ_{PSII}. If there are no changes

to σ_{PSII} during the course of the 10 min perturbations in irradiance, such decreases in τ_{PSII} due to sudden reductions in irradiance alone should remain stable. Yet τ_{PSII} did not remain constant throughout these transients, but instead increased slightly. This suggests that the rate with which electrons were generated by PSII increased as well during these transients and utilized some of the excess capacity in the PQ pool.

The concomitant increases in σ_{PSII} during the transients could explain the minutes-scale changes in electron flow dynamics. We noted two types of changes in σ_{PSII} occurred during these transients: an immediate response within the first minute, and a longer, minutes-plus increase that we just referred to. Thus, if these slower changes in τ_{PSII} over 10 minutes reflect changes in σ_{PSII}, it is reasonable to conclude that the sudden increases in σ_{PSII} keep τ_{PSII} from decreasing even more than it does in the first minute of the irradiance transient. The absolute decrease in τ_{PSII} during these transients differs considerably in *T. weissflogii* depending on its growth state. In a rapidly growing culture, τ_{PSII} during these transients decreased only slightly in the forenoon (Figure 3-7). This suggests that at this time of the day, concurrent increases in σ_{PSII} were able to maintain an electron supply to the PQ pool almost to the level of prior to the sudden decrease in irradiance. In the afternoon, stronger decreases in τ_{PSII} were observed, despite larger transient increases in σ_{PSII}, suggesting that the cell's ability to maintain an optimal supply of electrons to PQ has progressively been eroded over the course of the light period. Under nutrient-limiting conditions, similar transient decreases in τ_{PSII} were observed in the forenoon. These decreases were greater than those observed in the nutrient-replete culture, and were associated with smaller increases in σ_{PSII}, consistent with the idea that these nutrient-starved cells had less capacity to optimize light harvesting during the irradiance transients. When considering the entire experiment, the greatest decreases in τ_{PSII} during light perturbations were observed in the days immediately following the termination of dilutions (Figure 3-6 columns III-V). This corresponded to the period when these cultures experienced presumably strong nutrient limitation.

3.5.2.2 Rapid changes in fluorescence yields (F_m and F_o) and in F_v/F_m

The interplay between σ_{PSII} and τ_{PSII} appears to provide a good qualitative description of how the observed rapid changes in PSII could lead to the rapid enhancement in light-driven electron flow that were predicted by the numerical model. Changes in F_o, F_m, and F_v/F_m were also observed during these brief irradiance transients, but it is less clear how these changes might enhance or constrain photoacclimation. The FRR fluorescence method used in this study determines four PSII parameters (F_m, F_o, σ_{PSII}, and p) from the time course of a single measured property, the saturating kinetics of the apparent fluorescence yield F. The time scale of electron acceptors (τ_{PSII}) is retrieved from F kinetics measured during a separate relaxation period (Kolber and Falkowski 1992). Although the KPF98 physiological model of variable fluorescence presents F_m and F_o as being mathematically independent from σ_{PSII}, this does not mean that they are physiologically independent. For example, conversion of photoprotective xanthophylls into photoaccessory pigments can increase σ_{PSII} by reducing thermal dissipation of absorbed photons in the antenna bed (e.g., Oliazola et al. 1994). If the rate constants of thermal dissipation, photochemistry, and fluorescence sum to unity (Kiefer and Reynolds 1992), such an increase in σ_{PSII} might be expected to result in a concomitant increase in both F_m and F_o, especially at irradiances around E_K where the photochemical capacity of PSII is largely saturated. Identical percent increases in both F_m and F_o would not be apparent as changes in F_v/F_m, in contrast to how differential changes F_m and F_o would, e.g., resulting from the presence of an independent absorber or fluorophore (Cullen and Davis 2003). Assessing how transient changes in F_m and F_o may reflect aspects of rapid photoacclimation first requires determining whether or not these changes are measurement artifacts or actual physiological responses.

Both F_m and F_o decreased sharply during the 10 min perturbations in irradiance (Figure 3-7). These decreases could be explained in terms of the interplay between

σ_{PSII} and τ_{PSII}. Sudden decreases in irradiance increase the apparent rate at which a fixed PQ pool removes electrons from PSII, and so a rapid decrease in F_m or F_o simply reflects an increase in the relative availability of open PSII reaction centers, which are being serviced more frequently by the PQ pool. We also observed concurrent decreases in F_v/F_m during these transients, indicating that the relative decreases in F_m exceeded those in F_o. Our numerical model was originally designed to predict how physiological variability in PSII affects light-driven electron flow, independent of changes in biomass and over much longer time scales of hours to days. Over such periods, changes in F_v/F_m could be interpreted as reflecting changes in the fraction of functional reaction centers resulting from nutrient stress, or from midday inhibition of PSII function due to slowly reversible photodamage (Behrenfeld and Kolber 1999; Kolber and Falkowski 1993; but see Parkhill et al. 2001). Over the shorter 10-min transients, we assume that such processes are negligible in affecting F_v/F_m. If the observed short-term changes in F_v/F_m were important, we would expect that transient decreases in F_v/F_m would depress the electron flow rates. That predicted electron flow rates increased during these transients, despite concurrent decreases in F_v/F_m, suggests that changes in F_v/F_m are not a strong control on light-driven electron flow at these scales, compared to the interplay between σ_{PSII} and τ_{PSII}.

A final point to mention concerns the very rapid (< 1 min) changes in these fluorescence yields and in F_v/F_m that were observed in the rapidly growing culture, following each step decrease and increase in irradiance (e.g., Figure 3-7, arrows). It is difficult to explain these increasing, then decreasing, changes in fluorescence yield in terms of a directional photoacclimation response. It is more likely that these very rapid changes represent an inherent dynamical feature in PSII photophysiology, similar to the resonance observed at 59 s in a terrestrial macrophyte (Nedbal and Brezina 2002). Such features are similar to the response of an underdamped system to sudden perturbation, i.e., a “ringing” in photosynthetic dynamics. It is unclear why such very rapid responses in fluorescence yield and in F_v/F_m were largely absent in the nutrient-deplete culture. Such bidirectional responses in F_m and F_v/F_m may be of particular concern when examining irradiance-fluorescence relationships in natural light fields,

such as in sun-stimulated fluorescence. Such resonances inherently set a high-frequency limit on the time scales over which correlations between irradiance and fluorescence can be expected to hold.

3.5.3 Issues related to methodology or instrumentation

An unavoidable weakness of fluorescence techniques is that at *in vivo* temperatures, chlorophyll fluorescence emanates primarily from PSII, with little contribution from PSI. Thus, fluorescence techniques provide very little insight into the activity and photophysiology of PSI. At present, our inability to examine PSI makes it difficult to model how the complete photosystem photoacclimates to perturbations in irradiance. Better techniques to measure specific physiological properties of PSI and its activity *in vivo* are needed. Until those are developed, there are several possible avenues for improving how variable fluorescence can be used to examine rapid photoacclimation. Laboratory instruments with better signal-to-noise characteristics may more accurately resolve σ_{PSII}, p, τ_{PSII} and during very short irradiance perturbations. This is important especially for prokaryote phytoplankton, which have very low fluorescence yields *in vivo*. Instruments that can examine the variable fluorescence response over multiple turnover time scales may also provide useful information about processes downstream of PSII, but these techniques are in their infancy (Force et al. 2003; Kolber et al. 1998).

Inherent biases may arise when only a single, narrow excitation wavelength is used to stimulate variable fluorescence. If there are spectral biases in measurements of these various PSII properties that arise from probing their energetics with a very narrow range of wavelengths, as this instrument does (Raateoja et al. 2004), those biases may appear as well in the rapid responses in these parameters. For example, if PSII absorbs more strongly in the blue than the green, it is evident that the σ_{PSII} measured with a variable fluorometer using blue excitation may differ from the σ_{PSII}

measured with green excitation. Conversely, it follows that changes in the spectral absorption of PSII will introduce changes in σ_{PSII}, F_m, F_o, and therefore F_v/F_m, if these are measured with narrow band excitation wavelengths and if the spectral absorption of the sample changes. Being able to probe photosystem energetics at other wavelengths than only at ≈ 470 nm, as with this instrument, would be helpful (Gorbunov and Falkowski 2004).

The fundamental objective of these culture experiments was to separate the directional responses in PSII physiology to rapid changes in irradiance, from the longer scale changes in PSII physiology that reflect diurnal responses or circadian rhythms in this diatom. The time history of irradiance used to force these transient responses was not intended to replicate any "realistic" light field, as other systems purport to do in prior studies (Gocke and Lenz 2004; Kroon et al. 1992; Stramski and Legendre 1992). Instead, the incubation system we used was intended simply to provide a means to generate specific, idealized perturbations in irradiance. Considerable information about PSII responses to these perturbations can be obtained from the variable fluorescence transients of samples placed quickly in the dark, as was done in this study. However, being able to measure these photosynthetic properties in a similar incubator, but directly on cells exposed to the actual ambient light field, may provide a more accurate assessment of rapid photoacclimation in cultured phytoplankton.

Acknowledgements. We thank Dave Karl and the HOT team, especially Lance Fujieki, for support in collecting the PAR data during each survey cruise. We also thank the other co-PIs on the HALE-ALOHA project for support in the radiometry aspect of that mooring. Dr. E. Firing provided helpful comments regarding the vertical velocity field at Station ALOHA. Amanda Ashe and Kristin Landgren were invaluable during these culture experiments. Comments and criticisms by P. Wheeler, T. J. Cowles, J. R. V. Zaneveld, and T. Plant helped improve this manuscript considerably.

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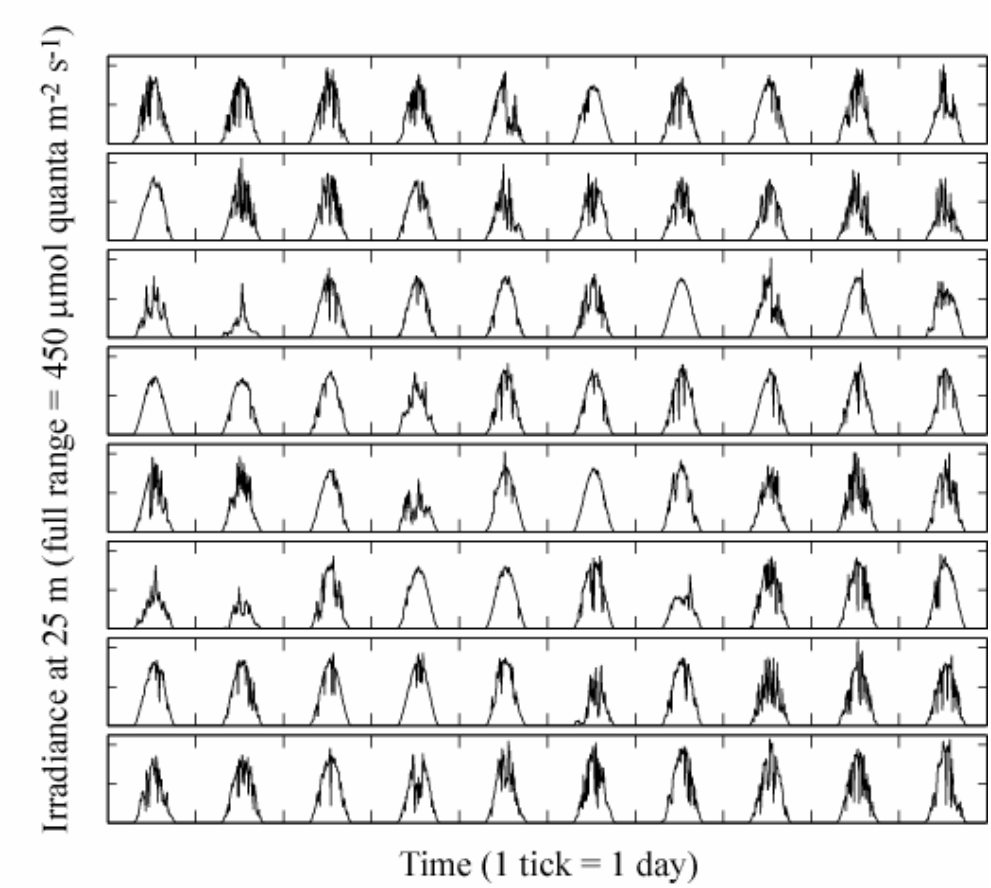

Figure 3-1. An 80 d irradiance time series collected at 25 m depth on the HALE-ALOHA mooring north of Oahu (22° 45'N, 158° W) from Nov 1998 – Feb 1999, at 10 min intervals.

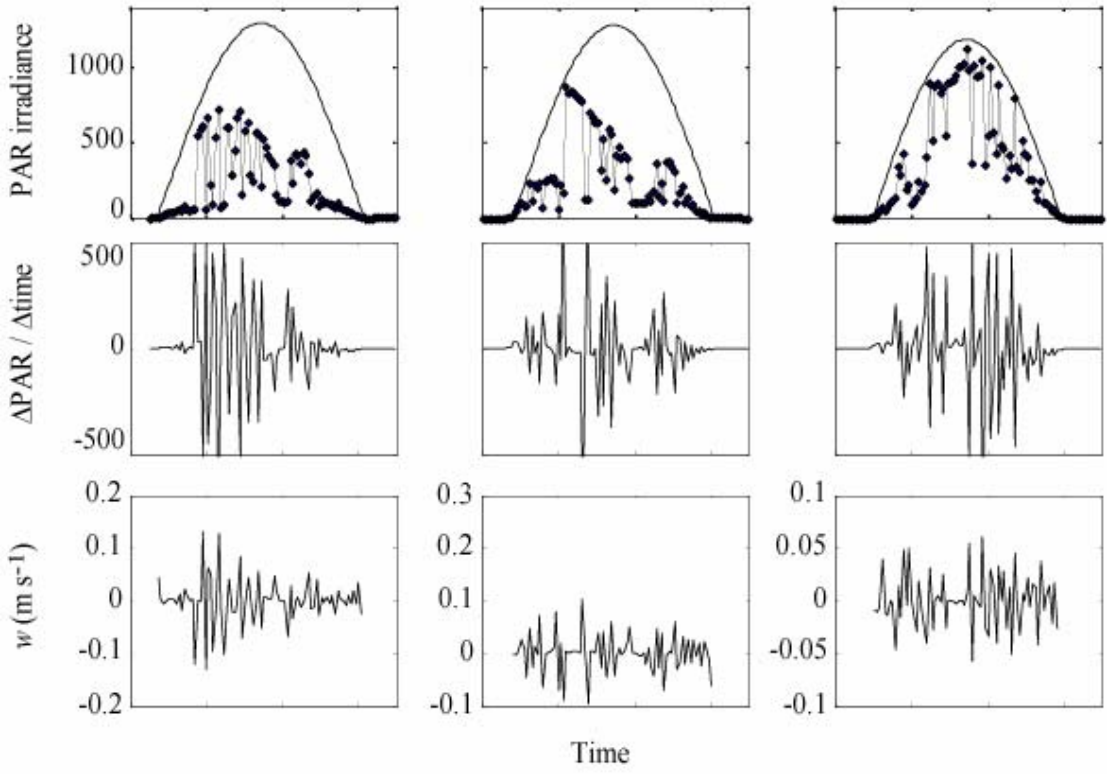

Figure 3-2. Three randomly chosen days of irradiance measured on the HALE-ALOHA mooring: (top row) measured PAR irradiance at 25 m depth (μmol quanta m^{-2} s^{-1}, marked lines) and the predicted clear-sky solar model, (middle row) rates of change in irradiance (μmol quanta m^{-2} s^{-2}) between each sample, and (bottom row) vertical velocities required to generate these cloud-induced changes in irradiance.

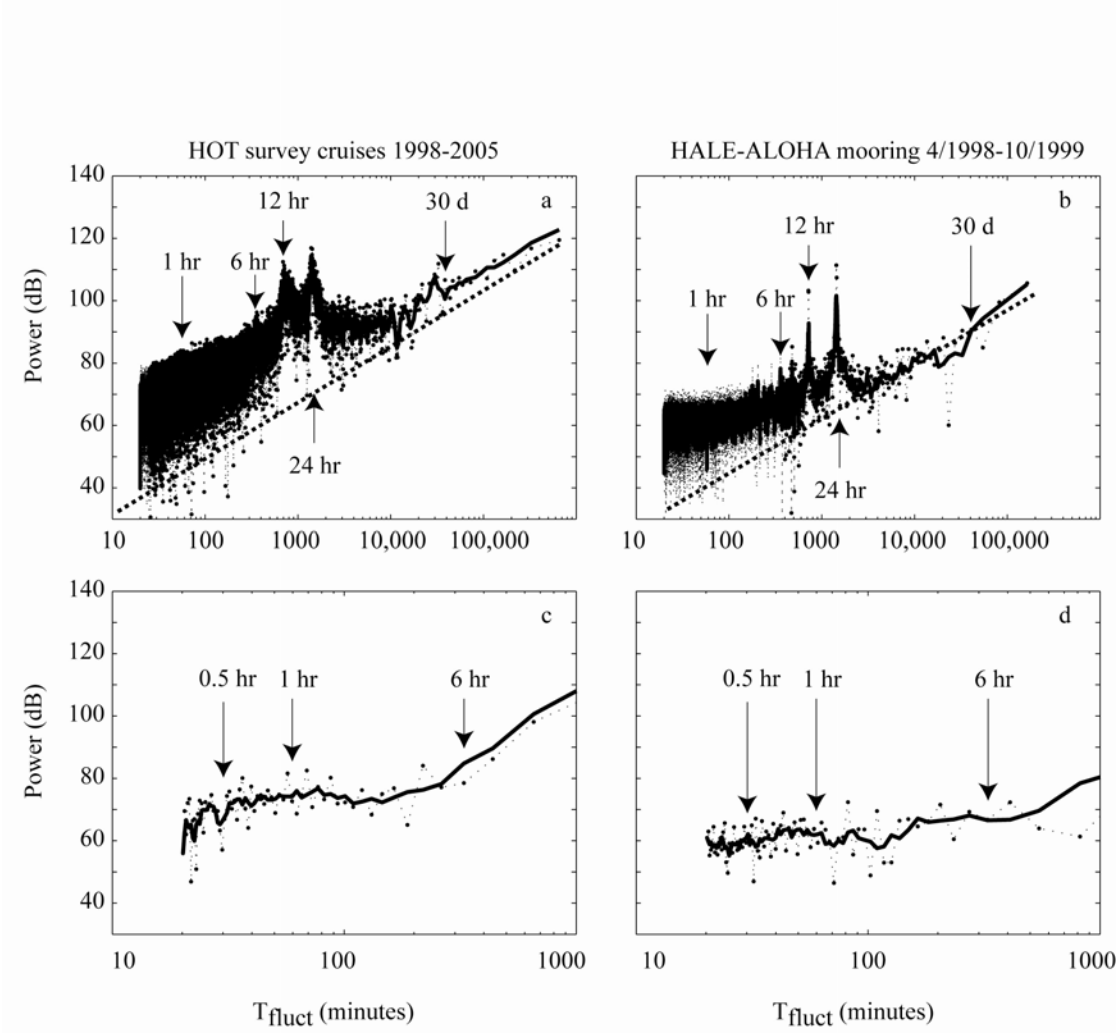

Figure 3-3. Power spectra of irradiance perturbations at Station ALOHA from data collected monthly during each HOT survey cruises (left column), and continuously by the HALE-ALOHA 25 m radiometer (right column). Dashed lines reflect general trends, not statistical fits. When these data are downsampled to identify the trend inside their envelopes, these power spectra appear roughly flat at periods below 6 h (bottom row).

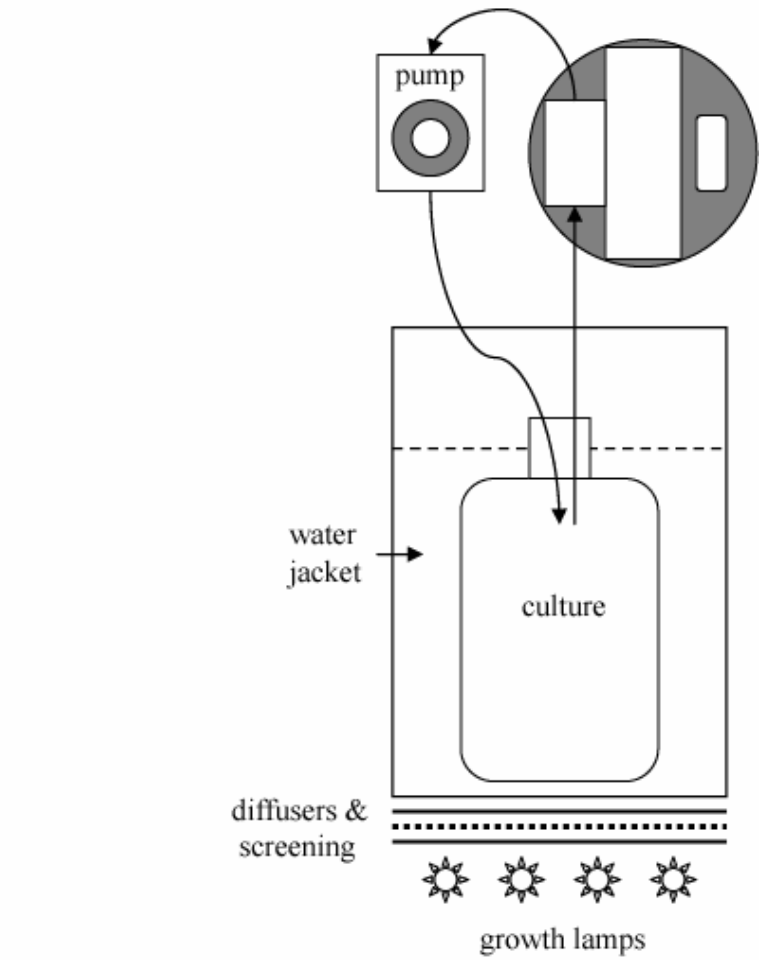

Figure 3-4. A diagram of the culturing apparatus, showing recirculation of sample through the fast repetition rate fluorometer.

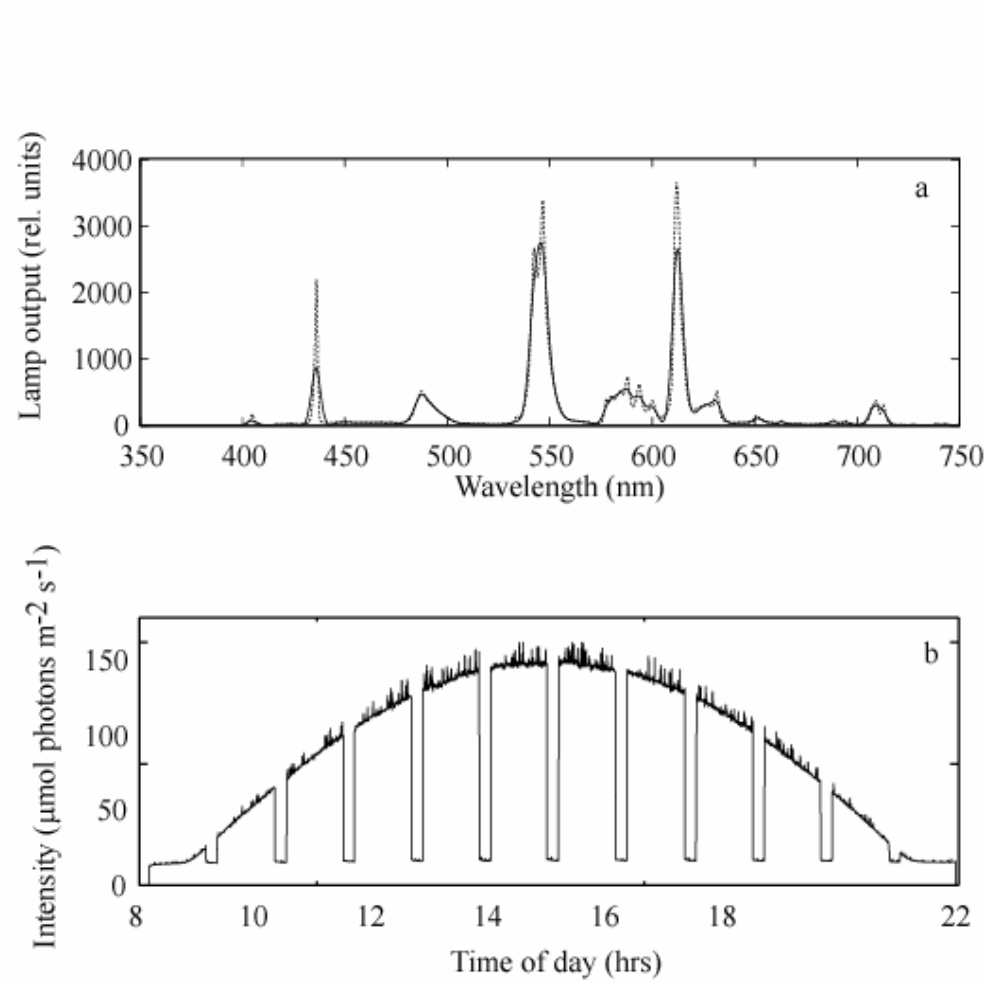

Figure 3-5. Characteristics of the growth irradiance provided to the cultures in these experiments: a) lamp spectral output at 0.4 nm (dots) and at 1 nm (solid) resolution, and b) a representative daily light profile for these cultures.

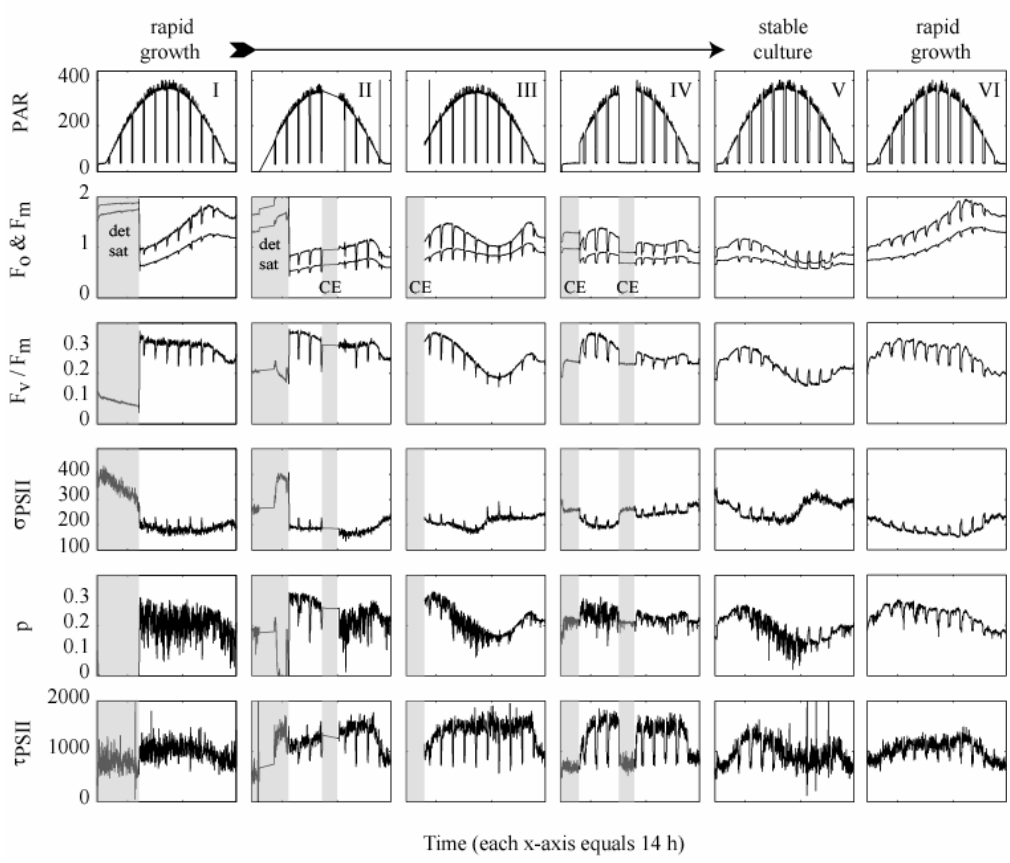

Figure 3-6. Six representative days of irradiance and PSII responses in the *T. weissflogii* culture. Columns I-V show the progressive change in diurnal patterns in PSII properties following a single dilution, as the culture experiences slowing growth due to nutrient limitation. Column VI shows an example day of rapid growth. Shading indicates periods when the instrument detector was saturated ("det. sat"), or when errors occurred in the lamp control system ("CE").

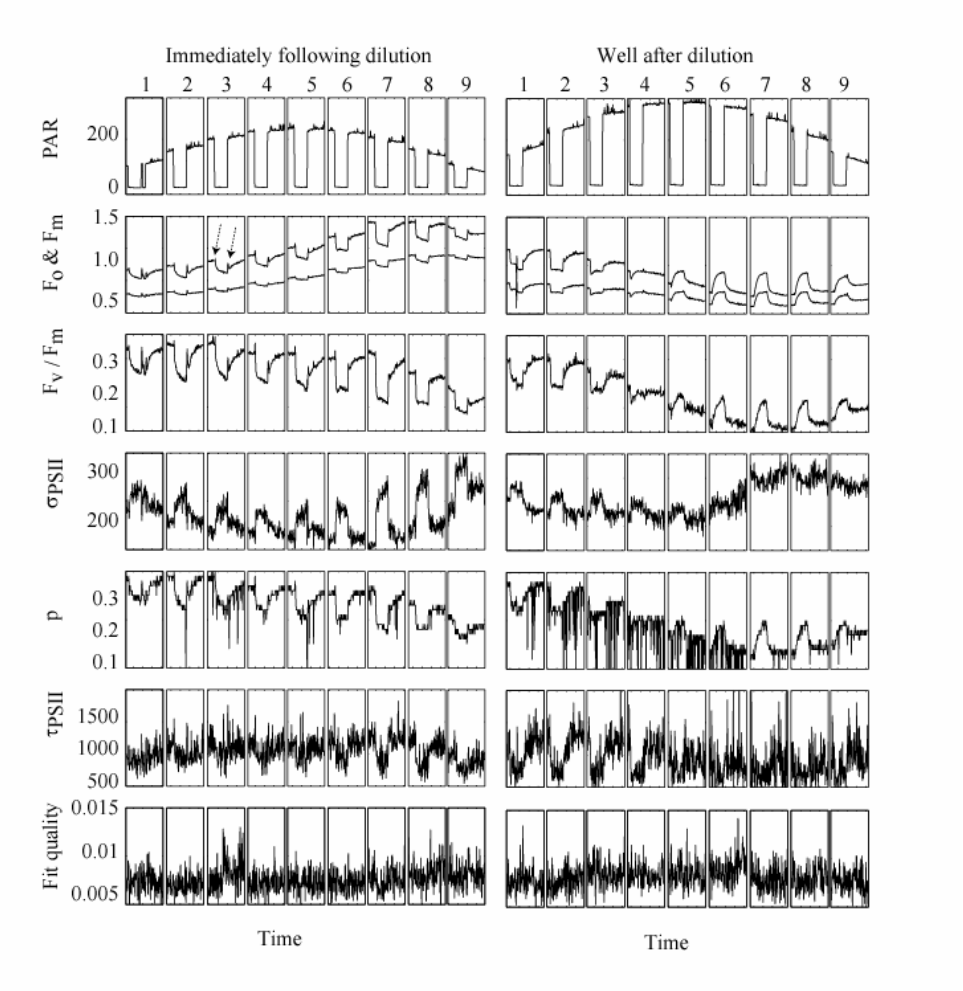

Figure 3-7. Details of the progressive changes in PSII properties during and immediately after 10 min transients in irradiance, for rapid growth (column VI) and stable nutrient-limited (column V) conditions of Figure 3-6. The numbers above each column are indices for each individual transient event during the diurnal period.

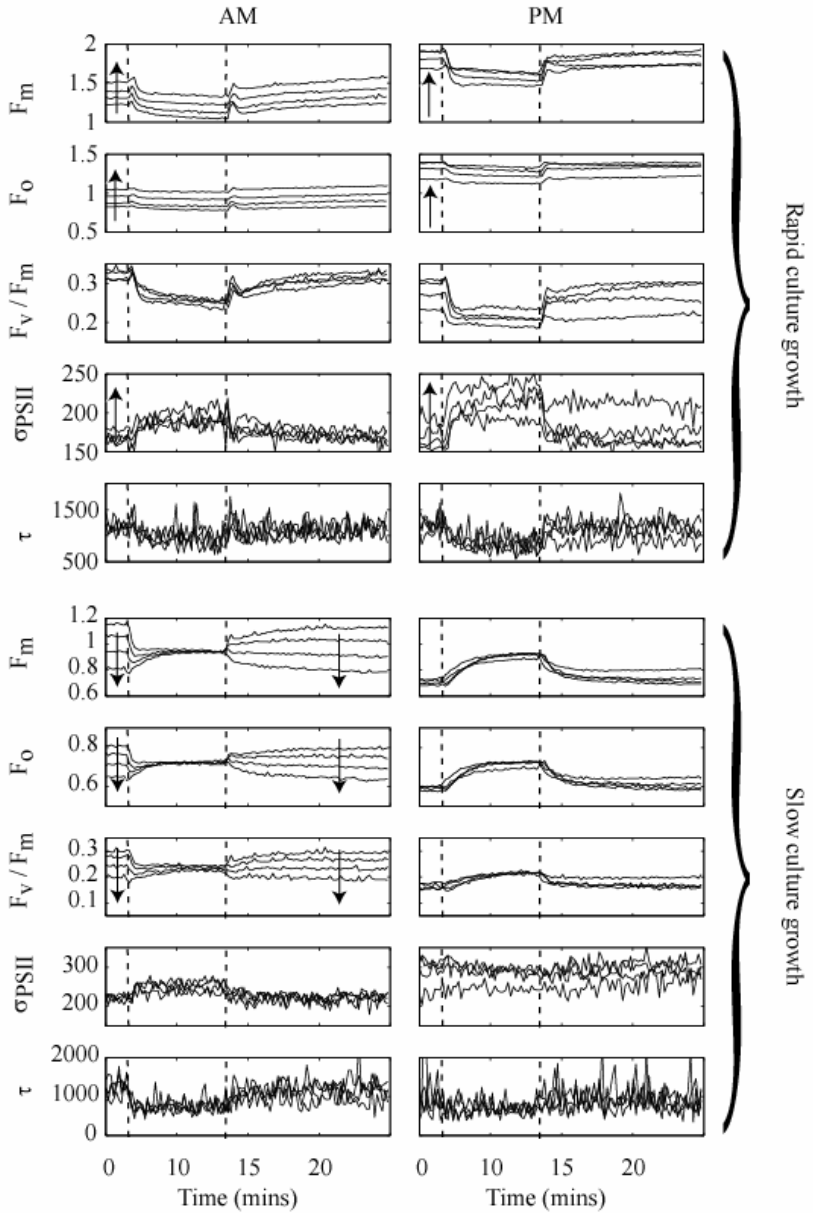

Figure 3-8. Composites of the transient PSII responses in Figure 3-7 for the rapidly growing (top panels) and slowly growing cultures (bottom panels), in the forenoon (left column) and afternoon (right column). Vertical dashed lines indicate the timing of the 10-min transient decrease in irradiance. Arrows indicate the direction over time of level shifts in PSII parameters that occur over the diurnal period.

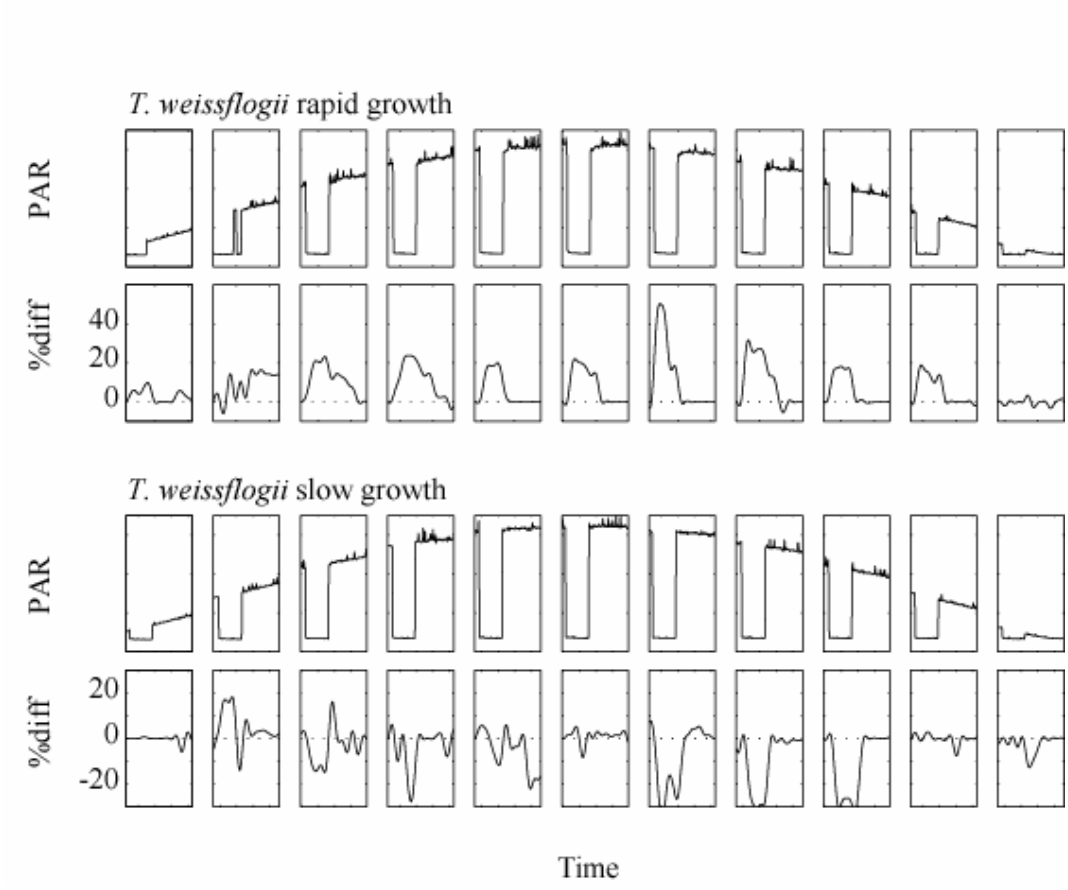

Figure 3-9. The enhancement to or constraint in light harvesting due to these rapid PSII responses during and after these 10 min transients, for the rapid growth and slow growth conditions in Figure 3-7 and Figure 3-8.

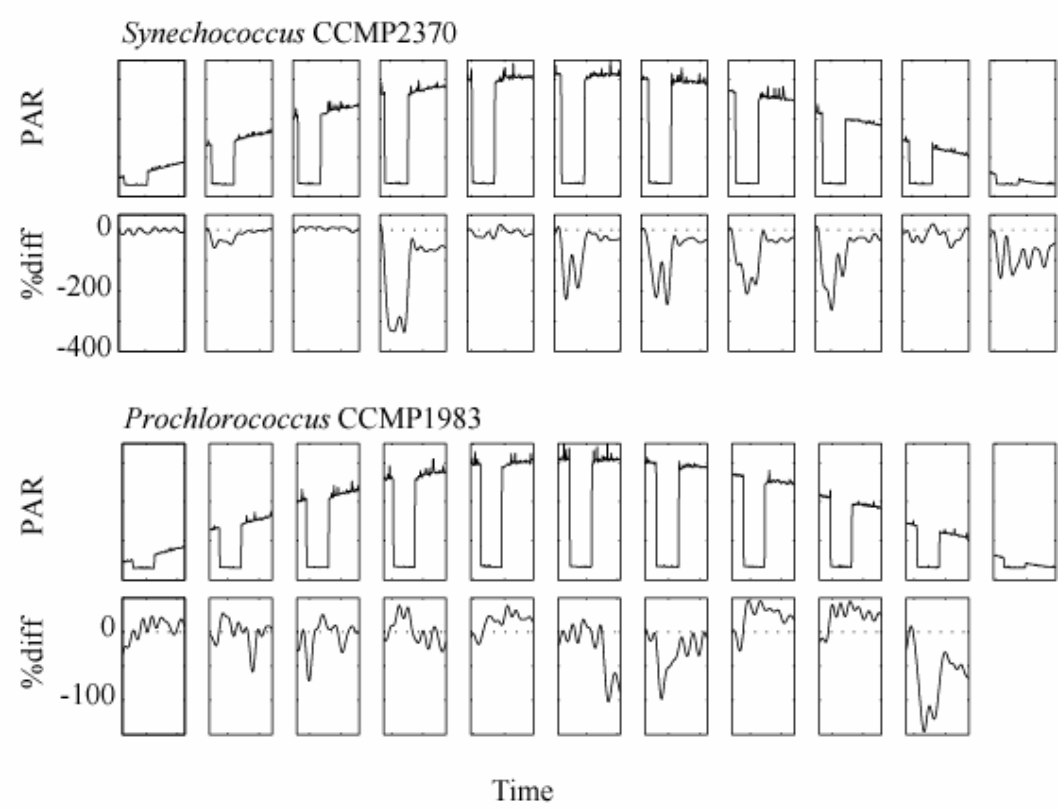

Figure 3-10. Preliminary assessment of how rapid photoacclimation to 10-min scale light perturbations affects light driven electron flow in a rapidly growing *Synechococcus* culture (CCMP2370), and in a slowly growing *Prochlorococcus* culture (CCMP1983).

4 Rapid photosynthetic responses in surface phytoplankton assemblages at Station ALOHA

4.1 Abstract

Rapid, minute-scale photosynthetic responses were examined in surface phytoplankton assemblages in the oligotrophic North Pacific. A single-turnover, variable-fluorescence method (fast repetition rate fluorometry) was used to measure physiological properties of Photosystem II, in surface seawater taken from a shipboard supply. A self-logging radiometer drifter was also deployed and recovered, to measure *in situ* changes in the natural fluorescence emission of these surface assemblages. These measurements were performed during two survey cruises at Station ALOHA in the fall of 2005, with temporal resolutions of a single minute or better. An automated filtering system was attached to the variable fluorometer to provide hourly measurements of the artifact signal that results from dissolved background fluorophores and instrument biases. This background contributed approximately one-third of the total fluorescence signal measured by this instrument on unfiltered samples, which indicates the need for robust corrective measures. Rapid responses in Photosystem II to short term perturbations in irradiance were observed in these phytoplankton assemblages. These assessments were limited to time scales only of about 5 minutes and longer, due to considerable measurement noise. These assemblages also displayed rapid shifts in photosynthetic state that were independent of rapid changes in irradiance, which reflected photosynthetic regulation during diel changes in irradiance. Similar irradiance-dependent and -independent changes were apparent in the natural fluorescence data. Separating these two forms of rapid photosynthetic responses in phytoplankton is essential for developing robust approaches to interpret variability in surface ocean phytoplankton assemblages, on the sub-diurnal scale.

4.2 Introduction

Over its life cycle, an individual marine phytoplankter can experience irradiance that ranges from a complete absence, to low levels insufficient to support growth, to intensities strong enough to inflict severe physiological damage. These strong fluctuations in irradiance availability are the result of numerous environmental processes: planetary rotation, atmospheric factors like clouds, hydrodynamics in the water column, and properties of the air-sea interface (Allen 1998; Harding et al. 1987). An important strategy of most if not all phytoplankton is to optimize their utilization of this essential, sometimes toxic, sometimes absent, resource. The wide range of photosynthetic responses that phytoplankton exhibit are typically interpreted as the result of evolutionary pressure, necessary to survive under such strong variability in resource availability.

Many photosynthetic responses involve structural adjustments to photosynthetic subcellular components. Macromolecular changes in the cellular quota or stochiometry of components of the light and dark reactions, are often referred to as *photoacclimation*. These responses are also referred to as *photoadaptation*, although adaptation and acclimation in an ecological sense refer to two distinctly different processes (Ricklefs 1990; Roughgarden 1998). For this discussion, photoadaptation refers only to genotypic changes in photosynthetic potential or behavior that occur in phytoplankton populations over evolutionary time scales. This usage is consistent with recent reviews by MacIntyre et al. (2002) and Raven and Geider (2003). Photoacclimation, in contrast, will refer to many – but not all – of the photosynthetic responses that a individual single phytoplankter can effect over its lifespan.

Photoacclimation has been well studied in phytoplankton, in terms of how physiological changes help phytoplankton adjuste photosynthesis in the continually varying marine light field (e.g., Falkowski 1984; Harding et al. 1987; Harris 1978; Prézelin 1981). Circadian responses to diurnal changes in irradiance are not typically included in this discussion because circadian photosynthetic cycles are generally not

considered to be photoacclimative, despite a strong similarity in many of their physiological bases (Harding et al. 1981; Suzuki and Johnson 2001). Since photoacclimation by definition involves alteration to pigment quotas and architecture, the time scale of photoacclimation is limited to that of biosynthesis, i.e., on the order of hours and longer. These > 1 h time scales correspond broadly with those of physical processes that displace phytoplankton vertically within an exponentially decaying light gradient (Denman and Gargett 1983; Lewis et al. 1984b). Vertical displacement and the scale of irradiance forcing (> 1 h) has received much attention in photoacclimation research (e.g., Cullen and Lewis 1988; Falkowski and Wirick 1981; Huisman et al. 1999; Marra 1978b; Marra 1980; Schubert et al. 1995).

However, most if not all phytoplankton exhibit additional physiological responses to changes in light that are independent of macromolecular changes to photosynthetic structure. These responses are generally referred to as photosynthetic *regulation* as distinct from photoacclimation. Examples of such regulatory mechanisms include enzymatic modification of xanthophyll pigments (Oliazola et al. 1994; Oliazola and Yamamoto 1994), and changes in the aggregation and conformation of Photosystem II (PSII) light-harvesting complexes (Horton et al. 1991; Wentworth et al. 2000). There are other less well understood responses that are also thought to adjust the energetic distribution between Photosystem I (PSI) and PSII and provide additional modes of regulation (Koblížek et al. 1998; Mullineaux et al. 1997; Sarcina et al. 2001). Whereas photoacclimation involves changes to fundamental photosynthetic structure and architecture at the transcriptional level, regulation involves primarily functional changes to a particular photosynthetic architecture that affects its dynamic behavior (Table 4-1). Thus, because these particular responses are independent of biosynthesis, they can occur very rapidly with time scales of seconds to minutes.

Table 4-1. A classification of some photosynthetic responses, based on whether these responses reflect changes in the fundamental structure of the light reactions, or more affect only their function independent of fundamental structure.

Structural responses (equal to time scales of biosynthesis)	**Functional responses (less than time scales of biosynthesis)**
Pigments: content & distribution	State of interconvertible pigments
PSI & PSII: number and stoichiometry	Aggregation of LHC with PS
Quota of electron acceptors & carriers	Mobility of phycobilisomes
Repair rates of photodamage	Energy redistribution among PSII & PSI
Thylakoid structure and position	Thylakoid structure and position (?)
Locational change (swimming, buoyancy)	(?)

Photoacclimation and regulation are distinguished by physiological differences, but the ecological role of these two types of photosynthetic responses, and their eliciting environmental stimuli, are in many respects quite similar. Both serve to optimize photosynthesis by adjusting rates of light harvesting and utilization. Both appear to respond to the same physiological trigger: the trans-thylakoid ΔpH that results from imbalance in the activities of PSI and PSII (Horton and Ruban 2005). Having identical physiological stimuli implies that both photoacclimation and photosynthetic regulation respond to identical forms of metabolic stress in the light reactions. Scale matching arguments would therefore suggest that the only ecological difference between the two is in their characteristic time scales of response. From this perspective, photosynthetic regulation presumably serves as a mechanism for optimizing photosynthesis in response to rapid, sub-hourly fluctuations in irradiance, whereas photoacclimation serves to adjust photosynthesis under more slowly varying

perturbations on the > 1 h scale. This scale-dependent distinction has been made before by several workers (Falkowski 1984; Ley 1980; Vincent 1979).

However, this view of rapid photosynthetic regulation – as a means to accommodate rapid changes in irradiance – does not fully describe its role. The same type of rapid changes in photosynthetic function can be observed in phytoplankton that grow in light fields that fluctuate gradually (Bruyant et al. 2005; Laney et al. 2005). In cultures of *Thalassiosira weissflogii*, sharp changes can be seen in its natural fluorescence yield and in specific physiological parameters of PSII, within a diurnal period having a smoothly varying light history (Figure 4-1). These rapid photosynthetic responses cannot be interpreted in terms of directional responses to any similar-scale perturbation in irradiance. Instead, these responses reflect the dynamical, inherently nonlinear behavior of light harvesting and utilization in the photosynthetic apparatus.

It has historically been difficult to reconcile these two apparently different modes of rapid photosynthetic response in phytoplankton. Sudden changes in photosynthetic properties in the absence of rapid light perturbations, indicate sudden shifts in photosynthetic dynamics as the light harvesting system attempts to maintain itself near the threshold between light-limited and light-saturated irradiance. That light harvesting is a complex process with nonlinear dynamics has important ramifications in phytoplankton ecology. The relationship between irradiance (E) and photosynthesis (P) in plants and algae has been long recognized as nonlinear, in the sense that there is photosynthetic saturation at high irradiances. However, even simple nonlinear systems exhibit complex nonlinear dynamics (May 1976; Strogatz 1994), and so the same photosynthetic architecture that leads to saturation at high irradiances may also exhibit simple nonlinear dynamics such as feedback, hysteresis, and thresholds. These dynamics can have important ramifications for higher-order, multiple generational processes, such as competition and exclusion, especially under non-equilibrium conditions (Levins 1979; Pascual-Dunlap 1995). Understanding how these nonlinear dynamics affect light harvesting and photosynthesis at sub-generational scales is also important, as responses of individual phytoplankton determine the outcome of such

multi-generational, population level processes (Abbott et al. 1982; Lande and Lewis 1989; Marra and Heinemann 1982).

The goal of this field study was to examine rapid photosynthetic responses in natural phytoplankton assemblages in the surface ocean and to assess how different aspects of photosynthetic physiology respond differently to rapid fluctuations in ambient irradiance. Specifically, we measured fluorescence properties of surface assemblages at the Hawaii Ocean Time-series (HOT) site at Station ALOHA (22° 45'N 158° W), 100 nm north of Oahu in the oligotrophic North Pacific. Arguably the dominant threshold behavior in these phytoplankton assemblages occurs near the transition from light-limited to light-saturated photosynthesis, which occurs at least twice daily at this site, in the morning and afternoon. However, intermittent cloud cover at this location is considerable, and could introduce additional transitions and provide the environmental forcing for rapid photosynthetic responses in the light reactions.

4.3 Methods

4.3.1 Variability in PSII properties in surface assemblages

Fast repetition rate (FRR) fluorometry was used to generate time series of PSII physiological properties during two HOT survey cruises at Station ALOHA (HOT174 and HOT175), in October and November of 2005. A commercial FRR fluorometer (Fasttracka FRRF, Chelsea Marine Systems, West Molesey, UK) was operated in a benchtop mode and connected to the ship's flow-through system, drawn from ≈ 3 m depth. The interval that seawater spent inside the ship's flow through system, before being sampled by the FRRF, was estimated to be less than a minute. Approximately five acquisitions were collected every minute, each consisting of 16 averaged, sequential flash sequences of 100 saturation and 20 relaxation flashlets. The saturation

flashlets were delivered over a period of ≈ 430 μs, whereas the relaxation flashlets were delivered over a period of ≈ 7.2 ms.

Phytoplankton biomass is very low in this region, as is the inherent fluorescence yield of the prokaryotes that dominate the upper water column. Thermal dissipation of absorbed light energy, a photoacclimative response, further decreases the absolute value of fluorescence yield that is measured in these surface assemblages. Consequently, it is especially important in such samples to correct for any artifacts in these variable fluorescence measurements that result from dissolved fluorescing sources, hardware biases in the instrument, or numerical weaknesses in the software used to estimate these PSII properties from variable emission kinetics (Laney 2003). An automated system was designed and built that periodically redirects the incoming seawater supply through a 0.2 μm pore size filter (CritiCap-50, Pall Gelman Sciences), before being sampled by the instrument. Under computer control, this system was used to measure the combination of background fluorescence and instrument artifacts every hour.

These measurements were used to correct the apparent fluorescence yield kinetics that were measured over the remainder of each hour. During each hourly filtering interval, approximately 19 excitation sequences ($EX[n]$) and emission sequences ($EM[n]$) of this filtered background and impulse response signal were collected. These 19 sequences were averaged to produce a mean hourly dissolved $EM[n]$ and $EX[n]$ transient, which was used to correct all the unfiltered fluorescence transients that were measured in the half hour preceding and following each of these hourly background determinations. Details of the mathematics used to perform these corrections can be found in Chapter 5 of this dissertation.

After correcting for background and artifacts, the $EM[n]$ and $EX[n]$ transients from unfiltered seawater were binned on 5 minute intervals. Custom fluorescence analysis software was used to fit the Kolber et al. (1998) physiological model of variable fluorescence transients to these corrected data. This software (v6, see Chapter 5 of this dissertation) better accounts for the instrumental and numerical biases that have been shown to introduce considerable artifacts into time series of PSII

parameters estimated using these FRR fluorometers (Laney 2003). The combination of impulse correction, applied earlier, and the more robust fitting process, improves the retrieval of PSII parameters that are most sensitive to the kinetics of the initial part of the saturation sequence, i.e., F_o, σ_{PSII}, and p.

In processing the variable fluorescence transients that we measured at Station ALOHA, we observed that the specific protocol we used did not maintain fluorescence saturation over the second half of the excitation flash sequence. Specifically, we observed a slow decrease in fluorescence yield. Such kinetics are not allowed in the Kolber et al. (1998) physiological model of variable fluorescence, which assumes that electron acceptor processes that relieve PSII of excitation pressure operate on comparatively much longer time scales. However, there is no physiological reason for this assumption, and such kinetics should be assumed for phytoplankton samples with rapid electron acceptors. Consequently, in addition to fitting the Kolber et al. (1998) model to these variable fluorescence transients, we also applied a modified version that included a decay term to account for concurrent relaxation of fluorescence yield during a saturation transient.

$$F(t) = F_0 + (F_m - F_0) \cdot C \cdot \left(\frac{1-p}{1-C \cdot p} \right) \cdot e^{\frac{-t}{\tau}} \qquad \text{Eq. 4-1}$$

The time scale (τ) in this modified model in theory is identical to that of the fast, order milliseconds acceptor-side time constant observed in the subsequent relaxation sequence.

A time series of local surface irradiance was measured using a different sensor, the “deck cell” of a profiling reflectance radiometer (PRR-600/610, BioSpherical Instruments, San Diego CA). This radiometer has six irradiance channels in the visible wavelengths, which were sampled with a temporal resolution of 6 Hz. Irradiance at these six wavelengths was numerically integrated to generate an estimate of the photosynthetically active (PAR) irradiance as a function of time. This PAR time series was merged with the FRRF physiological data later in post-processing.

4.3.2 Natural fluorescence kinetics in surface assemblages

During these two cruises a radiometer system (TSRB-I, Satlantic Inc., Halifax NS) was tethered to the free-drifting primary productivity array that is deployed by the HOT program each cruise. This radiometer system measured downwelling solar irradiance at 490 nm, and seven upwelling radiances at 412, 443, 490, 510, 555, 670, and 683 nm, at a 6 Hz rate. These radiometric data were averaged by the instrument internally every 10 s.

Measurements of upwelling radiance at the chlorophyll fluorescence emission wavelengths near 683 nm were separated into their backscattered and fluoresced components, using a fluorescence line height (FLH) algorithm modified from Letelier and Abbott (1996). This modification computed FLH at 683 nm from the wavelength doublet of 670 and 683 nm:

$$FLH = Lu683 - Lu670 * \frac{710 - 683}{710 - 670} \qquad \text{Eq. 4-2}$$

This algorithm differs from the one presented by Letelier and Abbott, which was developed for use with satellite-sensed data from the MODIS sensor. This sensor has a band at 746 nm in the near-infrared (band 15), which can be used to provide additional information, red-shifted of the fluorescence maximum, for computing a FLH baseline. The radiometer drifter used in this study did not have channels in the infrared, and so we used the assumption of Letelier et al (1997) that radiance is zero at 710 nm.

Given the very low fluorescence yields of the surface phytoplankton assemblage at Station ALOHA, it is possible that data from a discrete-wavelength radiometer may be inadequate to compute a robust measurements of FLH using Eq. 4-2. To determine this, we examined a separate data set from a radiometer buoy that was physically similar to the one used in this study, but which had a higher spectral

resolution of ≈ 3.3 nm (hTSRB, Satlantic, Halifax NS). These high-resolution data were collected on a prior HOT survey cruise at Station ALOHA (HOT154), off the stern of the vessel and well out of the ship shadow.

4.4 Results

4.4.1 Intermittent cloud cover at Station ALOHA

In Chapter 3 of this dissertation, a long-term time series of irradiance from Station ALOHA was examined. This analysis demonstrated that intermittent cloud cover was a major source of short-term variability in irradiance in this ocean region (Figure 3-1). There are spatial issues with cloud cover at Station ALOHA that the HOT time series at the HALE-ALOHA mooring does not reflect.

Using Level II, 4 km^2 chlorophyll data from MODIS, it can be shown that the number of cloud-free days in the immediate region around Station ALOHA is very low in general for the 2 months surrounding these HOT survey cruises (Figure 4-2). It is also apparent that during this time, Station ALOHA lay in a consistently clouded feature oriented ENE-WSW of Kauai. This feature presumably reflects the considerable orographic precipitation immediately windward of Kauai, as the trade winds from the northeast approach each island. In the channels between the individual islands in the Hawaiian chain, considerably more cloud-free days are observed along tracks northeast of these channels. In the precipitation features such as where Station ALOHA is located, only as few as 5 cloud-free days were observed in this two month period.

4.4.2 Trends in PSII physiology in Station ALOHA surface assemblages

Surface seawater spent approximately 1 min in the ship's flow through system, which is short compared to the relaxation scale of nonphotochemical quenching (several minutes and longer), but long compared to the relaxation of photochemical quenching (ms to seconds). We assumed that the nonphotochemical quenching of fluorescence changed minimally during the time spent in the ship's flow through system. Consequently, we interpret long-term, > 1 min changes in the properties of PSII measured using the FRRF as reflecting the physiological state of the phytoplankton as they were immediately before being taken into the ship's intake. It should be noted that for some taxa, this interval is enough to reflect considerable shifts in PSII energetics, but preliminary results using prokaryote cultures suggests a slower overall response in PSII energetics, on the order of several minutes (see Chapter 3).

The hourly variable fluorescence measurements on the 0.2 μm filtered samples provided a means to monitor the diurnal and longer-term variability in the background fluorescence of dissolved material at Station ALOHA. Roughly half of the fluorescence signal measured by the FRR fluorometer was artifact, due to the fluorescence of dissolved constituents and the scatter in the instrument's dark chamber. During HOT174, a clear diurnal pattern was observed in the ratio of emission to excitation, driven solely by changes in the background signal (Figure 4-3a). The magnitude of this fluctuation was 0.03 (in instrument units), which is approximately 20% of the full dynamic range exhibited by F_m. No similar diurnal pattern was evident on HOT175, and large transient changes were apparent immediately following periods when the ship's flow through system was switched off and on (Figure 4-3b). The amplitude of the background signal was roughly comparable to that observed during HOT174, between 0.15 and 0.2 (relative units).

The HOT174 cruise occupied Station ALOHA for three full diurnal periods between days 280 and 282 of 2005 (Figure 4-4, top panels). None of the three days was fully clear-sky, and there were consistent 5 min fluctuations in irradiance. The general trend in F_o and F_m involved a rapid increase in the early morning, followed by significant midday fluorescence quenching which was somewhat relieved in the late afternoon. Rapid, 5-min increases in both F_o and F_m were observed in concert with

sudden decreases in irradiance on day 281 and 282 (arrows). The highest value of F_v/F_m observed over these three days was ≈ 0.65. Over the diurnal scale, the general trend in F_v/F_m was one of substantial decrease around solar noon, although sudden decreases in irradiance resulted in similar-scale increases in F_v/F_m (arrows). Diurnal trends in σ_{PSII} were less clear; filtering these data on scales above 2 h revealed a sudden decrease at sunrise and a gradual recovery at sunset, but often with a midday increase. The sudden decreases in irradiance on day 282 coincided with increases in σ_{PSII} (arrows). Similar but weaker behavior was observed on day 281 following a series of more rapid irradiance fluctuations in the afternoon. During daytime, τ increased above its nighttime baseline value and also exhibited strong "spikes". Given the 5 min resolution of these data, it was unclear if these spikes reflected actual responses to irradiance transients, or were simply artifactual. This was difficult to ascertain with the FRRF used because of the relatively few number of points used to estimate τ from measured variable fluorescence transients. The irradiance decreases that occurred during days 281 and 282 did coincide with short-term increases in τ, however. Anomalously large values of the quality of fit indicator (X^2) were observed in this time series near 282.8, but no substantial anomalies were apparent concurrently in the PSII physiological properties.

The HOT175 cruise occupied Station ALOHA for three full diurnal periods on days 315 through 317 of 2005, and was immediately followed by a short, two-day visit to the HOT site on days 319 and 320 (Figure 4-4, bottom panels). Of these five days, day 317 displayed the closest approximation to fully clear-sky. Rapid responses in PSII physiology to light fluctuations were observed in the other 4 days. The diurnal and short-term responses in PSII physiology that were observed during HOT175 were similar to those observed in HOT174. Diurnal trends in F_o, F_m, and F_v/F_m show similar behavior in midday quenching. Short-term decreases in irradiance resulted in immediate increases in all three of these parameters (arrows). The maximum value of F_v/F_m observed during these three days was ≈ 0.61. Diurnal patterns in σ_{PSII} were generally similar to those observed during HOT174: a sudden decrease at sunrise and

a gradual recovery at sunset, but often with a midday increase. Diurnal patterns in τ were similar to those observed in HOT174. No anomalous patterns were observed in the quality of fit indicator.

Histograms illustrate the day-night differences in each of these PSII physiological parameters. Diurnal-nocturnal differences in the absolute values of these parameters reflect the general diel trends in F_v/F_m, σ_{PSII}, and τ when plotted as time series (Figure 4-5, top panels). When only the high-frequency variability in these parameters was examined (i.e., changes that occurred faster than 2 hours), it was evident that both F_v/F_m and τ exhibited more rapid changes during the daytime than during the nighttime (Figure 4-5, bottom panels). However, rapid changes in σ_{PSII} were only slightly greater during the day than during the night. These results suggest that primarily only F_v/F_m and τ responded rapidly to similarly quick changes in irradiance during the daytime. Changes in σ_{PSII} in response to irradiance transients were either absent, or small enough as to be invisible.

The correlation between very rapid changes in these PSII physiological parameters (faster than 30 mins) and similarly fast perturbations in irradiance were examined for the daytime-only data, using a Model II, geometric-mean method (Laws and Archie 1981). Data corresponding to small changes in irradiance less than 50 μmol photon m^{-2} s^{-1} were excluded from these correlations to place an emphasis on the larger magnitude irradiance fluctuations (Figure 4-6). In general, there was little correlation between light perturbations and rapid changes in PSII. The two parametes that showed the best correlation were F_m and F_v/F_m, although the strength of these correlations was ≈ 0.3 at best (Table 4-2).

Table 4-2. Correlation coefficients (r^2) between changes in irradiance on 30 min time scales, and PSII properties measured from variable fluorescence transients.

Cruise	F_m	F_o	F_v/F_m	σ_{PSII}	τ	p
HOT174	0.20	0.00	0.31	0.02	0.00	0.03
HOT175	0.12	0.03	0.13	0.02	0.01	0.01

4.4.3 Diurnal trends and rapid changes in natural fluorescence

The measured sun-stimulated fluorescence signal is very weak at Station ALOHA, due to low phytoplankton abundance and strong quenching of phytoplankton fluorescence. The upwelling radiance in the red wavelengths is at least two orders of magnitude weaker than that in the blue, despite roughly comparable intensities in irradiance (Figure 4-7a,b). The peak in sun-stimulated fluorescence yield was barely measurable around 680 nm at Station ALOHA (Figure 4-7c), but it is weak compared to identical measurements taken in a coastal transition zone along the Oregon shelf (inset).

Data collected by the radiometer drifter during these three 2005 HOT cruises show the same daily irradiance histories that were measured from the ship (Figure 4-8, top row). During HOT174, the drifter was deployed for only a single diurnal period on October 9. On HOT175 and HOT176, the drifter was deployed for two full diurnal periods on November 11 and 15, and on December 12 and 13. However, around solar noon on the first HOT175 deployment (November 11), the buoy became fouled in a way that resulted in the loss of all upwelling radiance data, starting approximately at noon. These data were omitted from the analysis.

The ratio of FLH to PAR radiance (*FLH/PAR*) is sensitive to overall changes in the light reactions that affect fluorescence emission. This ratio showed a weak diurnal trend of higher values in the early morning, followed by decreases over the course of the day (Figure 4-8, second row). An exception to this trend was evident in the HOT174 deployment data, where this ratio was observed to increase in the late afternoon (arrow). Visually, the general trend in irradiance during that HOT174 deployment was qualitatively different from the other four days, and so very broad similarities between the diurnal trends in *FLH/PAR* may not necessarily be expected.

The high frequency component of this ratio was computed by high-pass filtering these *FLH/PAR* ratios with a 4^{th} order Butterworth filter having a cutoff

frequency of 60 min. This high frequency component exhibited different characteristics among the three cruises (Figure 4-8, third row). If these rapid changes in fluorescence emission were directly correlated with changes in irradiance, the peak-to-peak amplitudes of these high-pass filtered *FLH/PAR* ratios would be very small. In contrast, when changes in FLH lagged changes in PAR by an amount much longer than the 1 min sampling interval, this high degree of correlation would disappear and the peak-to-peak amplitudes of this high-pass filtered signal would be higher. Of the three cruises, HOT174 was anomalous in displaying an unusually low amplitudes in its high-pass filtered *FLH/PAR* ratio. When plotted in irradiance space, only two of the days (Oct. 9 and Nov. 11) exhibited the 'dogleg' trend in fluorescence yield that was observed by Laney et al. (2005) in laboratory cultures of *T. weissflogii* and in coastal surface phytoplankton assemblages in the Gulf of Alaska. The remaining three days exhibit a markedly different diurnal trend in irradiance: increases in *FLH/PAR* with increasing irradiance (Figure 4-8, bottom row).

The power spectra of incident solar irradiance during these three radiometer deployments shows roughly flat distributions on time scales less than ≈ 3 h (Figure 4-9). These spectra are similar to those presented in Chapter 2, except that those data were limited to sampling intervals of around 10 min, whereas these data here are resolved to 1 min and thus provide a better indication of the variability in irradiance on short time scales. The rise in power in these plots at slightly above 2 h reflects the slow diurnal change in irradiance. Over time scales less than 2 h, distributions are generally flat, indicating that irradiance perturbations were not significantly weaker at short time scales of seconds to minutes.

This relationship between FLH and irradiance was also examined in terms of the high-frequency response of FLH responds to changes in irradiance (Figure 4-10). The correlation between the high-frequency components of FLH and PAR (high-pass filtered above 30 min) were strongly correlated in all cases, except for the Nov. 15 deployment which may have fouled in a manner similar to what happened previously on Nov. 11. The other four deployments exhibited correlations around or above 0.89,

indicating that rapid changes in FLH were strongly associated with changes in PAR, as expected (Table 4-3).

The same data were examined in terms of sample-to-sample differences. Since these high-pass filtered FLH and PAR data were sampled with 1 min resolution, a time series of these differences effectively acts to differentiate. Thus, these correlations would reflect the relationship between changes in irradiance and changes in FLH. However, these correlations did not differ materially from those in Table 4-3.

Table 4-3. Degrees of correlation between changes in PAR and FLH on less than 30 minute timeframes.

Cruise	HOT174 (Oct. 11)	HOT175a (Nov. 11)	HOT175b (Nov. 15)	HOT176a (Dec. 12)	HOT176b (Dec. 13)
Mean *FLH/PAR*	0.354±0.003	0.363±0.007	1.048±0.028	0.535±0.004	0.584±0.006
r^2	0.89	0.89	0.27	0.96	0.93

4.5 Discussion

4.5.1 Rapid photosynthetic changes at Station ALOHA

Rapid changes in photosynthetic physiology are widespread among the phytoplankton, although the specific physiological bases of these changes differ across taxa. These changes may serve as a response to cope with rapid perturbations in irradiance, similar to changes in irradiance over longer, hours-plus time scales. Photoacclimation has received considerable attention in phytoplankton ecology (e.g., Cullen and Lewis 1988; Falkowski and Wirick 1981; Huisman et al. 1999; Marra 1978b; Schubert et al. 1995), primarily because these > 1 h responses are on the same scale as the physical processes that displace phytoplankton vertically through light gradients (Denman and Gargett 1983; Lewis et al. 1984b). In contrast, the more rapid

physiological changes that we refer to as regulation have been largely ignored in phytoplankton ecology.

Regulation in photosynthesis appears to have two distinctly different roles. The first role, as discussed above in the context of photoacclimation, is as a response to environmental perturbations in light. Since many of the specific modes of photoacclimation involve redistribution of energy between PSII and PSI, regulation has often been thought of as a means to acclimate to changes in the color distribution of irradiance, primarily. A second role of regulation is apparent in phytoplankton growing in gradually changing light environments. Here, changes in photosynthetic physiology represent rapid shifts in the energetic dynamics of light harvesting. These physiological changes are not responses to rapid irradiance perturbations *per se*, but instead reflect photosynthetic optimization near the transition between light-limited and light-saturated photosynthesis. These regular but singular transitions are central events in the photosynthetic history of phytoplankton. Phytoplankton in the surface ocean can be guaranteed to experience at least two such transitions a day, once in the morning and in the afternoon, due to the solar cycle alone. As well, additional transitions may occur as a result of cloud cover or vertical mixing processes.

Vertical displacement is often considered to be the environmental process that most affects the irradiance that phytoplankton experience. Especially at open-ocean sites like Station ALOHA, vertical processes in the ocean are heavily red-shifted in the spectral sense, having high amplitudes on the long time scales of hours-plus but relatively weak amplitudes on the short time scales of seconds-minutes (Mann and Lazier 1996). Therefore, if vertical displacement is assumed to be the dominant factor contributing to variability in the irradiance that phytoplankton experience, in the open ocean these microbes would only experience small fluctuations in irradiance at short time scales. Consequently, rapid photoacclimation would be expected to provide only marginal enhancements in photosynthesis, which from a metabolic perspective may well be negligible (Lewis et al. 1984b).

Recent analyses on long-term irradiance time series from several open ocean sites indicated that this historical focus on vertical displacement may be misplaced,

given the effect that intermittent cloud cover has on the marine light field.
At Station ALOHA, rapid perturbations in irradiance due to clouds were considerable, equivalent to what phytoplankton would experience when displaced vertically by ≈ 50 m by inertial processes (Karl et al. 2002; Letelier et al. 2004). Cloud-driven variability occurs not only on the short, minutes-scale time scales, but well into the hours-plus time scales of vertical displacement (Figure 4-9). This source of strong, short-term variability in irradiance is typically not considered in the open ocean, which is assumed to be quiescent in terms of irradiance variability, compared to the coastal ocean (sensu Strzepek and Harrison 2004). Photosynthetic responses to irradiance fluctuations on the seconds to hour scale may be more important in the open ocean than has been previously assumed.

Thus, if a major role of photosynthetic regulation is to accommodate such rapid changes in irradiance, we should expect photosynthetic properties of phytoplankton in these regions to exhibit considerable physiological variability on these same time scales. Given its relatively clear water and the substantial short-term variability in irradiance there, Station ALOHA is in some ways an ideal location for examining such relationships between irradiance perturbations and rapid photosynthetic responses. However, the results from this study indicate that in practice, identifying rapid photosynthetic responses in surface assemblages at Station ALOHA was not straightforward, at least with the methods we applied. Low phytoplankton biomass in surface seawater, along with the strong fluorescence quenching of these cells, made it difficult to resolve time series of PSII properties on time scales faster than 5 minutes. Although individual transient responses to specific perturbations were observed (Figure 4-4), these responses were only apparent occasionally. On neither HOT cruise was it evident that there were general trends in directional responses to rapid perturbations in irradiance.

There are at least three reasons why only weak rapid photosynthetic responses were observed in these FRRF data from Station ALOHA. First, it is possible that rapid responses occur, but they are just too small to measure given the signal to noise of the FRRF we used. In laboratory studies with the prokaryotes *Prochlorococcus* and

Synechococcus, photosynthetic responses to light transients were evident in several PSII properties (Chapter 3), but these responses were small compared to those exhibited by a neritic diatom. These two prokaryotes form a large fraction of the phytoplankton biomass in surface assemblages at Station ALOHA (Karl 1999), and if their rapid responses *in situ* are similar in magnitude to the weak responses exhibited by laboratory cultures, the actual response at Station ALOHA may be too small to measure.

Second, it is also possible that rapid responses occur at Station ALOHA but that they were faster than could be observed in the 5 minute averages that were computed for this study. These averages were necessary to minimize the measurement noise from this FRRF. It may be that the majority of the transient responses occurred faster than this 5 minute scale, and thus should not be apparent in these averages. Preliminary laboratory results with *Prochlorococcus* and *Synechococcus* indicated that although their fluorescence changes were weak, they were in fact rapid enough to be effectively complete within a 5 minute time scale (Chapter 3). If this is the case with the surface assemblages at Station ALOHA, it may be that a large fraction of the rapid response occurred during the transit of the surface sample through the ship's seawater supply. This lag was estimated to be around 1 minute, which would also reduce the apparent correlation with irradiance if viewed only on 5 minute averages.

Third, it is also possible that surface assemblages at Station ALOHA do not exhibit substantial rapid responses at all. Being in the top optical depths, these assemblages experience considerable exposure to supraoptimal irradiance intensities. Rapid responses would be expected to be strongest at irradiances near the transition from light-limited to light-saturated irradiance, at much lower irradiances. At this transition, phytoplankton have an excess physiological capacity for optimizing light harvesting using rapid photosynthetic responses. However, at higher irradiances, little can be done to optimize photosynthesis rapidly, and photosynthesis is effectively light-saturated continuously. Under these conditions, photosynthetic regulation may not be expected, and techniques like FRR fluorometry will indicate no rapid changes in PSII.

Several of the above issues are effectively eliminated with use of a radiometric drifter. With such a drifter, the phytoplankton assemblage is monitored under natural irradiance conditions. There is no lag time associated with the sample being dark adapted in a ship's flow through system. Furthermore, sampling can be performed very rapidly, down to the time scales of sub-seconds. This improves the signal to noise ratio considerably through averaging, and allows higher temporal resolution in sampling. However, a problem with natural fluorescence is that it is inherently a function of irradiance, and thus a high degree of correlation should be expected between changes in irradiance and in F_{nat} over any scale (Figure 4-6). In the high-pass filtered (< 30 min) data examined here, changes in F_{nat} due to biomass are presumably minimized, except for those resulting from advection. Advective changes should be small, however, as the surface drifter was largely following a uniform patch of water over these 1 or 2 day periods.

The natural and variable fluorescence signals examined in this study provide separate but complementary information about rapid photosynthetic responses. The single-turnover, variable fluorescence approach of the FRRF provides a means to identify specific changes in photosystem physiology that act as first-order controls on the ability of phytoplankton to absorb and harvest photon energy. It is a physiological assessment of a specific but important component of the overall light harvesting process. The natural fluorescence emission measured by the drifter represents the other end of the spectrum, a "continual turnover" fluorescence yield that reflects the response of the entire light harvesting system to continually modulated light. Changes in these two signals (natural and variable fluorescence) result from the same photosynthetic responses and share the same physiological bases. Currently, there is not a common framework for interpreting the patterns that are concurrently observed in these two signals, although such a framework would be valuable for understanding the overall role of rapid photosynthetic responses, in both natural and artificial light fields.

4.5.2 Improving the assessment of rapid photosynthetic responses in field studies in the open ocean

The Hawaii Ocean Time-series (HOT) at Station ALOHA off Hawaii, and the Bermuda Atlantic Time Series (BATS) near Station S off Bermuda, have greatly expanded our understanding of the seasonal, interannual, and decadal dynamics of phytoplankton assemblages in the surface ocean (Karl and Lukas 1996; Karl and Michaels 1996). Yet these time series also indicate considerable variability in photoautotrophic processes and variables on shorter time scales, both between consecutive months and when comparing seasonal patterns between years (Karl et al. 2003; Letelier et al. 1996; Letelier et al. 2004; Winn et al. 1995). The approximately monthly intervals between sampling in both the HOT and BATS programs make it impossible to determine if month to month changes that are observed in autotrophic biomass and production reflect the actual assemblage kinetics, or instead represent artifacts due to inadequate sampling of dynamics on shorter time scales.

The actual causes of the outcome of ecological processes on monthly, multigenerational scales is a central issue in phytoplankton ecology. The outcomes are the result of interactions within and among individual phytoplankters on shorter, generational time scales of one to several days. Thus, understanding the factors that lead to the month-to-month variability in phytoplankton assemblage structure at HOT and BATS requires understanding how individual phytoplankters perceive and respond to environmental fluctuations on far shorter time scales. The marine light field is a classic example of an non-equilibrium environment, where the availability of a key resource (in this case light) fluctuates considerably over the generational scale of these organism. A major challenge in ecology has been to develop a robust theoretical framework for predicting how resource fluctuations at these short scales affect higher-level ecological processes in populations and assemblages, such as competition and exclusion (Levins 1979). Although there have been considerable advances developing theory for utilization of material resources by phytoplankton (i.e., nutrients, Tilman 1977), a similarly robust theory has not yet been developed with respect to their

primary energetic resource (light) (Grover 1997; Tilman 1990). The major factor that has limited the development of such theory is the lack of observational data with which to evaluate predictive theoretical models.

A major factor that is limiting our understanding of the role of rapid photosynthetic responses in the ocean is our inability to observe these responses on the appropriate time scales, throughout the water column. Long-term observational programs such as HOT and BATS have the potential to provide such observational data, although the current instrumentation at both sites is not adequate for this. Even the most basic environmental measurement related to photosynthesis – irradiance – has been collected on appropriate temporal scales only in a handful of instances. At present, moorings such as MOSEAN at ALOHA and the Bermuda Testbed Mooring provide only simple measurements that do not necessarily assess the fundamental ecological variables of interest, such as phytoplankton biomass and assemblage structure. In theory, these variables can be estimated from certain optical properties of seawater, but in practice there has been little success in using such bio-optical methods robustly in this fashion to measure these "pool" variables. Additionally, such moorings and observational programs typically lack the type of sensors that would provide physiological indications of photosynthetic responses, i.e., "rate" variables, such as radiometers with channels in the natural fluorescence bandwidths, or active variable fluorometers.

There are several possible avenues for improving our ability to measure these fundamental ecological properties in phytoplankton assemblages, on the scales required to understand better the extent of rapid photosynthetic responses in the ocean. Many of these improvements can occur within the context of these existing long-term ocean observational programs. Ocean gliders have the potential to operate in a station-keeping mode around ocean moorings or during brief survey cruises. Commercially available gliders can carry a range of lightweight optical sensors that can help to identify changes in assemblage structure and photosynthetic properties. The strength of using these approaches isn't in the actual interpretation of these optical properties *per se*, but in the climatologies that can be developed from having such extensive

spatiotemporal data sets. The near-monthly sampling by ship at these ocean sites fits well with the use of such gliders to maintain such on-station sampling, as optical sensors can be cleaned regularly.

Within the context of the existing ship-based sampling programs like HOT or BATS, there are also several enhancements that can greatly improve our understanding of regulation and photoacclimation in the oligotrophic ocean. If the culture studies in Chapter 3 are representative of the very rapid response scales of *Prochlorococcus* and *Synechococcus*, e.g., on the order of single minutes, then shipboard, flow-through systems may not be appropriate for resolving the rapid photosynthetic response of these organisms. Integrating sensors into existing drifting arrays, as was done in this study, provided a more direct assessment of the apparent photosynthetic variability on short scales, as evidenced in the natural fluorescence signal. However, the inherent physiological changes in light harvesting that led to these responses cannot be directly measured using simple radiometry. Adding variable fluorescence sensors to such drifters would greatly improve our ability to examine these responses *in situ* and in real time.

One key finding from this study is the fundamental nature of cloud-cover around Station ALOHA, and how it is not necessarily representative of the larger region around the Hawaiian Islands. The satellite data examined here showed that Station ALOHA lies in a region of anomalously high orographic precipitation. Such an observation was made possible only through the polar-orbiting ocean color satellites that regularly monitor cloud cover in this ocean region. It is important to keep in mind that the cloud-driven variability observed at this ocean site should not be taken as representative of the immediate area, and naturally not of the open ocean in general. Such meso-scale and global distributions of cloud cover may have a role in determining the types of photosynthetic responses that local phytoplankton assemblages exhibit, and how cloud cover and vertical displacements interact. This topic may be of interest in future studies, but will require attention to areas other than just the current sites at which long-term observing programs have been established.

Acknowledgements We would like to thank Dave Karl the Hawaii Ocean Time-series team, for their help on HOT154, 172, 173, and 175. Lance Fujieki and Blake Watkins were especially important to the optical program and the drifter deployments. This research was supported in part by the National Aeronautics and Space Administration (NAS5-31360 to MRA, and NNG04HZ35C to RML and MRA), by the National Science Foundation support for the HOT program.

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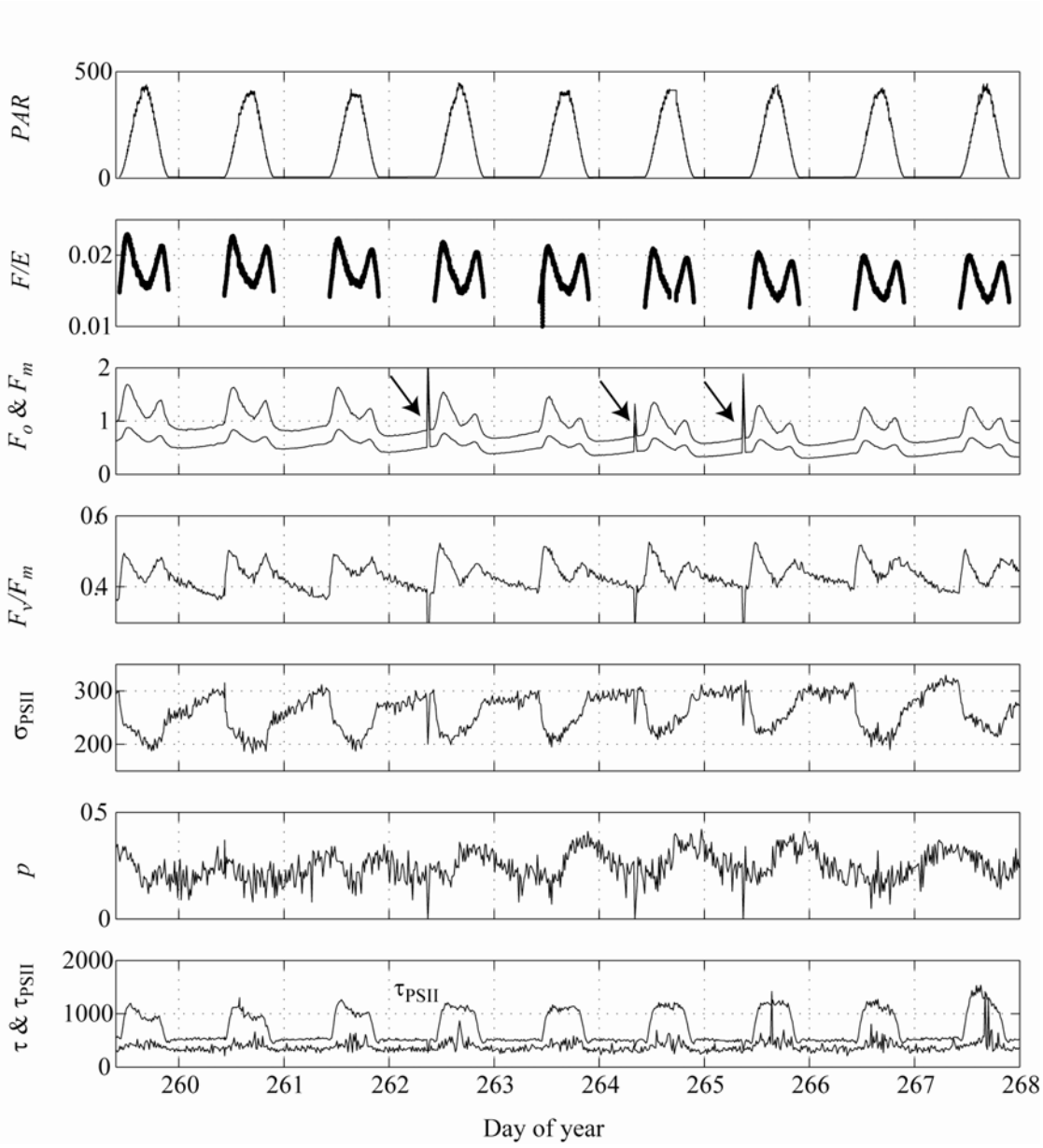

Figure 4-1. Rapid photosynthetic responses on sub-diurnal scales of *Thalassiosira weissflogii*, grown under regular light histories, of both F and FRRF. Panels, from top to bottom, show trends over the course of 9 diel periods in ambient PAR irradiance, F/E, F_o and F_m, F_v/F_m, σ_{PSII}, p, and τ and τ_{PSII}, where the former represents a more accurate relaxation time scale. Features indicated by arrows reflect sampling glitches, not actual changes in these and coincident parameters.

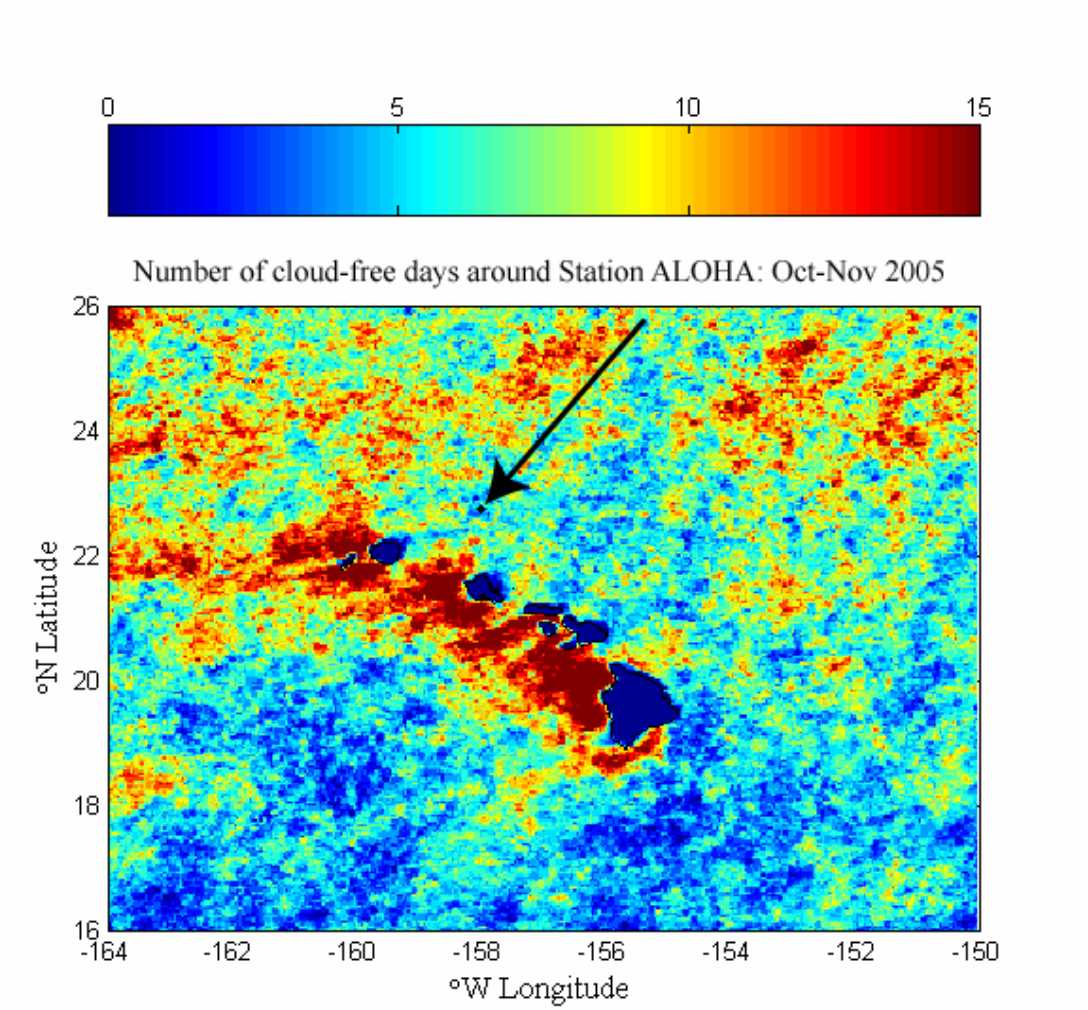

Figure 4-2. MODIS cloud-mask data for 61 days in October-November 2006, showing number of days in which each 4 km^2 pixel was cloud-free. The arrow indicates the location of Station ALOHA.

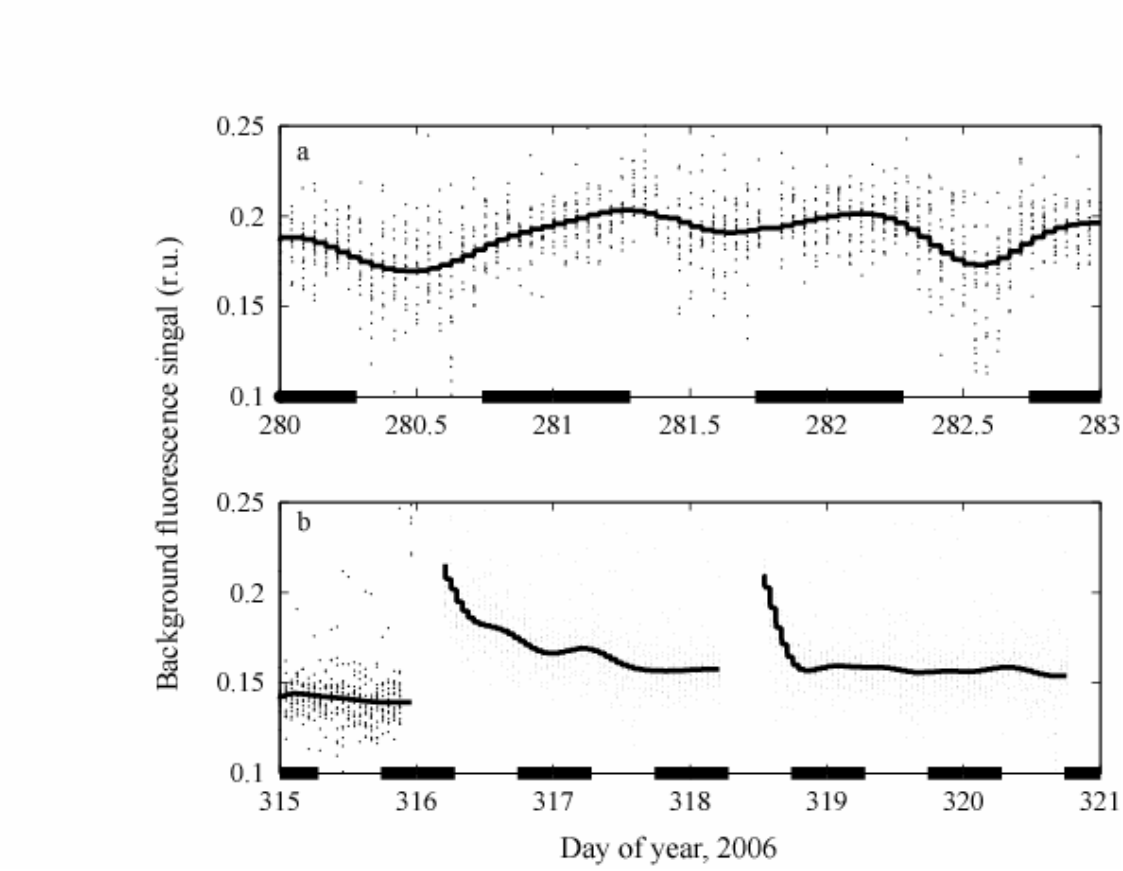

Figure 4-3. Changes over each cruise in the baseline background signal measured on 0.2μm filtered surface seawater.

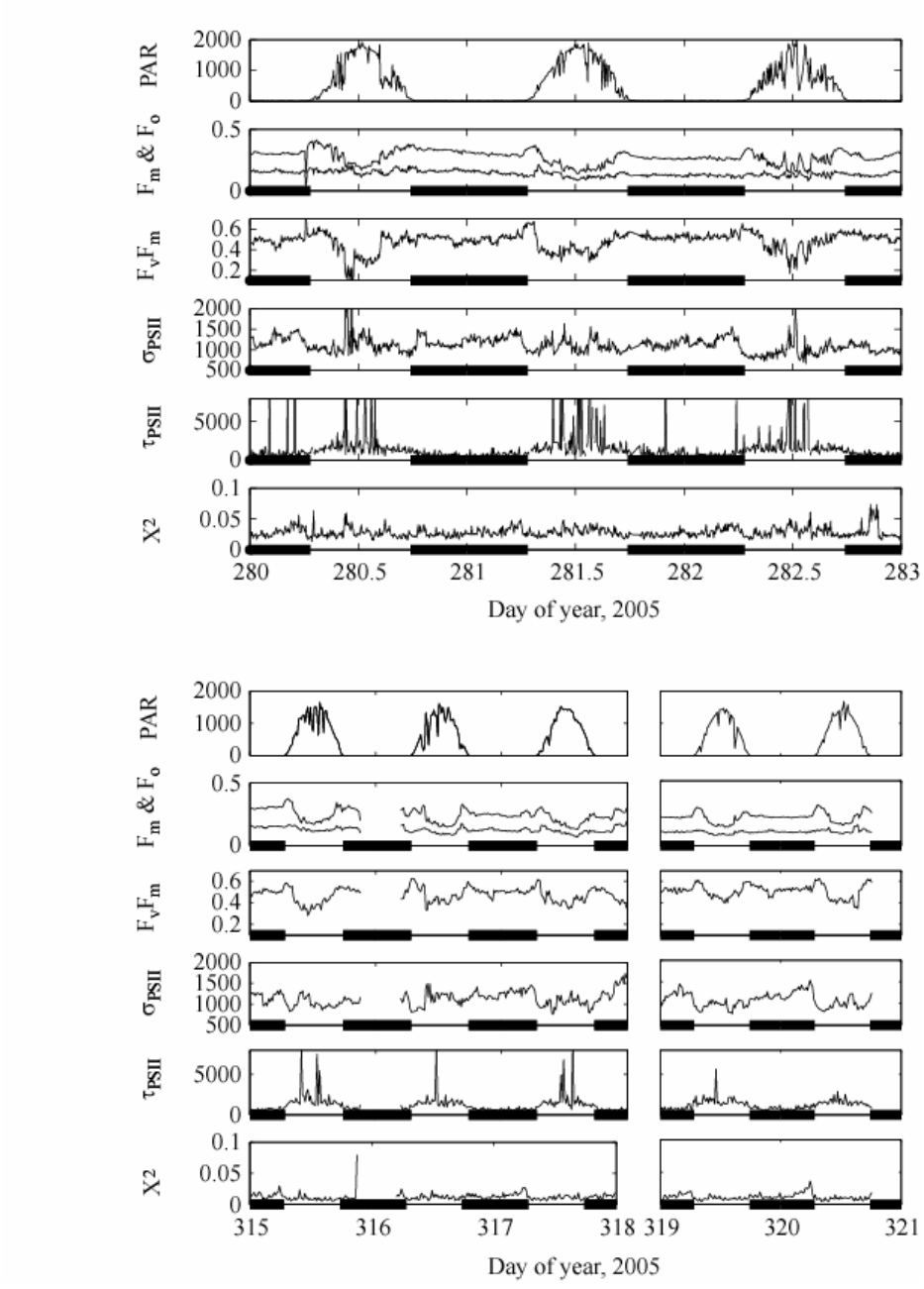

Figure 4-4. Time series of surface irradiance (top panel) and PSII parameters (remaining panels) in surface phytoplankton assemblages at Station ALOHA during HOT174 and HOT175: F_o and F_m, F_v/F_m, σ_{PSII}, and τ, as well as a quality of fit indicator.

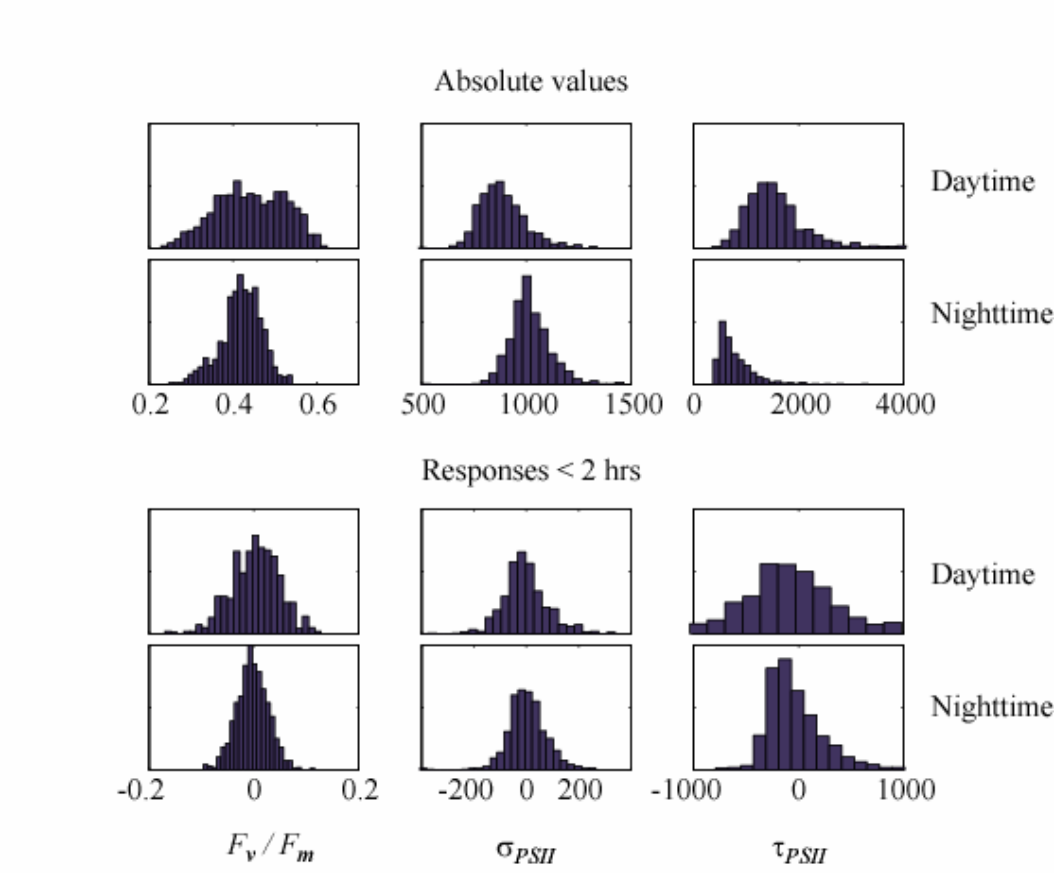

Figure 4-5. Histograms showing the distributions of F_v/F_m, σ_{PSII}, and τ as a function of daytime (top row) and nighttime (bottom row).

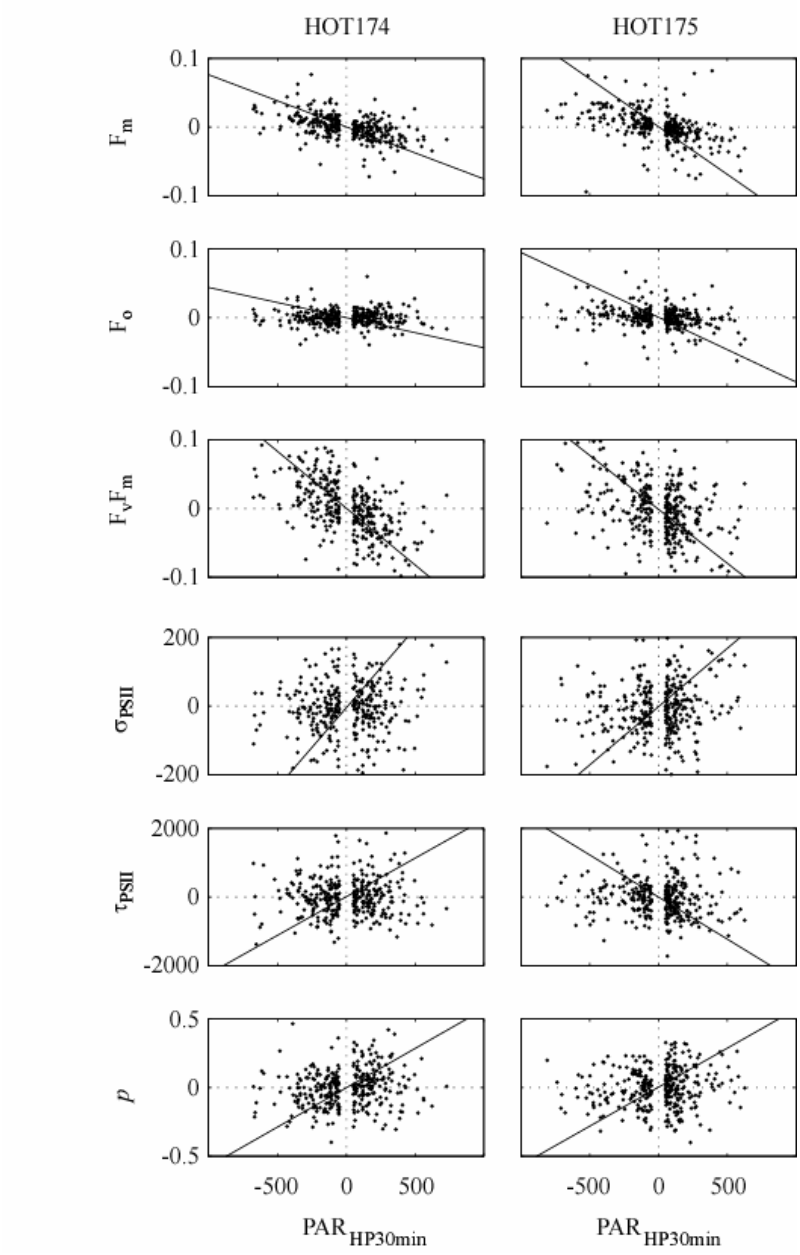

Figure 4-6. Correlations between the high-frequency components of PAR and F_o, F_m, F_v/F_m, σ_{PSII}, and τ (left column), as well as the sample-wise differences (bottom row).

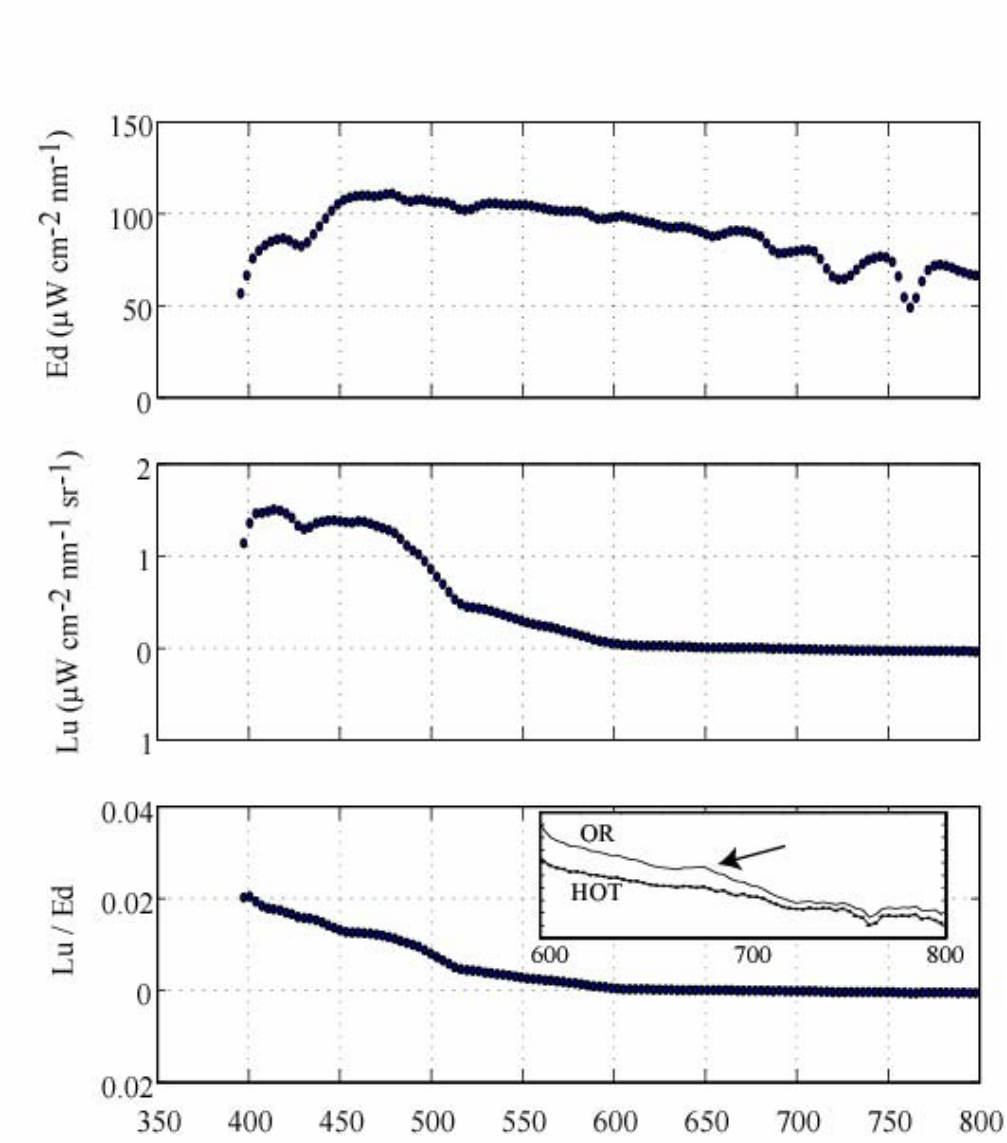

Figure 4-7. The average (a) incident spectral irradiance and (b) upwelling radiance at Station ALOHA for near-noon samples taken during HOT154 using a high-resolution radiometer. Their ratio (c) shows very weak natural fluorescence emission compared to that in the shorter wavelengths. Inset shows comparison with identical curves made in a coastal region off Oregon.

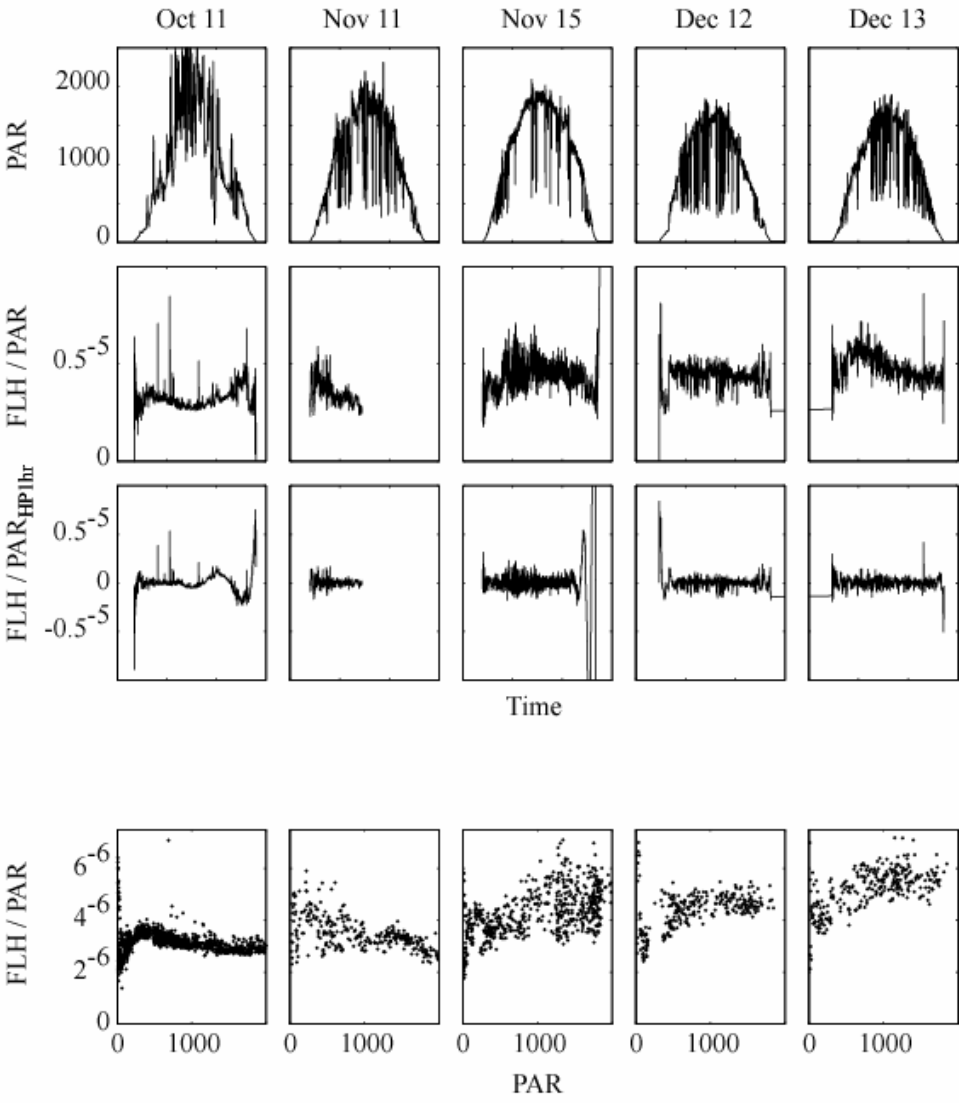

Figure 4-8. Radiometer time series from the HOT cruises 174-176, including (top row) PAR irradiance estimated from irradiance at 490 nm and (second row) the ratio of FLH to PAR irradiance, both as functions of time. Also, (third row) FLH as a function of PAR irradiance, and (bottom row) high-frequency fluctuations in FLH as a function of similar-scale perturbations in PAR.

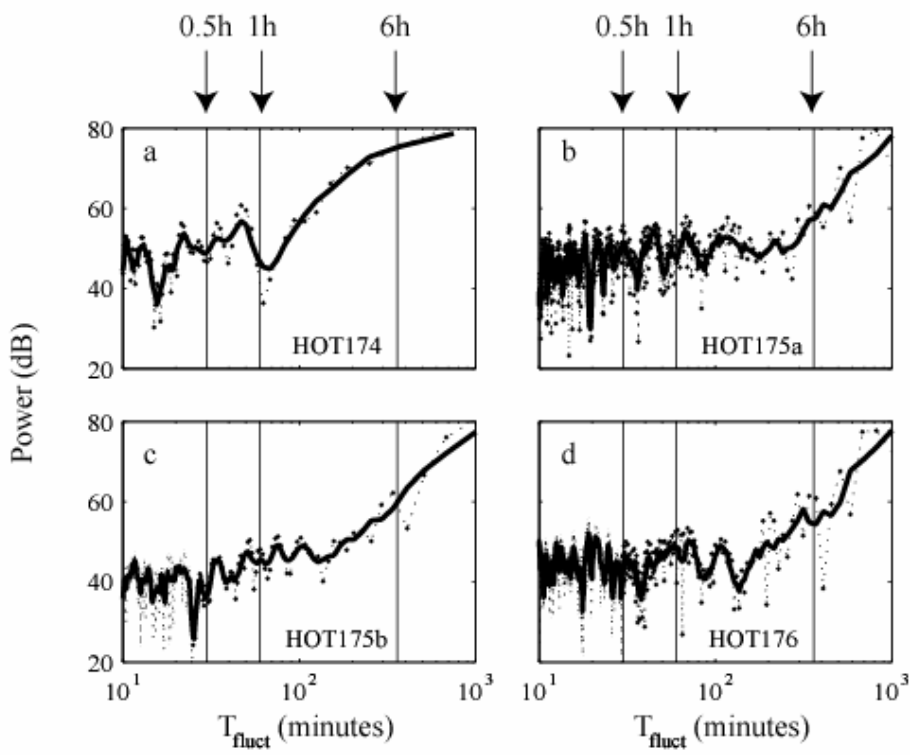

Figure 4-9. Power spectral of the 11-sec sampled radiometer data from different deployments during the HOT174-HOT176 cruises.

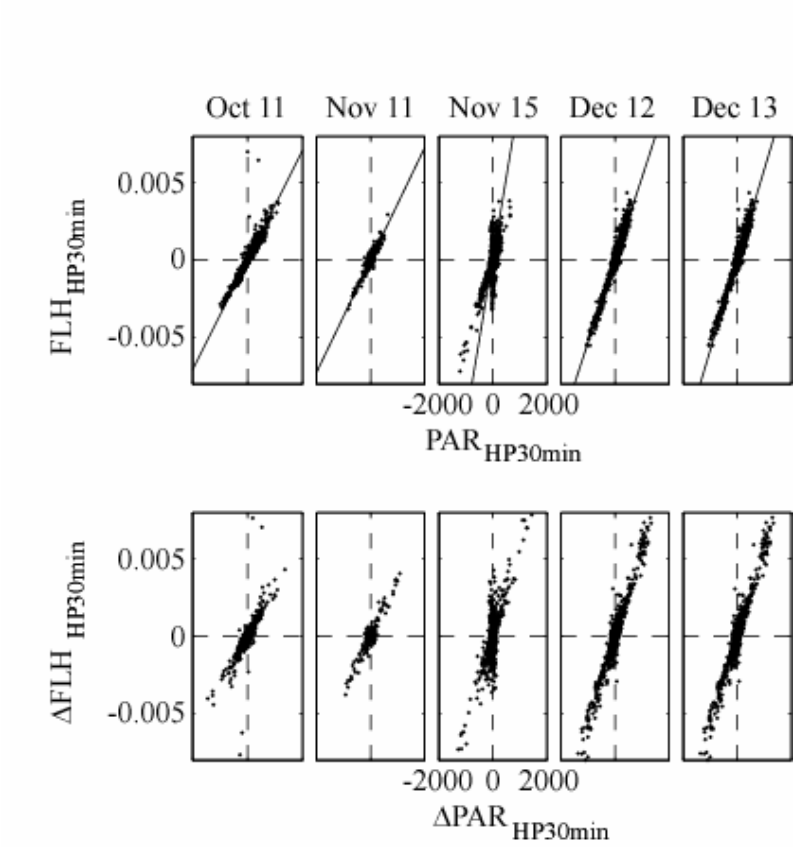

Figure 4-10. Correlations between the high-frequency components of FLH and PAR on time scales > 30 mins (top row), as well as the sample-wise differences (bottom row). Solid lines indicate best-fit linear regressions (geometric mean method).

5 Rapid photosynthetic responses in marine microalgae: summary, discussion, & future directions

5.1 Summary

The four chapters that form the main body of this dissertation each touch upon a necessarily narrow yet relevant aspect of rapid photosynthetic regulation in marine phytoplankton. Although certain modes of regulation have been recognized for over 50 years, we are only now beginning to develop a comprehensive understanding of their physiological bases, metabolic signals, and effect on light harvesting and utilization. There has been considerable speculation regarding their ecological importance to phytoplankton, but these hypotheses have been very difficult to confirm experimentally.

A few general conclusions about rapid photosynthetic responses can be drawn from this doctoral research. First, in these laboratory culture studies, *Thalassiosira* exhibited rapid responses to short, 5-min scale irradiance transients. A simple model of light harvesting indicated that these responses collectively enhanced the rate of primary photochemistry by around 30%. In this particular study the rapid transients in irradiance were intermittent but regular, occurring hourly. Thus the cumulative effect of these responses on photosynthesis and growth would be minimal for this particular light history. In a more realistic light field, however, with a greater degree of rapid irradiance perturbations, such rapid photosynthetic responses could in theory enhance growth and minimize photodamage considerably, even theoretically.

Second, when cultures of *Prochlorococcus* and *Synechococcus* were exposed to identical irradiance transients, rapid photosynthetic responses were also observed. In the prokaryote cultures that were examined, the details of these rapid responses were less clear: the inherently low fluorescence yield of these taxa made it difficult to measure these physiological changes in PSII accurately. Prokaryotes have not been well examined with respect to rapid photosynthetic responses. Being open-ocean species presumably adapted to quiescent conditions, canonical arguments would

suggest that these taxa should not require rapid photosynthetic responses to survive in their natural environment. Yet the responses observed in these prokaryotes were predicted to have an even larger effect on light harvesting than did the responses exhibited by the eukaryote *Thalassiosira*. It is possible that the physiological model used to make these predictions is not appropriate for these prokaryotes, and that the greater effect of these responses on light harvesting is misleading. These important marine photosynthetic prokaryotes appear to express substantial rapid responses to light perturbations. The extent to which these responses affect light harvesting, photosynthesis, and growth should be the focus of further research.

Third, analyses of long-term ocean irradiance time series suggest that the light environment in the open ocean is much more dynamic on the sub-hourly scale than has been previously demonstrated. Power spectra of surface irradiance are effectively flat down to the scale of minutes, indicating that intermittent cloud cover is a random process as expected. This finding makes it difficult to assert that the phytoplankton in the open ocean experience a generally quiescent light field on short time scales. I also observed a strong degree of spatial heterogeneity in irradiance, over spatial scales of 100 km, in cloudiness in the open ocean. Satellite data around the HOT site at Station ALOHA show that this region is not representative of the mesoscale with respect to insolation. Even at 100 nm north of Oahu, Station ALOHA lies in an anomalously cloud-covered region produced by orographic precipitation upwind from Kauai. Other broad features in cloudiness around Station ALOHA were observed but could not be attributed to orography.

Specific and persistent cloud features like the Inter-tropical Convergence Zone (ICTZ) have been well examined, as have other cloud features associated with land. However, in the context of the open ocean, very little has been done to examine how persistent intermittent cloudiness affects phytoplankton. Satellite imagery can provide the baseline data with which to make cloudiness climatologies of the open ocean, specifically designed for looking at the types of perturbations that would be expected to elicit photosynthetic responses in the phytoplankton. This type of analysis is feasible using existing datasets and sensors, and should be the focus of future research.

5.2 Discussion overview

As indicated by the findings of this study, much still is unknown about the physiological and ecological role of rapid photosynthetic responses in phytoplankton. Improving our understanding of these roles will require advances on several research fronts. The remainder of this summary chapter will expand specifically upon three general topics that are relevant to rapid photosynthetic responses in marine phytoplankton:

First, there are some widely held assumptions about the marine light field, the photosynthetic physiology of phytoplankton, and techniques used to examine both, which have important ramifications for how we examine rapid photosynthetic responses in the ocean. For example, a historical bias on vertical displacement processes in the ocean may have resulted in other important processes being ignored. In this dissertation the overlooked role of cloud cover has been examined in some detail, but there are still other processes that may introduce irradiance variability that affects phytoplankton assemblages. Similarly, variable fluorescence methods have been a staple of studies that examine the physiological bases of rapid responses to rapid changes in irradiance in the light reactions. Yet it is important to remember that fluorescence techniques provide only a limited view into the energetic dynamics of the light reactions, and that there are many important aspects of light reaction physiology that are not reflected directly as any change in fluorescence yield. An over-reliance on fluorescence methods, such as FRR fluorometry or similar techniques, is almost certain to miss some fundamental physiological responses in photosynthetic regulation that are only weakly associated with the few specific properties that these techniques measure. The first section of this conclusion will discuss these and other relevant trends in modern oceanography and phytoplankton ecology.

Second, the role of rapid photosynthetic responses is discussed in terms of how they contribute to changes in the structure and function of phytoplankton assemblages.

How environmental forcing on the scale of the individual shapes the structure and function of phytoplankton assemblages over many generations remains a very theoretical aspect of phytoplankton ecology. Rapid responses may be one of the fundamental factors that drive the trajectory of phytoplankton assemblages over many generations, along with predation and longer scale photoacclimation. However, such cross-scale linkages are very hard to identify or examine. Some general ideas will be discussed that might be considered for future investigations into this particular ecological role of rapid photosynthetic regulation in the ocean.

Third, issues related to the nonlinear nature of the photosynthetic light reactions are discussed in more detail. Throughout the course of this dissertation, the light reactions were described generally in terms of a nonlinear system. It was suggested that greater use could be made in plankton ecology of nonlinear systems analysis tools that have been developed in other fields of physical science, such as engineering. These were not well discussed in the main body of this dissertation. Some advances in nonlinear systems analysis will be discussed, which may provide new tools for understanding how different rapid responses work together in the light reactions to optimize photosynthesis under varying irradiance conditions. Other fields of ecology have begun to take advantage of specific techniques developed in fields such as signal processing and electrical engineering, for examining specific nonlinear interactions that occur in nonlinear systems. These same techniques may potentially find use for examining nonlinear interactions in light harvesting by marine phytoplankton.

5.3 Issues related to rapid photosynthetic responses in phytoplankton assemblages

5.3.1 What scales and sources of irradiance variability in the open ocean are important to consider?

> "To understand an organism you should learn to put yourself in its place, and in that context what it does usually makes perfect sense." (Roughgarden 1998).

It still is unclear if all of the important sources and scales of irradiance variability in the euphotic zone, that are relevant to phytoplankton photosynthesis, have been identified. Modern ocean observing programs collect a tremendous amount of data each day, yet even with irradiance – an especially easy parameter to measure – we continue to remain data-limited. There are still spatial and temporal scales of irradiance that we do not and cannot currently observe in the ocean. The scales that remain particularly intractable are those relevant to microbes, e.g., cm and below, and days and below. How phytoplankton respond to fluctuations in irradiance on these unobserved scales is potentially important to their growth and survival in the ocean. Understanding what occurs on those scales, and how those interactions affect phytoplankton assemblages, is an essential part of understanding the actual photosynthetic ecology of these key members of marine ecosystems.

Since it is very difficult for us to measure the environment adequately at the small or short microbial scale, we make assumptions regarding which scales of environmental perturbation affect phytoplankton the most. A claim made early in this dissertation was that there may easily be biases in modern phytoplankton ecology, resulting from an inability to observe phytoplankton dynamics and responses on temporal scales of less than a single generational period. This temporal bias is analogous to a spatial bias that Harris suggests arises from not appreciating phytoplankton dynamics on the appropriate microbial scale (Harris 1980). Knowing the irradiance variability that an individual phytoplankter experiences is the first step in understanding the ecological role of rapid photosynthetic regulation in the ocean. Roughgarden's suggestion (above) may appear anthropomorphic, but in fact expresses the only approach we have at present for forming hypotheses that indicate how phytoplankton respond to irradiance variability on the very short or small, unmeasurable scales.

There is a longstanding history of experimental apparatus that do not take this approach, and instead attempt to reproduce "realistic" irradiance histories in a laboratory or shipboard setting (e.g., Babin et al. 1994; Bruyant et al. 2001; Gallegos et al. 1980; Gocke and Lenz 2004; Huisman et al. 2002; Nicklisch 1998). At some level, all such experiments must be viewed as futile or overly simplistic, because they necessarily include an *a priori* judgment as to which scales of irradiance variability are the most ecologically relevant. Presupposing a particular scale of irradiance variability risks overlooking other, perhaps more relevant, scales of variability. The majority of the studies listed above started with a particular physical aspect of the water column (e.g., surface wave focusing, vertical displacement by tidal mixing, etc.) and extended this to a "realistic" environmental irradiance. The influence of atmospheric effects like cloud cover has been largely ignored, in general and particularly in the "realistic" systems listed above. Such studies tend to identify coherent or periodic structures in the environment, and many laboratory studies have employed very regular perturbations such as sinusoids, to mimic particular regular features such as wind-driven mixing (Flameling and Kromkamp 1997; Havelková-Doušová et al. 2004; Marra 1978a).

I used a slightly different approach in the culture experiments of Chapter 3: discrete step transients instead of such regular, smoothly varying light perturbations. The light history that phytoplankton experience in the ocean is probably better characterized as stochastic and unpredictable, due to cloud cover and turbulent mixing, than as regular and predictable, which would be thought to result from widespread coherent features like idealized Langmuir circulation. A major step forward in understanding the perturbation scales of irradiance that are important to phytoplankton assemblages will be to develop a means to characterize the stochastic nature of irradiance variability. In such a highly dynamic light field, a phytoplankter with a generational scale of a day or so essentially gambles that their current photosynthetic state will be adequately flexible enough to survive an unknown and highly variable future, in terms of light availability and excess. In this sense, a marine light field may be better characterized in terms of probabilities that irradiance will change

dramatically over different time scales in the future, instead of using the Eulerian or Lagrangian approaches that are commonly employed. There may be an application in this aspect of phytoplankton ecology of adapting principles from game theory or stochastic optimization to describe how phytoplankton may cope with such highly variable resource availability.

5.3.2 Getting a better look at cloud cover over the open ocean

Intermittent cloud cover is a highly random process in the environment, which has a material effect on phytoplankton photosynthesis and growth. A claim made in this dissertation is that intermittent cloud cover is one of the central sources of variability in the irradiance that phytoplankton experience, and that it has been largely overlooked in phytoplankton ecology. At Station ALOHA, intermittent clouds appear to be the dominant source of variability in irradiance, certainly on the short time scales of photosynthetic regulation, but also on the longer hours-plus scales of photoacclimation. Currently there are very few ocean observing stations that monitor insolation over the long time scales, with an adequately fast sampling resolution, to characterize the degree of intermittent clouds over specific ocean sites. To some degree, as oceanographers we have become used to seeing remote sensing images of the planet, from which all cloud cover has been removed (Figure 5-1). This step is necessary in order to visualize features of the ocean's surface, such as temperature and ocean color. An unintended byproduct of continually seeing such well-corrected ocean color images is that fundamental environmental processes like intermittent cloud cover can be forgotten (Figure 5-2). It is clear from such images that common notions of ours as a "blue planet" are not quite correct. Earth is largely covered by water, but the relative influence of each of its three phases differs considerably from place to place. Earth is more of a white planet, with clouds acting as severe filters on the intensity of incident light that enters the surface ocean. There is a need to assimilate better the role of atmospheric variability into our research into rapid photosynthetic responses.

A comprehensive, synoptic assessment of the role of cloud cover on the marine light field has yet to be made. Future research should focus on improving our collection of high-resolution time series of irradiance in more regions of the open ocean. Cloud cover over the ocean can be examined using atmospheric data from meteorological satellites. Sensing of clouds against the terrestrial regions or ice cover is challenging, as color and temperature alone are not robust discriminators of cloud presence or optical density. Over an ocean background, however, many of these problems are less important. Thus, for oceanography some relatively simple remote sensing analyses could be employed to generate a synoptic map of intermittent cloud cover over the ocean. Quality-control data from visible radiometers, e.g., SeaWiFS and MODIS, could be used to correlate poor pixel quality with crude degrees of cloud cover. These climatologies can be compared with derived data products for surface irradiance, such as the IPAR product from MODIS.

Although these polar-orbiting sensors provide only one scale of variability, e.g., the day-to-day, this scale of irradiance variability is itself largely under-examined and worth surveying. It is essentially identical to the generational scale of most phytoplankton. Geosynchronous weather satellites can provide higher temporal resolution, over the scale of many hours, over very broad spatial scales. These data are generally intended for forecasting, not research, and therefore are not typically archived. However, these sensors may provide additional data to examine intermittent clouds on large spatial scales, with some degree of diurnal resolution.

National ocean buoy programs do not typically measure insolation as a meteorological parameter, although deep-water research moorings such as at BATS and HOT do. Since there has been little interest to date in short, minutes-scale variability in irradiance in modern phytoplankton ecology, these research buoys typically sample irradiance only infrequently during the day, with ten to tens of minutes resolution. Irradiance data from surface drifters are often sampled at similar scales. This dissertation research indicates that irradiance fluctuations on these scales may be important to phytoplankton assemblages, but there is inadequate evidence to insist upon greater sampling resolution in irradiance in large ocean observing systems.

However, as additional studies in the future better examine the role of rapid photosynthetic responses, and as better conceptual frameworks are developed to explain how these responses affect photosynthesis and growth, relatively simple modifications of existing ocean observational systems could provide this irradiance data. Faster sampling is often performed by ocean gliders and autonomous profilers, and these platforms should be used more heavily in future studies.

5.3.3 Issues with examining photosynthetic responses with variable fluorescence techniques like FRR fluorometry

> "To paraphrase an old saying: Beware of the man of one method or one instrument, either experimental or theoretical. He tends to become method-oriented rather than problem-oriented. The method-oriented man is shackled; the problem-oriented man is at least reaching freely toward that which is most important". John R. Platt, 1964, *Science* 146.

Platt's advice in 1964 is especially pertinent to this dissertation and to any discussion about rapid photosynthetic responses. Variable chlorophyll fluorescence techniques, especially fast repetition rate fluorometry, were relied on heavily in this research to identify specific physiological changes in photosynthetic properties on short time scales. Physiological models used to interpret these responses from FRRF fluorescence transients form the conceptual basis of the stochastic model described in Chapter 2. Variable fluorescence techniques are considered useful for examining short-term changes in phytoplankton photosynthetic properties, because these techniques estimate aspects of the immediate photosynthetic "state" of the organism.

It is difficult to measure directly how rapid photosynthetic responses affect light harvesting and photochemistry. Instead, their effect on these processes of interest have been examined primarily through the three waste products that photosynthesis generates: oxygen, heat, and fluorescence. Because rapid changes in oxygen evolution and photoacoustic signal are too low to measure accurately in the natural environment,

fluorescence techniques by default have become the approach most used for examining photosynthetic physiological responses *in situ*, in natural phytoplankton assemblages.

However, there are several weaknesses associated with this particular technique, the conceptual models it is based on, and the instrumentation that exists for making these types of variable fluorescence measurements. There is a potential that these weaknesses affect some of the conclusions arrived at in this dissertation. There is also the potential that ignoring or dismissing these weaknesses may result in a misleading or biased view of the role of rapid regulatory responses in algal photosynthesis. Some of these weaknesses involve the manner in which the technique has been applied, and others involve fundamental aspects of variable fluorescence that complicate the types of physiological inferences that are drawn from its use.

Taxonomic differences in variable fluorescence kinetics – There has been considerable work over the last decade to understand how variable fluorescence in photosynthetic eukaryotes differs from that in cyanobacteria, with their fundamentally different light-harvesting architectures (Campbell et al. 1998). Such studies have identified several important methodological caveats for the use of traditional variable fluorescence techniques with these prokaryotes. Cyanobacteria do not use chlorophyll as their major light harvesting pigment, and what little chlorophyll cyanobacteria have is allocated to the reaction center complexes within the thylakoid membrane. Thus, chlorophyll in cyanobacteria is shaded by the phycobilisome structures set on the membrane surface. The effect of this architecture is that any fluorescence emanating from a closed cyanobacterial reaction centers is less likely to leave the cell and more likely to be absorbed by the phycobilisome.

Field studies have identified some of these caveats when using particular variable fluorescence instruments like FRR fluorometers in assemblages that are heavily weighted toward cyanobacteria (Raateoja et al. 2004; Suggett et al. 2001). The claim sometimes made that techniques like FRR fluorometry do not “work” with cyanobacteria is somewhat misleading, as fluorescence kinetic measurements are only

as robust as the conceptual framework used to interpret them. To date, the conceptual models for interpreting variable fluorescence transients measured with FRR fluorometry have been derived almost wholly from eukaryote models, e.g., *Dunaliella*, *Thalassiosira*, and *Chlorella*. Cyanobacteria, with their fundamentally different light harvesting apparatus, may exhibit fluorescence transients that are subtly different than those of eukaryotes. A more detailed examination of how these taxonomic differences are manifested in variable fluorescence transients would enhance the use of these techniques in a broader range of phytoplankton assemblages. It would also provide the physiological information needed to improve the physiological models of light harvesting and photochemistry like the stochastic one described in Chapter 2.

Concurrent changes in absorption – Another major problem with the use of variable fluorescence techniques in marine phytoplankton ecology is that concurrent changes in phytoplankton absorption are not typically measured and corrected for. Such variability can lead to considerable artifacts in time series of the physiological properties measured with techniques like FRR fluorometry. Since such techniques only measure the apparent fluorescence yield, changes in the absorption properties of phytoplankton will be directly translated into the photosynthetic parameters that are derived from measured variable fluorescence transients. The extent to which this sort of artifact affects the findings of this dissertation is not clear.

An obvious solution to this problem is to couple absorption measurements with fluorescence responses at the same time scales. In this study, we observed rapid, minutes-scale changes in the functional cross section of PSII in response to transients, in laboratory studies (Chapter 3). These transient responses changes over the course of the day, and may or may not be to diurnal changes in pigment complement. Instrumentation for measuring these variables exists, and these need to be integrated.

The effect of PSI is largely hidden in variable fluorescence signals – Although variable fluorescence techniques have long been recognized as being sensitive and

valuable for examining photosynthetic physiology, it is important to recognize that variable fluorescence provides only a limited picture of the overall dynamics of light harvesting and photochemistry, in the integrated photosystem. Virtually all of our insight into the energetics of photosystems and light harvesting arise from fluorescence into Photosystem II. This is because it is relatively easy to measure changes in two particular waste products of photochemical activity in PSII: oxygen and fluorescence. Most of the fluorescence emitted by phytoplankton at *in vivo* temperatures comes from PSII, and so the dynamics of fluorescence transients primarily reflect processes in and around this photosystem. Consequently, this technique is essentially blind to the photosynthetic activity of PSI.

Understanding how activity of PSI appears in measurements of variable fluorescence is an important part of being able to use these techniques robustly. If the light reactions were perfectly serialized and coupled, as suggested by the so-called "Z-scheme", understanding the kinetics of the collective photosystem would not require measuring the activity of PSI. However, the notion of a purely linear Z-scheme is outdated, and several cyclic and dissipative pathways have been identified since the linear Z-scheme was first developed (Falkowski et al. 1986a). Since one of the main effects of rapid photosynthetic regulation is to decouple activity of PSI from that of PSII, understanding the overall, integrated PSII-PSI systematic behavior of the light reactions is fundamental to understanding the role of regulation.

5.3.4 Rapid photosynthetic responses as apparent in the yield of natural fluorescence

As discussed earlier, measuring rapid photosynthetic responses in microalgae is difficult, even under laboratory conditions. These responses by definition occur over short periods of time, and they have generally subtle effects on photosynthetic light harvesting and utilization. Complicating factors are easily introduced by measurement artifacts. It is very difficult to measure these responses without in some way disturbing

or perturbing the organism under study. The process of measuring these rapid responses inherently elicits additional responses and changes the photosynthetic state of interest.

Variable fluorescence techniques like FRR fluorometry provide information about the photosynthetic physiology of PSII by interpreting a response to a fixed, discrete impulse of light. The deliberate manipulation of the energetics of PSII is at the core of this technique. In theory, with a more complete physiological model of variable fluorescence, it should be possible to go beyond the "single-turnover" scale of these discrete impulses of light, and assess photosynthetic physiology from the same organisms while they continuously turnover photons on much longer scales of seconds to minutes. These "continuous-turnover" fluorescence responses are manifested in nature as the natural fluorescence emission of phytoplankton.

There are several reasons to expect variability in the natural fluorescence signal to be a good indicator of rapid photosynthetic responses in phytoplankton assemblages. In Chapter 4, two seemingly different roles for these rapid responses were identified: as directional responses to rapid and immediate transients, and as sudden changes in response to slowly approached physiological thresholds. Prior research with *Thalassiosira* that was presented in this study (Figure 4-1) indicate that the latter type of rapid photosynthetic response could be readily observed in the natural fluorescence signature of these diatoms. Similar behavior was observed in field observations at Station ALOHA (Figure 4-8). What wasn't apparent, however, were the directional responses in natural fluorescence yield to the considerable degree of rapid irradiance fluctuation that occurred at this site. It appears as if the fundamental, regulatory aspects of these rapid changes in physiology occur, and the directional, acclimative behavior is absent.

A basic problem with identifying physiologically driven changes in natural fluorescence yield due to changes in irradiance is that the former is such a strong function of the latter. Especially in the open, surface ocean, fluorescence emission signals are very low inherently, due to low biomass, extensive nonphotochemical quenching, and the fundamental photosynthetic architecture of cyanobacteria. In

oligotrophic regions, most of the signal measured in the natural fluorescence band is not due to phytoplankton emission, but instead is a background reflectance. Simple radiometer detectors are not well suited for separating the natural fluorescence signal from the background, and spectrometer-based systems typically lack the sensitivity required to measure changes in this emission on the rapid scales of interest. Thus, identifying the relatively small deviations in natural fluorescence yield, in the oligotrophic ocean *in situ* remains somewhat of a challenge, despite the apparent ease of this simple radiometric type of measurement. However, addressing these problems is simply an engineering issue, and the technology exists to do so.

The more fundamental limitation with using natural fluorescence to examine photosynthetic responses in phytoplankton, rapid or otherwise, is that we lack a causal model to interpret changes in fluorescence yield in terms of specific physiological properties of the photosynthetic light reactions. With single-turnover methods, the task is simplified in some ways; the short, millisecond time scale of these measurements obviates the need to account for several important but poorly understood processes that are known to affect fluorescence yield over longer time scales.

5.4 Ultimately, cross-scale linkages: will we ever be able to tell if rapid regulation affects phytoplankton assemblages?

> "Well, there are two kinds of biologists, those who are looking to see if there is one thing that can be understood and those who keep saying it is very complicated and that nothing can be understood… You must study the simplest system you think has the properties you are interested in." Cy Levinthal, 1958 Conference of Biophysics, Boulder CO.

The claim made throughout this dissertation is that rapid photosynthetic regulation play an important role in phytoplankton, not only as a means to "fine-tune" of the light reactions, but as a central part of light harvesting in both highly variable and relatively stable light environments. All phytoplankton that have been examined

to date exhibit rapid changes in photosynthetic physiology on the seconds to minutes scale, following sudden changes in irradiance. Such a widespread distribution of different rapid responses across taxa is some of the strongest evidence that these rapid responses are essential to the photosynthetic ecology of phytoplankton.

These rapid physiological changes are presumed to reflect directional responses to the intermittent yet substantial irradiance perturbations that are introduced in the natural environment by clouds, internal wave packets, and other factors. A potential role for these rapid photosynthetic responses to participate in controlling the trajectories that marine phytoplankton assemblages take over multigenerational scales has been discussed before by several workers (e.g., Abbott et al. 1982; Marra and Heinemann 1982). There are several good theoretical reasons to expect a causal linkage from the physiological to the ecological scale. In other words, a differential ability to respond rapidly to light perturbations in an assemblage should affect higher order processes like nutrient uptake and competitive exclusion. However, the experimental evidence needed to prove this claim has not been collected either in the laboratory or in natural assemblages. Several factors make it very difficult to demonstrate these linkages within actual phytoplankton assemblages:

- An inability to measure rapid photosynthetic responses *in situ*
- Lack of knowledge of how these affect photosynthesis and growth
- Poor understanding of the actual variability in the marine light field
- Difficulty in parameterizing interspecific differences in ecological models
- Conceptual difficulty with describing light as a resource

It has been argued qualitatively that such linkages must exist, but just as with many useful concepts in ecology, such as niches (Pielou 1972), it has been difficult to measure these linkages directly. Variable fluorescence techniques provide a means to measure the time scales of specific changes to the photophysiology of PSII. However, it has been very difficult to associate these responses with changes in photosynthetic

rate or growth, let alone even higher order processes such as interspecific competition or exclusion.

Photosynthetic responses to fluctuations in irradiance are of interest to marine phytoplankton ecologists because such stochastic fluctuations in light provide a classic example of non-equilibrium resource availability. The ocean is quite nearly a perfect example of environmental disequilibrium. Species-specific variations in the temporal aspect of resource utilization is one of the simplest explanations for coexistence of disparate taxa under nonequilibrium conditions (Levins 1979). A major challenge in ecology has been to develop a robust theoretical framework for predicting how fluctuations in resources affect higher-level ecological processes in populations and assemblages, such as competition and exclusion (Levins 1979). The ecological responses of populations to changes in irradiance are intrinsically linked to the physiological responses of the individuals within those populations. Although there have been considerable advances in developing theory for utilization of material resources by phytoplankton (i.e., nutrients, Tilman 1977), a similarly robust theory has not yet been developed with respect to their primary energetic resource (light) (Grover 1997; Tilman 1990). Current competitive exclusion theory has been developed using arguments of environmental equilibrium or quasi-equilibrium, but these arguments are not in general appropriate to the marine light environment (Harris 1986).

The light reactions in photosynthesis are ultimately an uptake mechanism for an essential resource. Rapid photosynthetic regulation involves manipulating dynamic aspects of light harvesting and utilization, presumably to optimize the uptake of these photons. However, as applied to phytoplankton, resource utilization theory has been primarily concerned with how these organisms uptake solutes, i.e., nutrients (Grover 1997). It is clear that light and nutrients differ considerably as resources in a practical sense, in their availability to phytoplankton, and in the effects that they have on phytoplankton growth. For example, it is rare for a phytoplankter in the ocean to experience natural concentrations of a macronutrient resource high enough to be effectively toxic. With respect to light, however, the toxic effect of a superabundance of light is encountered daily in most if not all phytoplankton assemblages in the ocean.

These types of differences between the energetic and material resources of phytoplankton are central to any discussion of cross-scale linkages. Differences in resource utilization rates and efficiencies are a major mechanism for exclusion or preference of species in an assemblage, through competition. Recasting material resource theory in terms of irradiance is not straightforward as replacing terms for nutrients with terms for irradiance. Fundamental processes in nutrient resource limitation do not necessarily have meaningful analogs in terms of irradiance. For example, despite the fact that photons are as equally important as a resource to the nutrients that phytoplankton require for growth, resource theory is considerably more developed for nutrients than for light. One reason for this is that nutrients can become measurably depleted by phytoplankton growth and activity in the ocean. If depletion of a particular resource can be readily measured over time, development of a resource utilization is simplified, as is its experimental validation. The effect of the availability of a particular nutrient on nutrient growth can be examined directly using simple theoretical models and rather modest experimental designs, such as chemostats (Kubitschek 1970).

Ultimately, higher-order ecological processes like competition and exclusion on multigenerational scales result from the actions of individual members of populations, on the sub-generational scale. Thus, in order to develop a robust theory in ecology that describes how phytoplankton utilize energy from the ambient light field in terms of a resource, we must better understand two things. First, it is important to understand the nature of the underwater light field and how it varies on the sub-generational scale. Second, it is necessary to examine what physiological responses phytoplankton have evolved to cope with the characteristic variability in irradiance in the ocean. Since historically the focus has been on time scales of vertical displacement processes, i.e., hours and above, there is good reason to examine both the natural scales of variability in irradiance, and the physiological responses to irradiance on these scales.

In Chapter 3, clear differences were observed in the rapid responses of the *T. weissflogii* culture, and two important marine prokaryotes, *Prochlorococcus* and

Synechococcus. That both of the prokaryotes in this study exhibited rapid photosynthetic responses on the seconds to minutes scale may be a surprising observation. There sometimes is a presumption that such simple microbes, with very small genomes, should not display such sophisticated photosynthetic dynamics. Prokaryotes in the phytoplankton have been considered as having life strategies that do not require the ability to respond rapidly to environmental perturbations. Yet specific, rapid physiological responses in PSII are clear in both *Prochlorococcus* and *Synechococcus* (Figure 3-10), and the predicted effect of these combined rapid responses affected light harvesting considerably stronger in both *Prochlorococcus* and *Synechococcus* than in this *Thalassiosira* species. This evidence is very preliminary and warrants more detailed examination in further studies.

One potential test of how rapid photosynthetic responses affect assemblage structure might be performed by repeating an experiment of Turpin and Harrison, in which different mixed assemblages received the same total daily dose of nutrients, but at different periodicities (Turpin and Harrison 1979). There has been some recent work to repeat these experiments using irradiance fluctuations at relatively long, photoacclimation time scales (Litchman 2003). However, there have not yet been experimental tests using irradiance fluctuations at the rapid temporal scales that could only be accommodated by photosynthetic regulation. Such experiments may show different assemblage structures over multigenerational scales, as a function of irradiance variability. Interpreting these results will still be challenging without the type of detailed physiological assessment that has been performed in this study, to assess which particular aspects of light harvesting vary when irradiance conditions are perturbed.

5.5 Some benefits of thinking of light harvesting by phytoplankton in terms of a nonlinear system

> "When you can measure what you are speaking about and express it in numbers, you know something about it; but when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind". Lord Kelvin, 1883.

Photosynthesis in general, and light harvesting in particular, are nonlinear processes with respect to irradiance. At the simplest level, this means that there are situations where changes in irradiance are met with changes in photosynthesis, and there are also changes in irradiance that are not. On a more sophisticated level, this nonlinearity means that it is hard to predict where and when changes in irradiance decouple from changes in photosynthesis. Our canonical view of a static P-E curve works reasonably well over long time scales of many hours, or under very static environmental conditions. There, simple parameterizations of this nonlinearity such as α and P_{max} work reasonably well. However, over time scales of a single generation or when environmental conditions vary considerably, these simple parameterizations work only very poorly. Static models omit very basic aspects of nonlinear systems such as feedback, thresholds, and hysteresis.

It is easy to assume that because phytoplankton are small and unicellular, that their photosynthetic responses to environmental stimuli are also simple. Quite possibly the opposite is true. The highly integrated nature of unicellular organisms favors flexible physiologies with complex, nonlinear behaviors. Nonlinear aspects of light harvesting system represent very powerful regulatory mechanism, because small physiological changes to a nonlinear system can result in large differences in how that system functions dynamically. Nonlinear responses are very efficient for accomplishing substantial changes in system functionality through only minor energetic or material expenditures.

Nonlinear systems in ecology have been influenced considerably by the field of cybernetics. Cybernetics is the study of how information flows in systems, including aspects like feedback, control theory, network theory, and emergent phenomena. It coalesced as a discipline in the years following the Second World War,

and is identified with the work of Norbert Weiner at MIT who coined the term and published a seminal text on the subject (Wiener 1948). This text synthesized a number of phenomena under cybernetics that has been previously considered separate, but which fundamentally are not. Many of the ideas and conceptual problems that led to this coalescence came from the biological sciences, such as physiology and animal behavior.

Two decades later, Margalef suggested that many of the fundamental concepts in cybernetics had direct application to plankton ecology in describing the functioning of an ecosystem (Margalef 1968). He indicated how features in cybernetics theory could be used to describe the behavior of ecosystems, populations, and individual organisms. From these basic features, complex behaviors and properties of ecosystems, such as competition or stability, naturally arose from the inherent structure of the ecosystem. Many of the conceptual models that were used early on to illustrate cybernetic principles were derived from electrical engineering and computer science, because although biological systems were of more inherent interest, the engineering models were easier to characterize and understand.

Throughout this dissertation, the photosynthetic light reactions have been referred to as a nonlinear system. The stochastic nonlinear model described in Chapter 2 is only the simplest example of the types of nonlinear behaviors in the photosynthetic light reactions that can occur. Modern computers provide the processing power that is required to examine nonlinear systems in ways that were not possible only a decade ago. Very sophisticated state-variable models have been developed recently, that attempt to replicate some of the complex nonlinear behavior that is observed in the light reactions (e.g., Kroon and Thoms 2006). However, simple stochastic models appear to replicate some very fundamental nonlinear behavior in light harvesting, as demonstrated in Chapter 2, without requiring such a complex physiological description.

If the photosynthetic light reactions are thought of as a system in the signal processing sense, the measured responses to known inputs provide a means to identify the dynamics of the underlying system. Here, dynamics are used in the sense of

Jumars (1993), where kinetics refer to the manner in which responses apparently change, and dynamics refer to the underlying process that causes these changes. The dynamics of simple linear systems can be inferred from measurements of their stimuli and responses, and a range of numerical tools have been developed to do this ("transfer function estimation" algorithms). The physiological model of Kolber et al. (Kolber et al. 1998), used to interpret the fluorescence impulse response in fast repetition rate fluorometry, is an example of a limited transfer function. Ideally, if the Kolber et al. model were more complete, it could predict the natural fluorescence ("continuous-turnover") response of phytoplankton in the dynamic environment, instead of being limited to the time scale of ms.

A basic property of linear systems is that once the transfer function is known, responses to any complex, even random, perturbations can be predicted (Mitra 2001). This is not the case for nonlinear systems. Although tools and theory have been developed for nonlinear system analysis (Kantz and Schreiber 1997; Strogatz 1994), these are not as well developed as they are for linear systems and it remains very difficult to describe the dynamics of nonlinear systems from stimulus and response time series. However, since photosynthesis and light harvesting are strongly nonlinear processes, nonlinear systems analysis techniques must be used to examine how variability in the ambient light field affects light harvesting, through the physiological responses it causes in the photosystem. Perturbation experiments, like the one described in Chapter 3, help to assess to what extent and in which properties does photosynthetic physiology deviate from linear behavior.

Even though nonlinear phenomena such as feedback and thresholds have for a long time been independently recognized in photosynthesis, they have not been treated together as related dynamical features of a nonlinear system. Examining rapid photosynthetic responses to light perturbation has in general been challenging, both *in situ* and *in vitro*, and considerable assumptions have been required to minimize the need to measure these responses when examining phytoplankton photosynthesis in natural light fields. However, a number of recent methodological advances has made it now possible to examine certain aspects of phytoplankton photosynthetic physiology

on very short temporal scales. Recent applications of nonlinear analysis techniques from the engineering disciplines have identified a number of complex nonlinear dynamics in photosynthetic light harvesting, occurring with the time scales of minutes and less (Nedbal and Brezina 2002; Nedbal et al. 2003). This type of information is essential for developing a systems-oriented, dynamical model for light harvesting and utilization in phytoplankton. Such a dynamical framework would provide a more accurate picture of how environmental factors affect these processes in natural phytoplankton assemblages, in a way that current, static PE relationships cannot.

Frameworks derived from equilibrium or quasi-equilibrium theory have suffered much criticism because they cannot distinguish between coexistence, cohabitation, and co-occurrence (e.g., Harris 1978; 1980; 1986). Scale matching, a typically powerful tool in general ecology for identifying the environmental processes responsible for physiological responses, is of little use because nonlinear interactions cause these to fail (Abbott 1993). Nonlinear systems present some particular theoretical and experimental challenges to understanding how phytoplankton utilize their ambient supply of light energy in the dynamic marine light environment. Alternatives based on non-equilibrium conditions employ more realistic assumptions about the dynamic nature of natural environments, like that resources may be utilized by different species at different times, and that definitions of niches must include the effect of fluctuations in resources (Allen 1998). A fundamental conclusion of these initial examinations of non-equilibrium theory was that if ‘the resource utilization functions were non-linear then some complex properties ensued and persistent coexistence was possible even when no stable equilibrium existed’ (Levins 1979).

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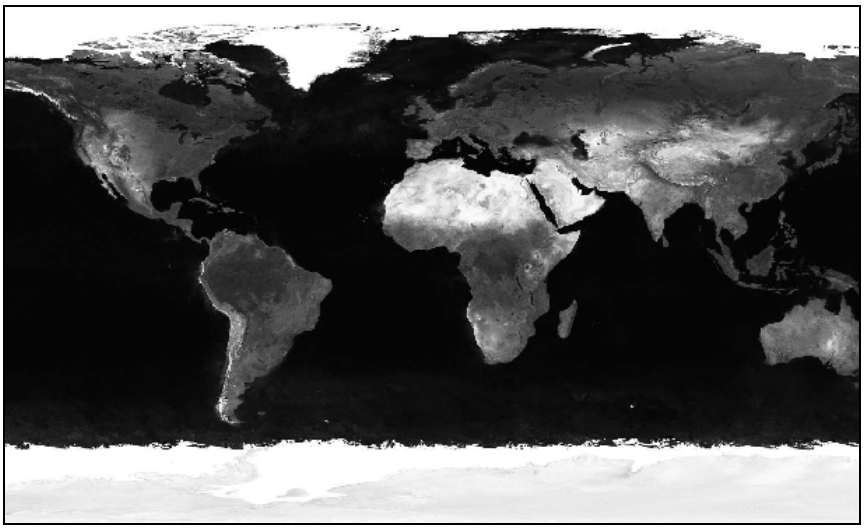

Figure 5-1. An example cloud-free composite remote sensing image.

Figure 5-2. The same image as in Figure 5-1, except without cloud removal.

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7 Appendix

7.1 When a simple blank is not enough: removing kinetic artifacts from phytoplankton variable fluorescence transients using a signal processing approach

7.1.1 Background

Flashes of light can be used to induce variable fluorescence transients in plants and algae. Analysis of light-induced variable fluorescence transients on the microsecond to millisecond time scale is the basis of several widely used oceanographic techniques for examining photosynthetic properties in phytoplankton, including the Fast Repetition Rate fluorometric method. Measurements of variable fluorescence kinetics on such rapid time scales also include instrument hardware artifacts. Correcting these data for instrument artifacts is a critical and necessary prerequisite for properly recovering physiological information from measured variable fluorescence transients. We present a theoretical framework, derived from signal processing principles, that describes how various time-variant and time-invariant artifacts affect the kinetics in measured variable fluorescence transients. This framework simplifies identification the correct numerical approach required to remove these artifacts from measured transients. This analysis demonstrates that a simple 'blank' is insufficient for correcting variable fluorescence transients for time-variant measurement artifacts, such as those that are exhibited by commercial Fast Repetition Rate fluorometers. We describe a simple, repeatable method for determining the instrument impulse response that is needed to correct these data. We also present an application of this signal processing based corrective approach using variable fluorescence field data collected in the oligotrophic Pacific, where algal biomass is very low and thus robust corrective approaches are critical.

When exposed to light, plants and algae will fluoresce at wavelengths around 680 nm in the red. The yield F of this fluorescence on a per-cell basis is not constant in phytoplankton but instead varies, due to physiological factors related to the

biomass, structure, and function of the light harvesting apparatus. On time scales much shorter than those of chlorophyll synthesis, *F* can be experimentally manipulated between two extremes that reflect the functional state of the primary quinone electron acceptor Q_A: a minimum *F* representing a population of Q_A in the oxidized state (F_o), and a maximal *F* representing a Q_A population that is wholly reduced (F_m). The difference between these two endmembers in fluorescence yield is defined as the *variable fluorescence* $F_v \equiv (F_m - F_o)$. Since the underlying process that drives such a change in *F* is ultimately photochemical in nature, this variable fluorescence is also often referred to as *photochemical fluorescence quenching* (Hall and Rao 1992). An extensive nomenclature exists to distinguish between *F* yields that result from different experimental or environmental conditions (e.g., van Kooten and Snel 1990).

Measurements of F_v in plants provide valuable insight into their photosynthetic physiology, and several techniques for inducing and measuring F_v in marine phytoplankton have been developed for oceanographic use. Herbicides such as DCMU can be used to inhibit photosynthetic electron transport, because they behave as analogs to secondary electron acceptors (Q_B) and block binding sites on the D1 protein. Measuring differences in steady-state *F* measured before and after addition of DCMU is a chemical means to measure F_v (Parkhill et al. 2001). However, similar shifts from F_o to F_m can also be induced by exposing photosynthetic organisms to a brief flash of photosynthetically saturating irradiance. If an adequate amount of light is delivered to photochemically reduce all available electron acceptors, light harvesting will remain effectively inoperable for a period of time commensurate with the turnover time of the plastoquinone pool. The "pump-and-probe" technique for assessing photochemical yield in plants and algae relies on this principle, by initially measuring F_o in response to a weak probe flash, and then measuring F_m with a similar probe flash immediately after a strong "single turnover" pump flash that effectively reduces all available Q_A (Falkowski et al. 1986b; Mauzerall 1972).

For very intense single turnover flashes, the rate of photon delivery to the sample is very rapid and leads to a near-instantaneous reduction of electron acceptors

and a similarly rapid increase from F_o to F_m. By reducing the intensity of these saturating flashes so that the rate of energy delivery to the photosystems only slightly exceeds their inherent rate of electron throughput, the kinetics of the variable fluorescence yield change from F_o to F_m can be measured. These *F(t)* kinetics reflect how an impulse of photon energy loads the light harvesting apparatus and progressively closes a finite population of photosynthetic reaction centers. These kinetics can be described using cumulative one-hit Poisson functions to estimate specific physiological properties that govern these kinetics, from measured *F(t)* transients (Ley and Mauzerall 1986). Several functionally similar techniques have been developed or adapted for oceanographic use that induce single turnover transients for the purposes of examining photophysiology in phytoplankton (e.g., Johnson 2004; Koblížek et al. 2001; Kolber and Falkowski 1992; Kolber et al. 1998; Olson et al. 1996).

Although this process of recovering physiological information from variable fluorescence transients is conceptually straightforward, a number of theoretical, instrumental, and experimental issues make it challenging to determine PSII properties from measured *F(t)* kinetics in practice. The measure of fluorescence emission signal *EM(t)* does not represent only the fluorescence emitted by the algal sample, as other sources may contribute to this signal. These sources can include ambient or excitation irradiance that is scattered by water or particles, which leaks past emission blocking filters, or fluorescence contributed by non-phytoplankton sources such as dissolved organic matter. Such artifacts are effectively time-invariant on the μs-to-ms time scale of the single turnover flash sequence employed by fast repetition rate (FRR) fluorometers and functionally similar instruments. In theory, artifacts that are time-invariant over the scale of the measurement can be corrected for algebraically, by measuring "blanks" and subtracting them from the signal of interest. In certain ocean environments these sources of artifact in F_v can be considerable, and ignoring them can potentially bias the ecological interpretation of variable fluorescence data (Cullen and Davis 2003).

Other artifacts in measured *EM(t)* are not time-invariant on the single turnover time scale and thus will not be readily corrected by applying a static blank. An example of such an artifact is the impulse response that have been documented in commercial Fasttracka FRR fluorometers (Laney 2003). For other functionally similar single turnover techniques or instrument prototypes, published data are insufficient to determine to what extent such impulse responses affect data collected with those instruments. These time-variant artifacts appear to be more evident in *EM(t)* than in the recorded single turnover pulse *EX(t)*, reflecting unavoidable limitations with high-speed measurement of weak light signals such as chlorophyll fluorescence (Figure 7-1). Measuring *EM(t)* on single turnover time scales requires high-speed detectors and circuits with maximally flat responses, minimal overshoot, and undue damping. Engineering such detectors remains a nontrivial problem despite continual advances in electronics. As a result, such time-variant artifacts in measurements of *EM(t)* are unavoidable in any real instrument and at best can only be minimized with careful design.

An empirical method for identifying, quantifying, and correcting variable fluorescence kinetics measured in single turnover applications was presented by Laney (2003). This method has been implemented in numerous oceanographic field studies in which commercial FRR fluorometers were used to assess phytoplankton photophysiology (e.g., Cermeño et al. 2005; Corno et al. 2006; Moore et al. 2003; Raateoja et al. 2004). This method improves the accuracy with which photosynthetic parameters were retrieved using these instruments and in theory is also applicable to any functionally similar technique (e.g., Johnson 2004; Koblížek et al. 2001; Olson et al. 1996). However, the approach outlined in Laney (2003) has several limitations. First, because the method is purely empirical, it provides no physical or mechanistic explanation for the time-variant sources of artifact (i.e., the instrument response functions) that are observed in these instruments. Second, the method requires extensive and cumbersome characterization of these response functions, over a wide range of protocols and gain settings. Third, these empirically determined response functions inherently contain measurement noise, even after considerable averaging,

which is added to the signal of interest during the correction process and can make fitting the physiological model to the observed fluorescence transient more difficult.

Correcting measured *EM(t)* kinetics for instrumental artifacts is a critical and necessary prerequisite for properly interpreting variable fluorescence transients in terms of photosynthetic physiology. Cullen and Davis (2003) demonstrate clearly how specific time-invariant biases in variable fluorescence measurements can inadvertently bias ecological interpretations of environmental variability in derived photosynthetic properties, primarily F_v/F_m. Approaches for correcting for such time-invariant artifacts were presented by those authors for the purposes of improving oceanographic assessment of F_v/F_m, particularly in highly oligotrophic regions. However, the treatment of Cullen and Davis (2003) did not address how time-*variant* artifacts such as the instrument response affects measurements of F_v/F_m, or of the other physiological parameters that can be recovered from *EM(t)* using single turnover variable fluorescence techniques. Strictly speaking, such artifacts cannot be corrected for using algebraic approaches, and more sophisticated approaches must be used to remove the effect of these artifacts from measurements of *EM(t)* transients.

7.1.2 Discrete-time descriptions of variable fluorescence transients

Phytoplankton variable fluorescence measurements on the μs time scale are almost always presented as discrete-time sequences, not as continuous analog measurements. This is either explicit, as with the integrated ‘flashlets’ in FRR fluorometry (Kolber and Falkowski 1992), or implicit in the digitization of analog signals (e.g., Johnson 2004; Koblížek et al. 2001; Olson et al. 1996). Consequently, the kinetics of variable fluorescence emission $EM(t)$, measured in response to an excitation impulse $EX(t)$, are more correctly described as discrete-time sequences

$EM[n]$ and $EX[n]$, where the index $[n]$ represents the location of a particular sample within a sequence.

A graphical method commonly used in signals and systems analysis provides an effective means for identifying and quantifying how phytoplankton physiology, sample artifacts, and instrument-specific biases each contribute individually to the measured transient fluorescence emission $EM[n]$ elicited by a single turnover light pulse $EX[n]$. Such diagrams show how time-variant signals propagate through linear systems and how different factors affect such signals both in magnitude and in time. Factors that affect only the magnitude of signals, independent of time, are represented by triangles; a scalar value A quantifies the gain associated with these elements. Factors that affect the magnitude of a signal in a time-dependent manner are represented by boxes and are described by a transfer function $h[n]$. The $h_I[n]$ of the instrument would contain information about how the kinetics of the signal are modified during measurement, as well as how the magnitude is affected, such as across different instrument gain settings. In practice, transfer functions can be scaled so that their overall gain is unity, i.e., 0 dB. Thus, a scaled $h[n]$ would contain information only on the shape of the signal is affected by the measurement system. A general overview of these and other basic aspects of signals and systems theory can be found in textbooks such as Mitra (2001).

In a system diagram, the fluorescence of an ideal inert fluorophore resulting from a single turnover flash would be represented as in Figure 7-2a. A discrete-time excitation signal $EX[n]$ is absorbed by a fluorophore and reemitted as a fluorescence. For ideal inert fluorophores, the absorption and reemission of $EX[n]$ can be considered instantaneous and time-invariant, so that the scalar multiplier A represents the fluorescence yield of this fluorophore. Consequently, the actual fluorescence signal emitted by this fluorophore is a scalar multiple of $EX[n]$, or $EM[n] = A \cdot EX[n]$. However, if the instrument measuring this fluorescence has a non-ideal, discrete-time impulse response $h_I[n]$, the apparent fluorescence emission reported by the instrument is actually the convolution of $A \cdot EX[n]$ by $h_I[n]$, or $EM[n] = A \cdot EX[n] * h_I[n]$. Correcting for time-variant instrument artifacts, to recover the actual fluorescence

yield of interest, requires deconvolution of the instrument impulse response from the measured signal (Doebelin 1990). This corrective approach is commonly used in a wide range of measurement situations where time-dependent instrument artifacts occur on the same time scale as the signal of interest, such as with profiling the physical microstructure in lakes and in the ocean using thermistors (e.g., Nash et al. 1999; Oldham 1994).

In more complex fluorescing systems the relationship between $EX[n]$ and $EM[n]$ can also be described using similar system diagrams, providing that the systems themselves are linear and time-invariant. For example, the fluorescence emitted by an optically dilute suspension of phytoplankton in a scattering and fluorescing medium is shown in Figure 7-2b. In this more realistic system, the $EM[n]$ measured by a non-ideal detector represents the optical signal of three independent components. Separate time-invariant gain elements represent the apparent fluorescence due to scatter A_S and the fluorescence contribution of chromophoric dissolved material A_C. A second discrete-time transfer function $h_P[n]$ represents the variable fluorescence kinetics of the phytoplankton which, like the transfer function ascribed to the instrument $h_I[n]$, includes information about how the phytoplankton contribute to changes in the gain and shape of $EX[n]$.

Since these three elements (A_S, A_C, and $h_P[n]$) can be considered to act independently in the optically dilute case, according to the linearity principle the overall system can be equivalently represented by a linear combination of these individual discrete-time sequences, each separately convolved with $h_I[n]$ (Figure 7-2c). Thus, any rearrangement of the network diagram that does not violate the linearity principle is mathematically correct, although some alternatives may be more practically useful. For example, the ultimate parameter of interest in oceanographic measurements of phytoplankton variable fluorescence transients is $h_P[n]$, and the impulse response of this element alone is what should be fit to physiological models of phytoplankton variable fluorescence. Figure 7-2d shows how the effect of both A_S and A_C can be removed simultaneously from measured $EM[n]$ transients, simply by subtracting the $EM[n]$ measured on a "blank" sample that has both scattering and

fluorescing artifacts. Then, the correction procedure requires only one further deconvolution of the instrument response function $h_I[n]$, in order to arrive at the ultimate physiological kinetics of interest $EM[n] = EX[n] * h_P[n]$.

Such a system analysis framework makes it readily apparent that a simple corrective approach based on subtracting blanks is insufficient when the $EM[n]$ transients are measured by instruments having non-negligible impulse responses. A second and similarly important ramification is that it is more correct to remove the impulse response from the actual measured $EM[n]$ transients, not the derived yields $F[n]$ that can be computed by dividing each $EM[n]$ by its corresponding excitation impulse $EX[n]$. It is tempting to first compute this yield and then attempt to correct it for instrument and measurement artifacts. However, as these signal network diagrams illustrate, for all $n > 1$ the $EM[n]$ measured is not solely a function of its pairwise $EX[n]$, and $EX[1{:}n\text{-}1]$ may affect $EM[n]$. Accounting for the effect of $EX[n]$ on $EM[n]$ is more correctly done by deconvolution, not subtraction.

The overall goal of this study was to improve correction of variable fluorescence transients for time-variant instrument artifacts. We developed a method for determining the impulse response $h_I[n]$ of the most commonly used single turnover variable fluorometer in oceanographic studies, the Fasttracka FRRF (Chelsea Marine Systems, West Molesey, UK). We examined variability in measured $h_I[n]$ both within and between different instrument gains and assessed how these differences affect photosynthetic properties derived from $EX[n]$ and $EM[n]$ measured with these instruments. We also demonstrate an application of this corrective approach on near-surface phytoplankton assemblages in the subtropical North Pacific, where algal biomass is very low and therefore robust corrective approaches are required when using variable fluorescence methods (Cullen and Davis 2003).

7.2 Materials and Methods

7.2.1 Measuring instrument impulse response

The impulse response of the Fasttracka FRRF examined in this study (SN 014) was measured over the entire dynamic range of four of its detector gain settings (x1, x4, x16, and x64). Impulse responses at the most sensitive gain setting (x256) were not examined due to very low signal to noise levels at that gain. Impulse responses were collected with the instrument oriented horizontally, with its "light" sampling channel facing upward and with its sun block removed. A 25 mm glass prism (Edmund Optics NT32-337) was placed in the sample area so that excitation irradiance was redirected directly into the fluorescence emission aperture (Figure 7-3). Since the optical blocking of the excitation irradiance by the emission filters within the instrument is not perfect, leakage of excitation irradiance $EX[n]$ into the fluorescence detector is measured as an apparent fluorescence $EM[n]$. Different combinations of 2" square neutral density filters (Edmund Optics, kit p/n G54-460 or G55-222), inserted in the optical path, were used to attenuate the intensity of the *EX* light the prism redirected into the detector. Between 5 and 9 different filter combinations were used to attenuate the coupling between *EX* and *EM* within each of the 4 gains examined, to assess any differences in step response within the dynamic range of each gain setting. The combination of prism and neutral density filters represents the simplest case of an inert substance that transduces single turnover excitation irradiance into the emission wavelengths of the detector, represented in network diagram form by Figure 7-2a.

The optical head was covered with a dark cloth during these measurements to minimize the influence of ambient room light on the measured step response, and the instrument was allowed to thermally stabilize for 60 min before conducting these measurements. The apparent measured transients $EM[n]$ were zero padded to length $2n$ -1 so that $A_{filter} \cdot h_I[n]$ could be computed by deconvolving $EM[n]$ from $EX[n]$. Thus, several instances of $A_{filter} \cdot h_I[n]$ were determined for each gain setting. To assess potential differences in $h_I[n]$ within the dynamic range of each gain setting, a single

idealized $EX[n]$ of uniform intensity was convolved with each of these measured $A_{filter} \cdot h_I[n]$ to predict the $EM[n]$ transient that would be expected from the particular neutral density filter used with the prism. The actual excitation flashlet sequences produced by the instrument decay considerably with time to about 80% at $n = 100$ of their value at $n = 1$. Using an idealized excitation sequence makes it easier to assess the effect of $A_{filter} \cdot h_I[n]$ on measured $EM[n]$ transients.

7.2.2 An application to field measurements of variable fluorescence

We applied this signal processing approach to correct field measurements of single turnover variable fluorescence for instrumental and environmental artifacts. The same Fasttracka examined in the characterization study was deployed on a 6 d cruise in November 2005 at Station ALOHA, 100 nm north of Honolulu in the North Pacific. In this ocean region phytoplankton biomass is very low, and the fraction of the total variable fluorescence signal due to instrument artifact and background fluorescence can be considerable. In this study the instrument was operated in benchtop mode and was connected directly to the ship's uncontaminated seawater supply. Variable fluorescence transients were measured continually over a five day period, collected at ≈ 13 s intervals where each transient represents the average of 16 different acquisitions, internally averaged by the instrument. We designed a computer-controlled valve system that periodically redirected the supply of seawater to the instrument, through a 0.2 μm pore size filter (CritiCap-50, Pall Gelman Sciences). This operation occurred automatically every hour, for 4 min, for the entire duration of the cruise. The signal measured on these filtered samples contains information about all dissolved constituents that contributed to artifact as well as the instrument impulse response. Consequently, this signal represents what would be measured due to factors in the top signal path of Figure 7-2d.

Approximately 19 $EX[n]$ and $EM[n]$ sequences were collected during every 4 min intervals each hour. These were averaged to generate a mean hourly dissolved $EM[n]$ and $EX[n]$. Next, $EX[n]$ was deconvolved from $EM[n]$ to compute $(A_S + A_C) \cdot h_I[n]$, which were then normalized to make the overall gains of these transfer functions unity (0 dB). Normalizing in this manner thus characterizes how $h_I[n]$ affects the shape of $EM[n]$, independent of any effect on the overall final amplitude of EM.

These hourly normalized impulse responses were used to correct all the fluorescence transients measured in the half hour preceding and following each determination, as illustrated in Figure 7-2d. First, the dissolved $EM[n]$ blank corresponding to the nearest hour in time was subtracted from each unfiltered $EM[n]$. Next, the normalized impulse response was deconvolved from result of this operation to compute the $EM[n]$ that would be expected without the time-variant artifacts introduced by the instrument. These corrected $EM[n]$ transients and their corresponding $EX[n]$ were analyzed using software designed to estimate the photosynthetic parameters F_v/F_m and the Photosystem II functional cross section σ_{PSII} from the saturation flashlet sequences. This software ("v6") is a further development of the open-source fluorescence transient analysis software presented in detail by Laney (2003).

7.3 Assessment

7.3.1 Character of the instrument impulse response

Reconstructing $EM[n]$ from an ideal excitation sequence of n identical flashlets provides a model for the instrument response $h_I[n]$ that cannot be determined by simply computing the fluorescence yield transient $F[n]$. By computing the $EM[n]$ that would result from a sequence of n excitation impulses of uniform intensity, we predicted a series of apparent fluorescence transients that would be expected for an

ideal excitation sequence, but still with real instrument impulse response. These predicted $EM[n]$ sequences were well described by an analytical function containing three weighted time constants:

$$EM[n] = EX[n] \cdot EM[\infty] \cdot \left(1 - \alpha_1 \cdot e^{(-\frac{n}{\tau_1})} - \alpha_2 \cdot e^{(-\frac{n}{\tau_2})} - \alpha_3 \cdot e^{(-\frac{n}{\tau_3})} \right) \qquad \text{Eq. 1}$$

In this function, the amplitude of measured apparent fluorescence emission $EM[n]$ is a function of the intensity of the concurrent excitation flashlet $EX[n]$, a gain term $EM[\infty]$ that represents the eventual maximal emission at long times into the transient, and a series of weighted exponentials τ_1 - τ_3 that describe how the impulse response of the instrument to samples prior to n affect the measured at n. These weighting of these exponentials is such that the sum of the weights α_1, α_2, and α_3 is unity. The first time constant in this model is defined to be very short (0.001 of a time step), to replicate the near-instantaneous initial rise in *EM* that is observed in actual measurements. The two longer-scale time constants describe the kinetics of the step response, i.e., how *EM* eventually attains $EM[\infty]$. Other analytical equations could in theory be fit to these predicted *EM* transients, but this function provides a time-continuous description. Equations functionally similar to Eq. 1 are commonly used to characterize the step response of detector systems that have multiple response scales, such as photodiodes (Graeme 1996) and photomultiplier amplifier circuits (Kume 1994).

Apparent variable fluorescence transients due to the instrument impulse response were fit to Eq. 1 using nonlinear fitting methods (Figure 7-4) and estimates for the parameters in this equation were determined. The FRR fluorometer in this study exhibited spurious measurements of *EM* for $n = 1$ (Figure 7-4, green traces), an artifact that has also been reported also by other workers. In this study, we did not attempt to correct for this particular source of error. Since Eq. 1 is nonlinear, determining confidence intervals for these fitted parameters is not trivial and

statistically weak (Press et al. 1992). Instead, we performed a one-way analysis of variance on each of the 3 time constants and 3 weights and found that only the medium-scale time constant τ_2 and the long-scale weight α_3 differed significantly among gain settings at the 0.05 level. In general, the instrument responded to variable fluorescence impulses to a magnitude of approximately 82% of the final signal required, at the first flashlet. The remaining rise in variable fluorescence to $EM[\infty]$, characterized by the two time constants τ_2 and τ_3, accounted for ≈ 18% of the entire magnitude of the signal, and these two time constants contribute equally these kinetics (Table 7-1).

The magnitudes of $EM[\infty]$ that were predicted from Eq. 1 provide a means to assess any differences in impulse response within a single gain setting. A geometric-mean, Model II linear regression (who) was fit to the relationship between filter optical density and $EM[\infty]$ for each group of gains (Figure 7-5a). For the impulse responses collected at instrument gains x1, x4, and x16, the computed slopes of these regressions were indistinguishable, whereas the slope of the relationship at gain x64 differs significantly from those of the three lower gains. These predicted $EM[\infty]$ can also be used to compute actual differences in instrument gain between gain settings. Although the nominal gain settings of the instrument are set in factors of four (e.g., x1, x4, x16, etc.), the actual gains obtained at each setting are not so perfectly spaced. By replotting the relationships in Figure 7-5a as a function of transmittances (a linear scale), instead of optical densities (a logarithmic scale), differences in the estimated slope of the regressions indicate how the actual gain setting compares to its nominal setting (Figure 7-5b). From these regressions we computed actual gains of 1.00, 2.71, 6.90, and 23.98 for gain settings x1 through x64. By comparison, a prior factory calibration of this instrument indicated the same range of actual gains to be 1.00, 3.09, 9.36, and 27.04, although the method used to generate these factory calibrations has not been published.

7.3.2 Results from a field study in a highly oligotrophic region

The variable fluorescence time series collected in this study covered ≈ 5.5 d, including ≈ 1.5 d that are not representative of Station ALOHA due to transit back and forth from port. In this 5.5 d period, the automated filtering system provided 151 instances of 0.2 μm filtered blank sample (Figure 7-6a). The magnitude of the apparent variable fluorescence measured on these blanks ranges between ≈ 1300 and 1900 (in instrument units). For comparison, with the excitation sequence parameters used in this study, the maximum signal was ≈ 18000 (Figure 7-6b). This means that the blank due to dissolved fluorescing constituents in these oligotrophic waters accounts for approximately 10% of the total dynamic range at instrument gain of x64. However, since the average *EM* signal recorded during this cruise rarely exceeded 5000 at this gain setting, the dissolved constituents were a substantial fraction of the measured $EM[n]$ signal, effectively representing about a third of the total signal measured. There was no clear long term change in the $EM[\infty]$ over the course of this cruise although there were substantial long term shifts in the magnitude of the dissolved contribution to *EM* (Figure 7-6b).

Noticeable kinetics in $EM[n]$ were universally evident in the apparent variable fluorescence of the dissolved fraction, despite the absence of any physiological source of variable fluorescence kinetics (Figure 7-6a). Even with the considerable effective averaging that each hourly "blank" sample contains (n_{ave} = 304), the signal to noise ratio of these blanks was low enough that it was generally impossible to fit the three-exponential impulse model of Eq. 1 to the sequences in Figure 7-6a. An abbreviated two exponential equation could be fit with success, where again the first time constant τ_1 component represents the near-instantaneous rise to $EM[1]$, and the second time constant τ_2 characterizes the overall instrument impulse response on the time scale of the single turnover measurement. These fitting results indicated that the instantaneous response of the instrument raised the *EM* signal to ≈ 94% of $EM[\infty]$ by the first

flashlet. The average τ_2 computed for these blanks was 40.78 ± 16.26 μs, which is comparable to the average τ_2 of 32.55 ± 7.37 μs that was computed for the same instrument using Eq. 1, from the characterization study using neutral density filters. As with the estimates of $EM[\infty]$, there were no clear diurnal patterns in τ_2 for this cruise although there were some differences in instrument response across the cruise (Figure 7-6c).

The effect that the additional correction for instrument impulse response on time series of two variable fluorescence parameters, F_v/F_m and σ_{PSII} is evident in both parameters. Correction for static blanks alone leads to an overestimate of F_v/F_m by roughly 10% in these data (Figure 7-7a), which additionally correcting for impulse response demonstrates. For σ_{PSII}, correcting for static blanks has negligible effect on improving the parameter estimates, which remain underestimated by up to 30% from their more accurate values when both static and impulse response artifacts are considered. There appear to be no significant diurnal or long term trends in these errors.

7.4 Discussion

Variable fluorescence techniques provide a means to estimate several key photosynthetic properties of phytoplankton that are directly related to the physiological aspects of light harvesting and primary photochemistry. These properties in turn cast light on the underlying physiological bases for photoacclimation and photoadaptation responses of phytoplankton in the dynamic marine light environment. Of the several instruments that have been developed to perform single turnover variable fluorescence measurements on marine phytoplankton, the Fasttracka fast repetition rate fluorometer (FRRF, Chelsea Marine Systems, West Molesey UK) is most widely used in oceanographic field studies, primarily to assess distributions of photosynthetic properties in natural assemblages in situ.

Yet several fundamental questions remain unaddressed regarding the accuracy and precision of the estimates of F_v/F_m and σ_{PSII} that these instruments generate, despite these instruments being commercially available for over ten years. A study by Laney (2003) discussed several factors that can contribute to error in estimates of F_v/F_m, σ_{PSII}, and other properties that can in theory be recovered from variable fluorescence kinetics measured with these in situ FRR fluorometers. These factors generate error in these measured properties by introducing artifacts into the fluorescence yield transients $F(t)$ on which physiological models for variable fluorescence are fitted. The artifacts affect $F(t)$ primarily by varying either the magnitude or kinetics of the fluorescence emission signal $EM(t)$ that is measured.

The importance of measuring and applying static blanks to variable fluorescence measurements has been discussed at length by Cullen and Davis (2003) with respect to natural ocean systems. Without correcting variable fluorescence measurements for artifacts due to non-phytoplankton sources of fluorescence emission, estimates of parameters like F_v/F_m will be biased as can be shown by simple algebraic manipulation. Failure to account for the sometimes subtle variability in these blanks can materially affect the ecological inferences drawn from physiological properties that are estimated from variable fluorescence, such as F_v/F_m. In our field measurements at Station ALOHA in the North Pacific Subtropical gyre, we see considerable variability in the magnitude in the apparent fluorescence at 683 nm of surface seawater filtered through a 0.2 μm pore size filter, over time scales of one to several days, up to 50%. As expected, variability in the background apparent fluorescence had a considerable effect on estimates of F_v/F_m and less so on estimates of σ_{PSII}. The automatic valve system developed for this study provides a means to measure these blanks continually to provide better resolution of the correction data needed.

To what extent additional correction for kinetic artifacts in single turnover variable fluorescence transients are needed in studies that employ these and similar fluorometers is less well understood. Instrument artifacts that occur on the single turnover measurement scale are likely to differ among various designs of single

turnover fluorometers, being largely the result of the specific electro-optic design of the fluorescence detector and associated circuitry. This study demonstrated how a highly repeatable method for assessing the instrument impulse response can be performed, using prisms and neutral density filters. The kinetic artifacts we observed in this particular Fasttracka were well described by a typical instrument step response function used widely to characterize the response kinetics of a range of high speed detectors. The importance of accounting for the impulse response of single turnover fluorometers can be shown clearly from first principles using a signal processing framework (Figure 7-2). This finding confirms the claim of Laney (2003) that although instrument artifacts are visually similar to the kinetics that would be expected from the photophysiological factors that control $F(t)$, they are in fact materially different numerically and that robust numerical fitting algorithms are needed to identify these subtle yet important functional differences in variable fluorescence kinetics.

7.5 Comments and recommendations

Signal processing theory provides a rigorous framework for describing how various static and transient sources of artifact can affect the kinetics that are measured by variable fluorometers, not only on the single turnover time scale but on any scale over which instrument artifacts occur. These frameworks simplify the process of identifying the proper combination of deconvolution and subtraction that is required to recover the variable fluorescence kinetics that reflect physiological sources alone. Properly correcting the time-variant artifacts in single turnover fluorescence yields by accounting for the impulse response of these instruments is a necessary complement to correction approaches that use analytical blanks to correct for other sources of artifact that are time-invariant on the single turnover time scale. Studies that employ commercial or prototype instruments to measure single turnover fluorescence transients should be viewed with caution, if they do not include documentation of how

these transients were affected by measurement artifact kinetics or can be considered negligible due to careful instrument design.

Acknowledgements The authors would like to thank Dave Karl and the Hawaii Ocean Time-series program for support of our efforts to collect variable fluorescence time series at Station ALOHA on HOT175. Support for this research was provided by the National Science Foundation.

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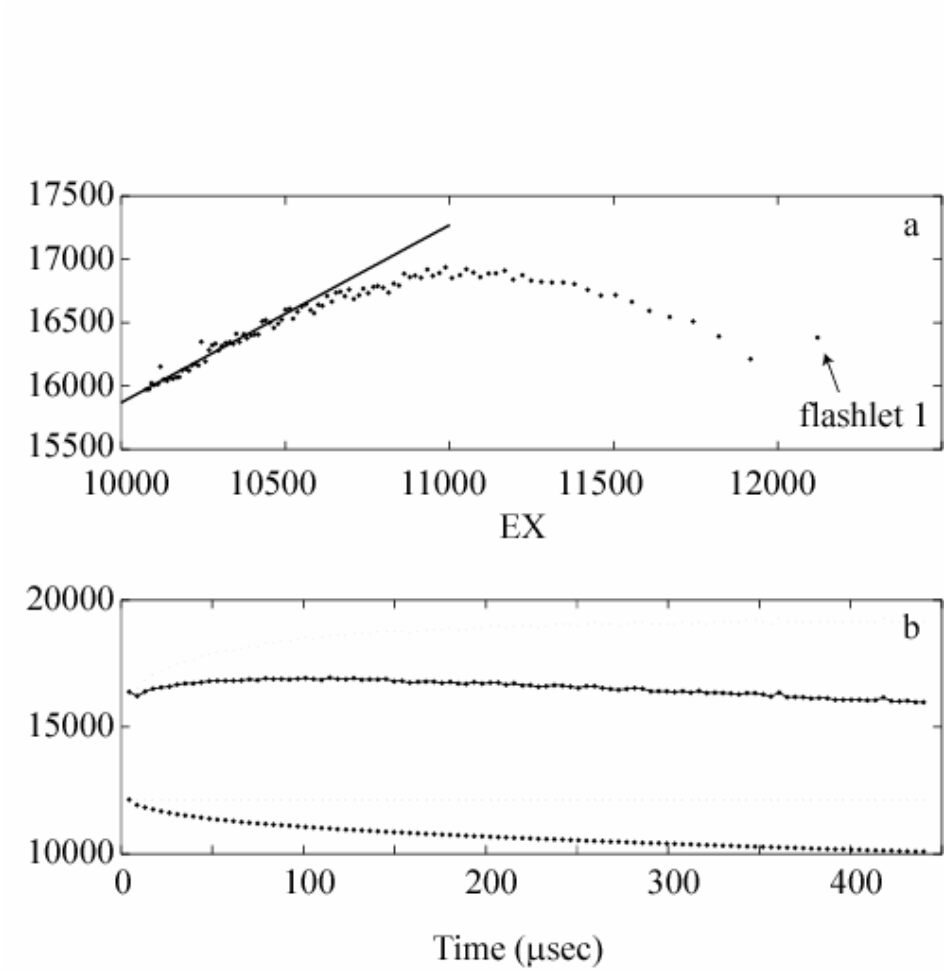

Figure 7-1. a) The relationship between $EX[n]$ and $EM[n]$ for a typical variable fluorescence transient measured by a Fasttracka FRRF on an inert fluorophore. Only the second half of this 100 flashlet sequence shows a linear correlation between $EX[n]$ and $EM[n]$ ($n = 40$, $r^2 = 0.94$). The first half of the $EM[n]$ emissions are disproportionately low compared to their excitation. The first emission sample $EM[1]$ exhibits particularly anomalous behavior that is not due to the excitation intensity of this flashlet. b) The same variable fluorescence transient, plotted instead as a function of time, with $EX[n]$ in blue and $EM[n]$ in red. The response $EX[n]$ (EM_{ideal}) to an idealized excitation sequence with identically intense flashlets (EX_{ideal}) can be predicted by calculating the impulse response $h_I[n]$ of the instrument from the measured $EX[n]$ and $EX[n]$.

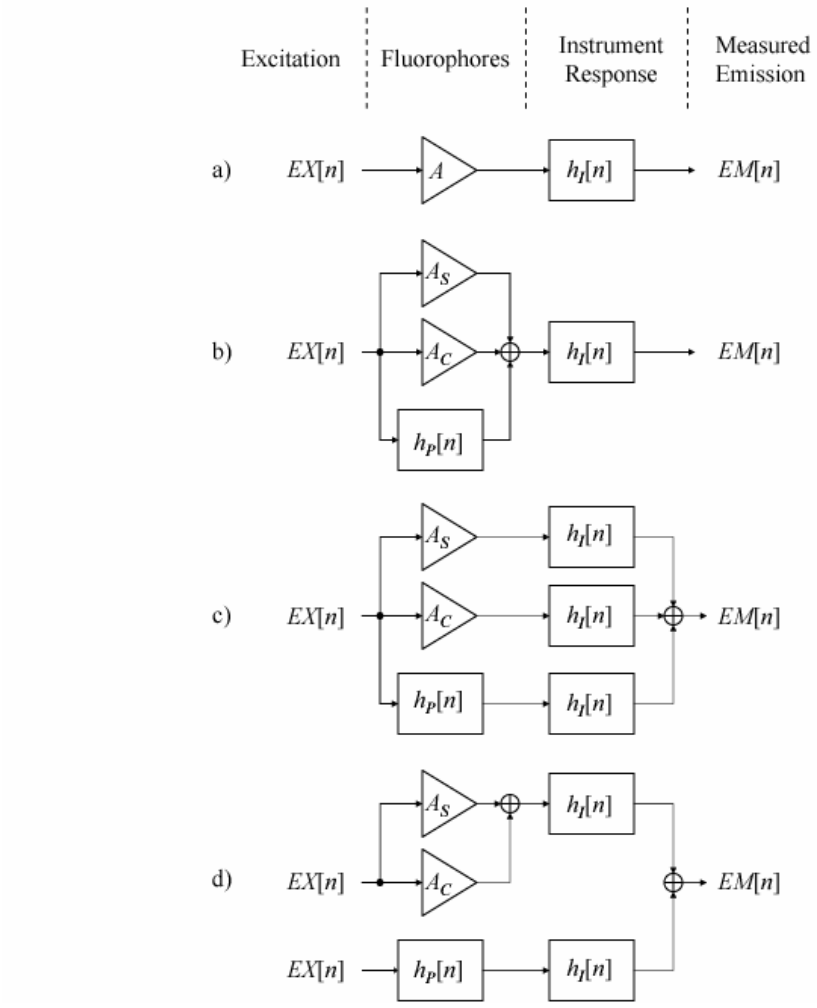

Figure 7-2. System diagrams showing how time-variant measurement artifacts affect the measurement of variable fluorescence transients $EM[n]$ that are stimulated by an excitation sequence $EX[n]$ and measured by an instrument with a non-ideal impulse response $h_I[n]$. Diagram a) shows a system where the sample excited has no inherent fluorescence transient behavior, such as a dye. Diagram b) shows a more oceanographically realistic system in which the measured emission transient $EM[n]$ includes a physiological control $h_I[n]$, in addition to contributions from scatter G_S and the fluorescence of dissolved matter G_C. In linear systems, the result of the sum signal of all fluorescence contributions passing through the instrument can be equivalently represented c) by the sum of each component signal after passing separately through a representation of the instrument. Linear equivalents to diagrams such as c) demonstrate how the subtraction of a simple 'blank' measured in the absence of a phytoplankton sample d) does not correct the inherent time-dependent biases.

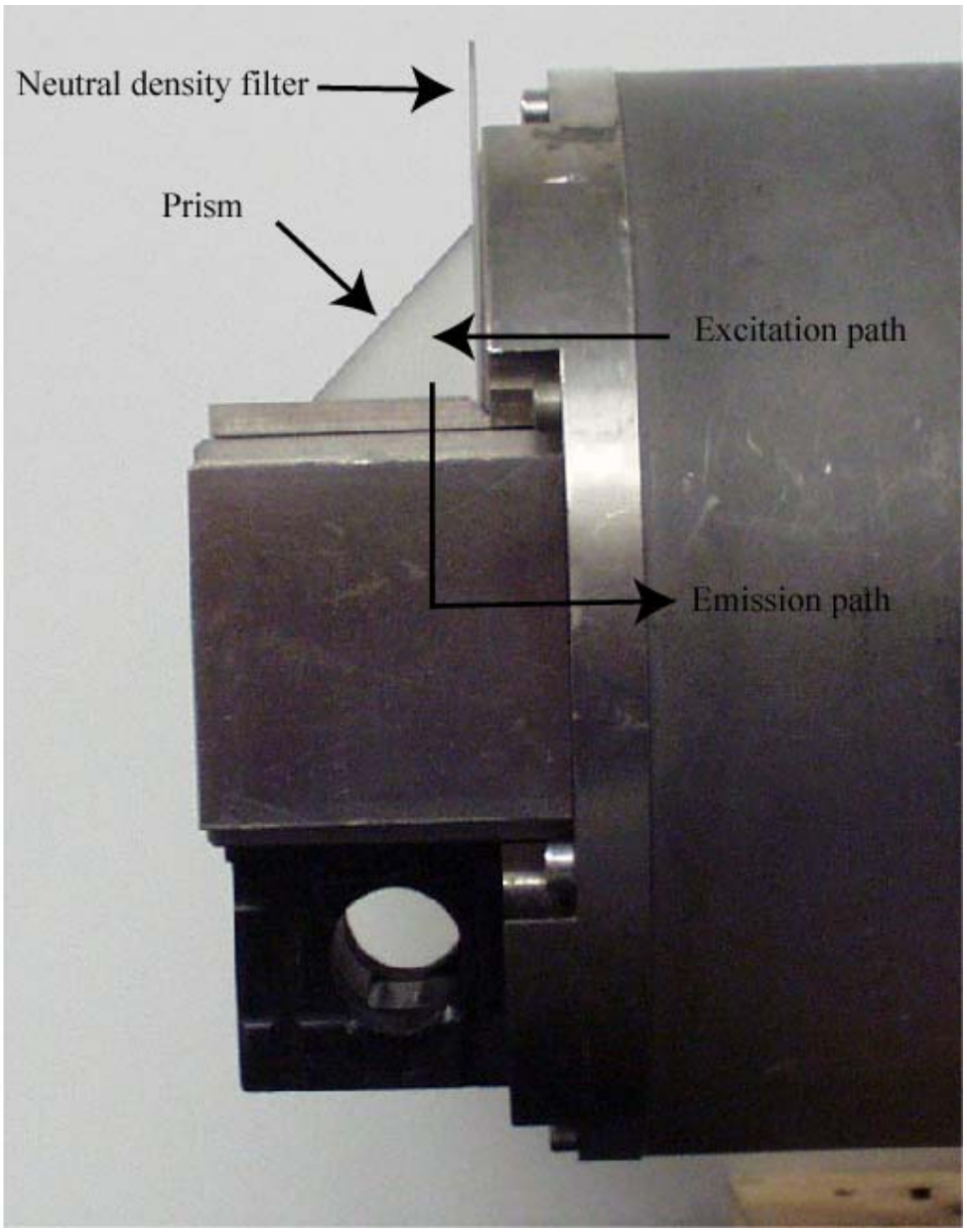

Figure 7-3. The optical apparatus used to determine $h_I[n]$ of the Chelsea Marine Systems Fasttracka fluorometer. A prism is used to redirect excitation irradiance into the fluorescence detector. Different combinations of neutral density filters are used to further attenuate the coupling between the source and the detector.

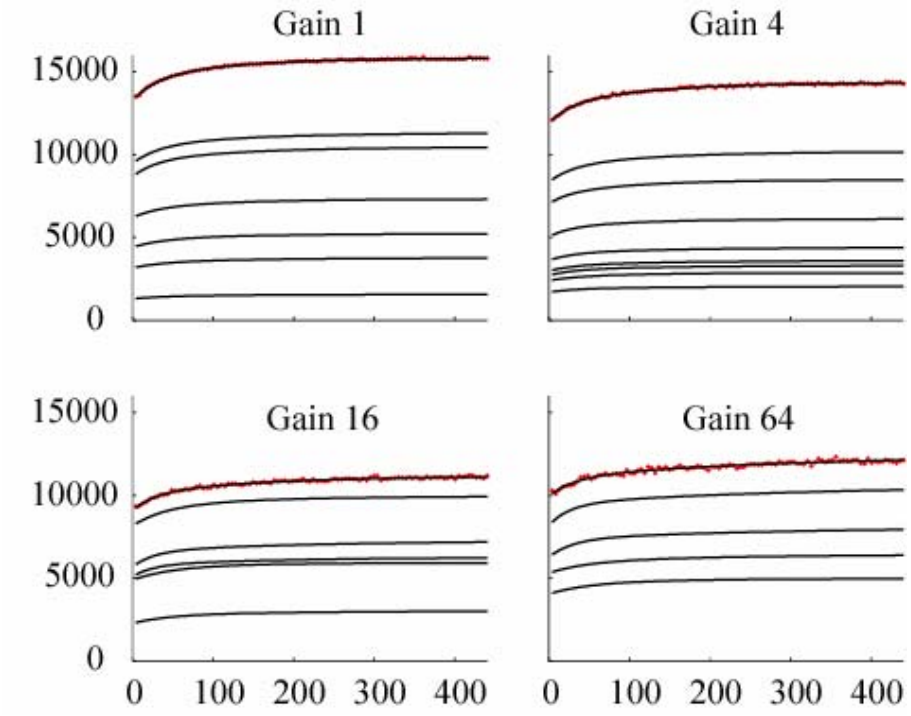

Figure 7-4. Plots of all the predicted EM and fits of the analytical response for all gains and ranges.

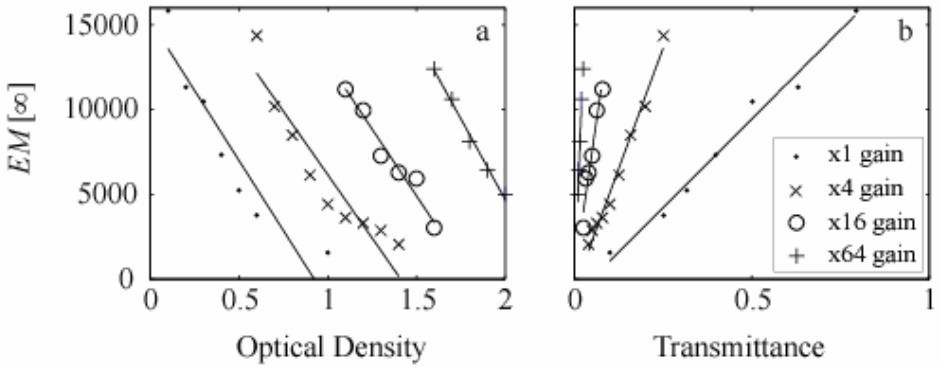

Figure 7-5. Relationships between *EM*[∞] and a) optical density and b) transmittance, as a function of gain for each characterization assay. Lines represent the results of Model II geometric mean regressions.

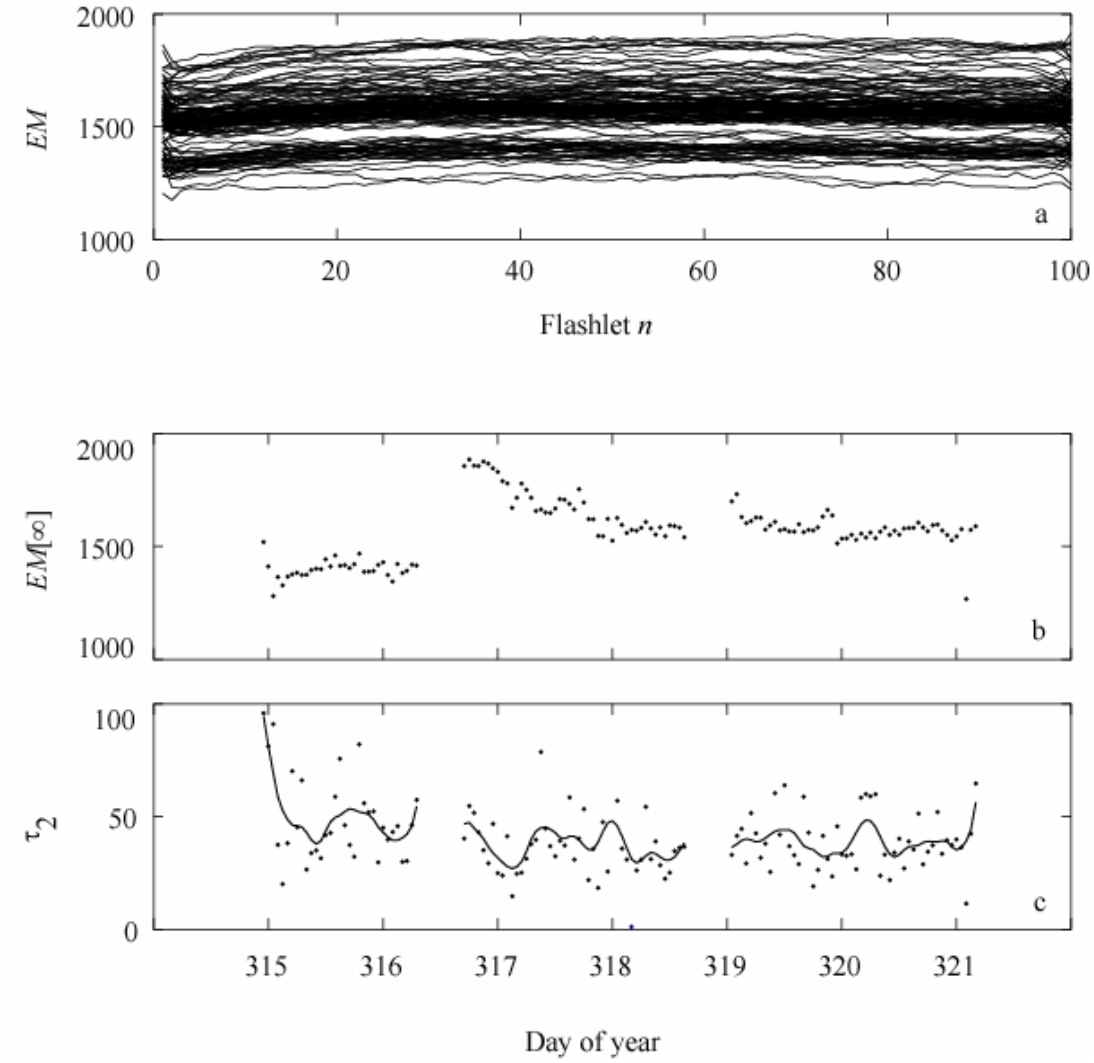

Figure 7-6. The apparent variable fluorescence kinetics measured on 0.2 μm filtered seawater at Station ALOHA as a) a function of flashlet number for all blank samples, b) for fitted EM[∞] as a function of time for the 6 d duration of the cruise, and c) for fitted τ2 as a function of time.

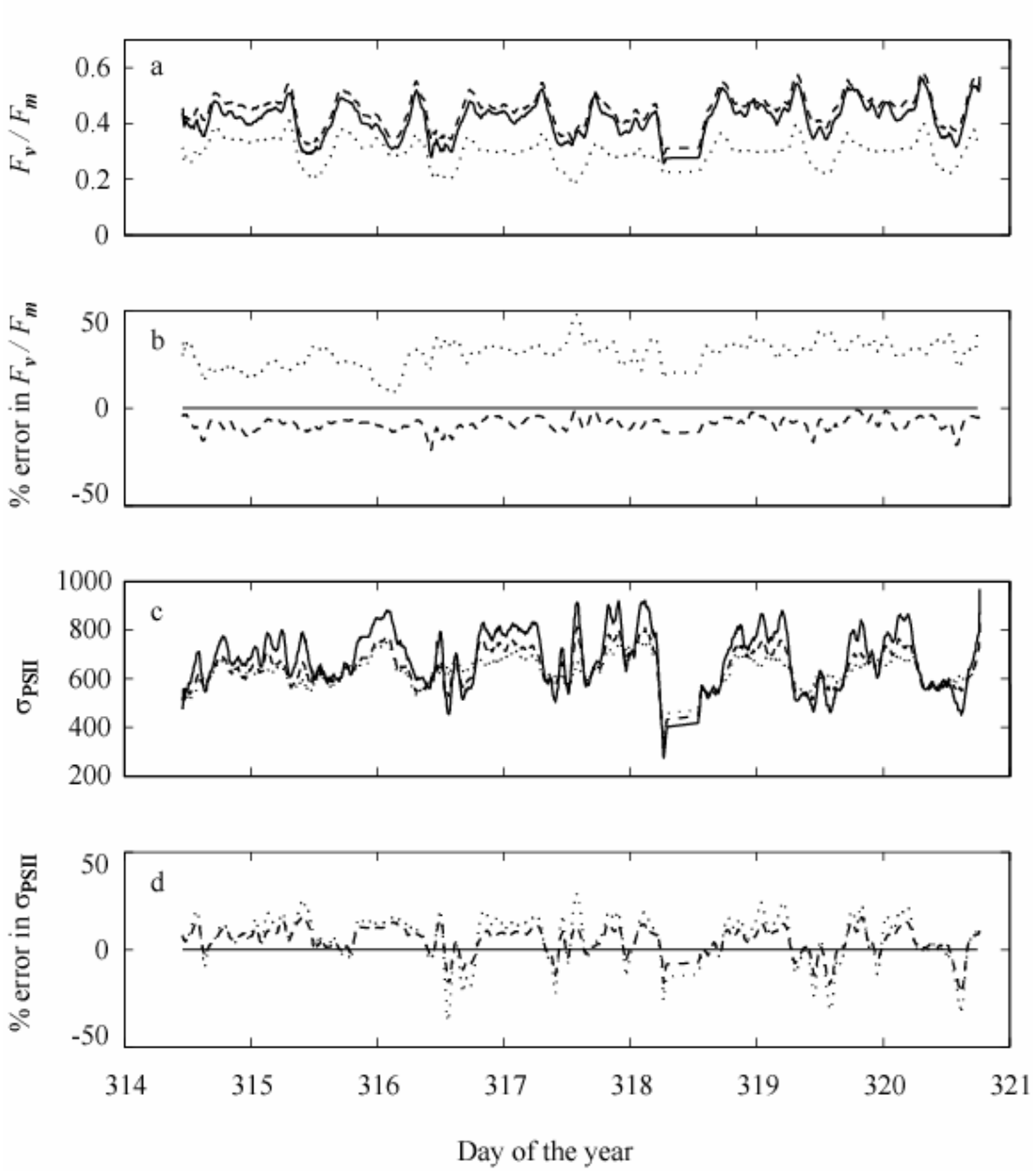

Figure 7-7. Time series of a) Fv/Fm and c) σPSII for 6 d at Station ALOHA, showing differences between uncorrected, blank corrected, and fully corrected parameters. The percent error between the fully corrected time series, and the uncorrected or partially corrected data, are shown in b) and d) as dotted and dashed lines, respectively.

Table 7-1. A table of the exponentials for each IRF. Checkmarks above a column indicate that a one-way analysis of variance indicated significant differences in this fitted parameter between gain settings ($\alpha = 0.05$). Values of $\tau 3$ in italics indicate fitted values that were constrained by a parameter bound and likely represent incorrect fits; the mean and standard deviation computed when omitting these data are also indicated by italics.

Gain	Filter (OD)	τ_1	τ_2	τ_3	α_1	α_2	α_3
			✓				✓
1	0.1	0.001	25.48	104.53	0.84	0.07	0.10
	0.2	0.001	32.95	133.28	0.84	0.09	0.07
	0.3	0.001	26.95	122.45	0.83	0.08	0.09
	0.4	0.001	33.29	122.59	0.85	0.08	0.08
	0.5	0.001	37.94	133.23	0.84	0.09	0.07
	0.6	0.001	31.28	107.63	0.84	0.07	0.10
	1.0	0.001	43.59	102.53	0.84	0.08	0.08
4	0.6	0.001	25.23	113.87	0.83	0.07	0.10
	0.7	0.001	28.75	121.22	0.82	0.09	0.10
	0.8	0.001	23.54	98.31	0.83	0.06	0.11
	0.9	0.001	26.34	106.02	0.82	0.08	0.09
	1.0	0.001	29.85	171.99	0.82	0.10	0.08
	1.1	0.001	30.94	164.65	0.82	0.10	0.08
	1.2	0.001	28.13	119.14	0.82	0.09	0.09
	1.3	0.001	32.34	86.71	0.84	0.06	0.11
	1.4	0.001	37.14	192.26	0.83	0.11	0.06
16	1.1	0.0011	27.28	159.28	0.81	0.09	0.10
	1.2	0.0011	40.75	141.11	0.82	0.11	0.07
	1.3	0.001	25.37	208.17	0.78	0.13	0.09
	1.4	0.0011	24.83	181.64	0.81	0.11	0.08
	1.5	0.001	46.09	100.89	0.82	0.11	0.07
	1.6	0.001	45.11	182.38	0.75	0.16	0.09
64	1.6	0.001	30.69	269.40	0.80	0.09	0.11
	1.7	0.001	25.63	*300.00*	0.77	0.12	0.11
	1.8	0.001	29.03	*300.00*	0.77	0.13	0.09
	1.9	0.001	42.57	164.44	0.83	0.09	0.09
	2	0.001	47.68	156.44	0.81	0.13	0.07
Mean		0.001	32.55	154.23 *(142.57)*	0.82	0.10	0.10
Standard deviation		0	7.37	58.64 *(42.57)*	0.02	0.02	0.01