PHOTOSYNTHETIC ENERGY CONVERSION EFFICIENCY

IN THE OCEAN

By

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ABSTRACT OF THE DISSERTATION

Photosynthetic energy conversion efficiency in the ocean

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The foundation of almost every ecosystem on Earth relies on photosynthetic organisms to convert sunlight energy into chemical bond energy. In aquatic ecosystems a diverse group of single celled organisms called phytoplankton are the prevalent gateway for biological energy, responsible for nearly half of Earth’s net primary production. Consequently, phytoplankton play a vital role not only in the dynamics of their respective environment, but also in global geochemical cycles. The first step in the photosynthetic process is the absorption of light energy, which can then drive a photochemical reaction, or alternatively dissipate via fluorescence or thermal dissipation. The efficiency of each pathway and the partitions between them collectively denote the physiological state of phytoplankton, which ultimately controls phytoplankton primary production. The research presented in this dissertation examines the mechanisms by which phytoplankton physiologically acclimate and adapt to rapid variations in nutrients and light. The methodological approach in this research relies on simultaneous measurements of chlorophyll a variable fluorescence and fluorescence lifetime in a laboratory study and in two oceanographic cruises. With this approach both the photochemical and fluorescence emission pathways efficiencies are directly measured, and the thermal dissipation efficiency is inferred. In chapter 1, I present a review of the topic. In chapter 2, I examine the role a family of LHCx proteins plays in photoprotection and regulation of the light harvesting complex functional size in diatoms. In chapter 3, focused on the West Antarctic
Peninsula, I demonstrate the potential simultaneous measurements of the photochemical and fluorescence efficiencies have as a rapid diagnostic tool for *in situ* assessments of phytoplankton physiology in response to iron limitation. In chapter 4, I examine dynamics in phytoplankton physiology across the Equatorial Atlantic Ocean in response to infrequent upwelling events.
Prior Publications

Chapter 2 was published in its entirety and has the following citation: “Buck, J. M.*, J. Sherman*, C. R. Bártulos, and others. 2019. Lhcx proteins provide photoprotection via thermal dissipation of absorbed light in the diatom *Phaeodactylum tricornutum*. Nat. Commun. 10: 4167. doi:10.1038/s41467-019-12043-6”

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Dedication

For my wife Adi and children, Eitan and Yali, the wind in my sails and a safe harbor from the storm
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Chapter 1: Introduction to Photosynthetic Energy Conversion Efficiency

This dissertation is focused on understanding the variations in photosynthetic energy conversion efficiency in marine phytoplankton in the global oceans and their causes. In that effort, I have worked both in the laboratory and at sea, using two, distinctly different, chlorophyll fluorescence techniques, to quantify photosynthetic energy conversion efficiency from a biophysical perspective. In this introductory chapter, I review and outline my experimental research.

1.1 The evolution of photosynthesis and phytoplankton

Life on Earth is driven by solar energy which can be converted by photosynthetic organisms to chemical bond energy. In this process, inorganic carbon (e.g., CO₂) is chemically reduced to organic carbon (Blankenship 2014), which is used to build organic matter (i.e., biomass). As inorganic carbon is thermodynamically stable, absorbed photon energy (hν) provides the free energy needed to drive a series of photochemical reactions that lead to the oxidization of an electron donor (Falkowski and Raven 2007; Fischer et al. 2016). Anoxygenic photosynthesis, which evolved ~3.5 billion years ago (Ga), uses electrons provided by the oxidation of various molecules such as H₂S, HS⁻, H₂. In contrast, oxygenic photosynthesis uses electrons derived from the oxidation of water (the only biological process capable of such a feat), which also produces molecular oxygen (O₂) as a by-product (Falkowski et al. 2004a; Blankenship 2010; Fischer et al. 2016). All oxygenic photosynthesizers ubiquitously utilize chlorophyll a (Chl a) as their primary pigment. The
evolution of oxygenic photosynthesis between 3.2 and 2.7 Ga and the introduction of O$_2$ to the atmosphere at 2.4 Ga paved the way for the evolution of multicellular animals (Falkowski 1994; Falkowski and Raven 2007; Blankenship 2014). In this review, photosynthesis denotes oxygen evolution.

From an ecological perspective, the energy and biomass produced by photosynthesizers forms the base of the global food web, and as such is termed primary productivity. Approximately 45% of the global net primary production today occurs in aquatic systems (Field et al. 1998) primarily by phytoplankton, a group of polyphyletic single cell organisms (Falkowski 1994), which account for less than 1% of the global photosynthetic biomass (Field et al. 1998; Falkowski et al. 2004a). Modern phytoplankton include cyanobacteria, the only extant prokaryote capable of oxygenic photosynthesis, and eukaryotic phytoplankton which are distributed across several phyla (Falkowski et al. 2004a).

Photosynthetic eukaryotes are hypothesized to result from a series of endosymbiotic events (Margulis 1970). The first photosynthetic eukaryotic cell engulfed a photosynthetic cyanobacterium, the primary endosymbiosis event. Over time, through gene loss processes, the cyanobacterium was reduced into the chloroplast, a membrane-bounded organelle (plastid) housing the photosynthetic apparatus where the photochemical reactions occur. This resulted in the evolution of three clades of photosynthetic eukaryotes, differentiated by the accessory pigments incorporated in the plastid alongside Chl $a$. The first clade, the green lineage, which also gave rise to all land plants, utilized Chl $b$. A second clade, the red lineage used phycobilins. Following the primary endosymbiosis additional secondary and tertiary endosymbiosis further diversified the lineages and introduced the use of additional chlorophylls. In the modern ocean the vast majority of photosynthetic eukaryotes belong to the red lineage. These include among others; cryptophytes, which use Chl $c$ and phycobilins, haptophytes (e.g., coccolithophores) and heterokonts (e.g.,
diatoms) which both use Chl c and fucoxanthins (a type of carotenoid) (Margulis 1970; Falkowski et al. 2004a; Blankenship 2010).

1.2 The photosynthetic reaction and apparatus

In general, the photosynthetic process comprises of two coupled steps. The first, a series of light dependent reactions, provides reductants that are used in the second series of light independent reactions which lead to the fixation of CO₂. Here I will focus on the light dependent reactions.

The photosynthetic machinery where the light dependent reactions occur is composed of an ensemble of protein-pigment structures associated with a lipid bilayer membrane, the thylakoid. These protein-pigment structures are either peripheral, anchored to one side of the thylakoid, or span the width of the membrane (i.e., integral). The spatial organization and orientation of the photosynthetic apparatus within the thylakoid membrane are critical to facilitate a vectorial flow of excited state energy, electrons, and protons during the photochemical reactions. This in turn creates gradients of electrochemical potential and protons across the membrane which is then coupled to the production of ATP and NADPH (Cramer and Knaff 1990).

The first step in photosynthesis is the absorption of light energy by a protein-pigment light harvesting complex (LHC). The LHC functions as an antenna, and as such efficiently harvests and transfers excitation energy to a reaction center (RC), where the photochemical reaction occurs (Green and Parson 2003). A photon that is successfully absorbed creates an excited electronic state in the absorbing pigment. The excited state energy is then transferred by non-radiative excitation energy transfer (EET) mechanisms through the LHC until it is eventually dissipated by one of three competing pathways (Butler 1978). The primary dissipative pathway uses the excited state energy to drive photochemistry, and the ensuing photosynthetic process. Alternatively, the excited state
energy can dissipate via fluorescence emission or by non-radiative thermal dissipation. These two pathways may seem as waste products, as they don’t contribute to photosynthesis, however they represent crucial pathways to dissipate excess light energy, thereby providing photoprotection for the reaction centers.

In all oxygenic photoautotrophs, there are two photochemical reaction centers. The first, denoted photosystem II (PSII), is where water is oxidized. For photochemistry to occur in PSII, the excitation energy needs to be transferred from the LHC to an open (i.e., reduced) PSII RC, a specialized Chl $a$ dimer (P680, named after its absorption maximum of 680 nm) that serves as the primary electron donor. Once trapped by a PSII RC the excited state energy causes a charge separation event in P680 which loses an electron to an adjacent quinone acceptor (QA). To replenish the missing electron in P680, water is ultimately oxidized, producing O$_2$ in the process. In order to avoid a charge recombination, in which the electron returns to the oxidized P680, and the absorbed solar energy is lost, the electron on the reduced QA is spatially separated by a rapid electron transfer chain to a secondary quinone (QB) and then onwards to a cytochrome $b_{6}f$ complex. The electron is transferred to a second reaction center, photosystem I (PSI), where it is further excited to a more negative electron acceptor and ultimately is combined with protons to NADPH, a biological hydrogen carrier. In addition to providing electrons, the oxidation of water also results in the buildup of protons in the inner part of the thylakoid membrane (the lumen), creating a transmembrane pH gradient. Additional protons are transported into the lumen via plastoquinones. This provides a proton motive force that is used to produce ATP.

To summarize, the first step of photosynthesis uses a light driven oxidization of water to produce ATP and NADPH used in the reduction of CO$_2$. Below, Figure 1.1 provides an illustration of the photosynthetic process and the facilitating components.
1.3 Photon energy harvest and transfer in the photosynthetic unit

Chl $a$ based photosynthesizers absorb in the visible portion of the solar spectrum (wavelengths between 400-700 nm) where the solar irradiance reaching Earth’s surface is highest. This spectral region is colloquially termed Photosynthetically Available Radiation (PAR).

Surprisingly however, sunlight is a very dilute source of energy (Mauzerall and Greenbaum 1989), particularly in the ocean where the intensity and spectral quality decreases with depth. To cope with this fact, phytoplankton LHCs are remarkably diverse, incorporating a verity of pigments able to absorb various wavelengths under a multitude of light environments (Collins et al. 2012).
(Figure 1.2a). The diversification of the pigments incorporated in the LHC is in contrast to the near ubiquitous use of Chl a as the terminal acceptor of excitation energy.

![Absorption spectra of photosynthetic light harvesting pigments. Reproduced from (Mirkovic et al. 2017).](image)

**Figure 1.2 Photosynthetic pigments and energy funnel.** a) Absorption spectra of photosynthetic light harvesting pigments. Reproduced from (Mirkovic et al. 2017). b) Schematic representation of the funnel model. Excited state energy is transferred from high energy absorbing pigments (blue light) to lower energy absorbing pigments (red light). From (Blankenship 2014)

The vital role antenna complexes play in transferring energy to a RC was first hinted at in experiments carried out by Emerson and Arnold in 1931. They estimated that roughly 2500 Chl a molecules were required to produce one molecule of O₂ following a saturating pulse of light. At the time this result was not fully understood. By 1936 Gaffron and Wohl proposed that the large number of chlorophylls act to transfer energy to a center where photochemistry occurs (Blankenship 2014; Mirkovic et al. 2017). Gaffron and Wohl argued that the harvest and transfer of excitation energy by an LHC is crucial for effective photosynthesis since the reaction center alone cannot harvest a sufficient number of photons to keep pace with the multi-electron requirement of photochemistry, a theoretical minimum of 8 photons per O₂ molecule (Mirkovic et al. 2017).
In a simplified manner, the LHC is viewed as an energetic funnel (Figure 1.2b). The outermost pigments absorb higher energy short wavelengths (blue light) and the pigments closer to the RC absorb lower energy long wavelengths (red light). In this manner, energy transfer between the pigments flows energetically downwards towards the RC.

Two main mechanisms have been proposed to explain the highly efficient transfer of energy within the antenna bed towards the RC, Förster theory for EET and molecular excitons. In Förster theory, a resonant overlap in the excited state wavefunction of a donor pigment (D) and acceptor pigment (A) drive energy transfer. The overlap manifests as a Coulombic dipole-dipole interaction of D and A (∥ D and ∥ A). The rate of energy transfer is dependent on the donor excited state lifetime and fluorescence quantum yield, the donor-acceptor relative orientation (k), distance (R\text{DA}), and spectral overlap (i.e., overlap of the donor fluorescence emission and acceptor absorbance spectrums). Förster reasoned that the dipole-dipole energy transfer rate is the square of the electronic coupling, V

\[ V^2 = \left( \frac{1}{4\pi\varepsilon_0} \frac{k |\langle \mu_D | \mu_A \rangle|^2}{R_{DA}^3} \right)^2 \text{ Equation 1.1} \]

Hence it is clear that the Förster energy transfer rates represent localized energy transfers, as there is a highly inverse distance dependency (R_{DA}^-6) (Olaya-Castro and Scholes 2011; Mirkovic et al. 2017).

The classical Förster theory holds true in the case that R_{DA} is large relative to the pigment’s size (tens of Å), resulting in a weak electronic coupling. In the case of strong electronic coupling, occurring at distances smaller then 10 Å, electronic excitation is coherently shared, through a superposition of excited state wavefunctions of several pigments, resulting in a delocalization of the excited state (Chenu and Scholes 2014). An additional element of EET emerged with the observation of long lived and significant quantum coherence in photosynthetic systems not only in
vitro at (77K) (Engel et al. 2007) and room temperature (Thieme et al. 2014) but also in vivo (Tiwari et al. 2018) based on 2D electronic spectroscopy (2DES) measurements. In quantum coherence mediated EET it is thought that the system is not only poised in terms of the probability for a certain state, but also in terms of the wavefunction amplitudes. Rapid excitation causes a superposition of the wavefunctions, and as they dephase the wavefunctions interact, undergoing constructive and/or destructive interference which facilitates or suppresses EET. In this manner, the excited state “senses” all possible deexcitation pathways and “chooses” the most efficient one, thereby steering the energy migration (Engel et al. 2007).

Regardless of the exact EET mechanism within the LHC, we see that the specific spatial organization of each pigment within the scaffolding protein and the surrounding complexes in the LHC play a critical role for photosynthesis. More importantly, this spatial organization can be rapidly regulated in order to control the flux of energy to the RC, as will be discussed in this dissertation.

1.4 Chlorophyll a fluorescence

As mentioned above, two other pathways compete with photochemistry to dissipate the excited state energy, namely fluorescence, and heat. The efficiency of each pathway to dissipate the absorbed energy and the partitions between them collectively denote the physiological state of phytoplankton which is critical in regulating primary productivity. Spectroscopic measurements of fluorescence have proven vital to our understanding of photosynthesis for nearly a century.

1.4.1 Chlorophyll a variable fluorescence
As early as 1834, Sir David Brewster observed that alcohol extracts of laurel leaves emit red light upon exposure to a strong beam of light at a shorter wavelength. In 1852, Stokes termed this phenomenon fluorescence. During the 1930s and later, it was noted that in intact, living leaves, the intensity of Chl $a$ fluorescence is variable over the timespan of picoseconds to minutes (later termed the Kautsky effect) (Figure 1.3) (Kautsky and Hirsch 1931; Mauzerall 1972; Govindjee 2004). In the years since, Chl $a$ fluorescence has been widely used to study a plethora of aspects relating to phytoplankton biomass, photosynthetic energy transfer rates and efficiency (Falkowski, Koblizek, Gorbunov, & Kolber, 2004).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.3.png}
\caption{The Kautsky effect and variable fluorescence. a) A schematic representation of changes in fluorescence intensity over time as seen by a naked eye. From (Kautsky and Hirsch 1931). b) Time evolution of fluorescence yield measured at 685 nm in Chlorella following a single saturating pulse of light. From (Mauzerall 1972)}
\end{figure}

A notable advancement in the study of phytoplankton photophysiology was the introduction of pump-probe type fluorometers to study the variable nature of Chl $a$ fluorescence \textit{in-vivo}, followed by the development of fast rate repetition fluorescence. The basis of the variable fluorescence method is derived from Butler’s model of competing pathways for dissipation of light induced excited state energy (Butler 1978). Butler showed that the quantum yield (efficiency) of each pathway, denoted by $\Phi$, which is the ratio between the number of photons used in a specific
pathway to the total number of photons absorbed, was dependent on the rate constants of each pathway; photochemistry, fluorescence, and heat ($k_p$, $k_f$ and $k_T$ respectively). In this formalism, the $\Phi$ of a certain pathway is equal to the ratio of that pathway’s rate constant and the sum of the three rate constants.

Following Butler’s formalism, the fluorescence yield of low light acclimated photosynthetic organisms with open PSII RCs (i.e., the primary electron acceptor, $Q_a$, is oxidized) is expressed as

$$\Phi_{F_0} = \frac{k_F}{k_F + k_T + k_P} \quad \text{Equation 1.2}$$

In this case, fluorescence yield is minimal ($F_0$) since photochemistry is maximal (photochemical quenching of fluorescence). However, once a saturating pulse of light causes a closure of all PSII RCs (all $Q_a$ are reduced), $k_p$ diminishes to zero and fluorescence yield rises to a maximum ($F_m$) and is expressed as

$$\Phi_{F_m} = \frac{k_F}{k_F + k_T} \quad \text{Equation 1.3}$$

Since all fluorescence at room temperature arises from PSII, the ratio of the variable fluorescence ($F_v = F_m - F_0$) and the maximal fluorescence ($F_m$) provides the quantum yield of PSII photochemistry ($\Phi_{PSII}$), more commonly referred to as simply $F_v/F_m$

$$\Phi_{PSII} = \frac{F_v}{F_m} = \frac{k_P}{k_F + k_T + k_P} \quad \text{Equation 1.4}$$

In addition, the rate of fluorescence rise between $F_0$ and $F_m$ is proportional to the effective absorption cross section of PSII ($\sigma_{PSII}$), which represents the probability of an absorbed photon (at a specific wavelength) to successfully result in a photochemical reaction in PSII (Kolber et al.}
1998). Abstractly, $\sigma_{\text{PSII}}$ represents the size of the LHC antenna actively harvesting and transferring light energy to a PSII RC (Falkowski and Raven 2007).

Several other photophysiological parameters can be derived from variable fluorescence. For example, changes in $F_v/F_m$ in response to increasing light intensity (PI curves) are used to calculate the electron transfer rates through PSII ($\text{ETR}_{\text{PSII}}$) (Kolber et al. 1998). Similarly, the kinetics of fluorescence relaxation indicate the rate of $Q_a$ re-oxidation, and can also be used to derive the electron transfer rates (Gorbunov and Falkowski 2020).

Aside from dynamics in photochemistry, variable fluorescence provides an avenue to study an additional source of variability in fluorescence yields ($\Phi_F$), non-photochemical quenching (NPQ). Discovery of this quenching mechanism originates in field observations that fluorescence varied throughout the day, while Chl $a$ concentrations remained consistent (Owens et al. 1980). NPQ represents an umbrella term for different mechanisms that functionally cause an increase of $k_T$ at the expanse of $k_F$ and $k_P$. NPQ mechanisms play a vital role in protecting the photosynthetic system from photooxidative damage under high light levels (Kuzminov and Gorbunov 2016). For example, NPQ mechanisms have been shown to drive a reduction of $k_T$ in diatoms, thereby reducing excitation pressure on the reaction center at higher light levels (Buck et al. 2019). Figure 1.4a provides an illustration of a variable fluorescence transient, measured by a Fluorescence Induction and Relaxation instrument (FIRe), and the associated photophysiological parameters derived from it.

1.4.2 Chlorophyll $a$ fluorescence lifetimes

Until recently, the majority of research focused on understanding the nature of $\Phi_{\text{PSII}}$. However, in order to fully “close” the energy budget of absorbed photons a second pathway needs
to be measured concurrently to $\Phi_{\text{PSII}}$. While direct measurements of $\Phi_F$ are extremely hard to collect (Falkowski et al. 2017), $\Phi_F$ can be derived from fluorescence lifetime analysis.

As early as the 1950s picosecond ($10^{-12}$ s) light pulses were used to excite chlorophyll molecules and measure the kinetics of fluorescence lifetimes (Brody and Rabinowitch 1957; Holzwarth 1986). Fluorescence lifetime is the average time a molecule spends in an excited electronic state before returning to the ground state via fluorescence emission. With further technological advancement and the introduction of time correlated single photon counting it became possible to measure, with remarkable accuracy, fluorescence lifetimes \textit{in vivo} (Holzwarth 1986; Lakowicz 2006; Lin et al. 2016) and even in native phytoplankton communities in the ocean (Lin et al. 2016). In practice, Brody and Rabinowitch (1957) showed that the fluorescence lifetime is quantitatively related to the absolute quantum yield of fluorescence, $\Phi_F$, by

$$
\Phi_F = \frac{\tau}{\tau_0}
$$

Equation 1.5

Here, $\tau$ is the measured lifetime and $\tau_0$ is the natural lifetime. The natural lifetime, which is constant for a specific molecule, denotes the time it would take fluorescence emission to dissipate the excited state if it was the sole dissipative pathway. The natural lifetime of Chl $a$ is 15 ns (Brody and Rabinowitch 1957). It is important to note however that the natural lifetime cannot be measured directly. Rather, the fluorescence decay, $1/\tau_0$ (excited electronic state $\rightarrow$ ground state), is related to the reciprocal absorption reaction (ground state $\rightarrow$ excited state). The absorption is described by a molar extinction coefficient at a certain wavenumber, $\epsilon(\bar{\nu})$ (Birks and Dyson 1963). In this manner, the natural lifetime is calculated by integrating the absorption curves of a specific molecule (Chl $a$ in our case) (Brody and Rabinowitch 1957). Brody (2002) summarized the calculation as follows
\[ \frac{1}{\tau_0} = 8\pi n^2 c \ln 10 \int \varepsilon_m(2\nu_0 - \nu)^2 \frac{d\nu}{\nu} N 10^{-3} \]  

Equation 1.6

Here, the integration limits cover the absorption spectrum of the first excited state.

Since there are a number of possible competing deexcitation pathways (particularly \textit{in vivo}), \( \tau \) is inherently shorter then \( \tau_0 \) (Brody 2002; Falkowski et al. 2017) and reflects the physiological state of the cell. For example, when PSII RC are open and photochemistry is maximal (\( F_0 \)) \( \tau \) is short (low \( \Phi_F \)), and when the RCs are closed (\( F_m \)) \( \tau \) increases (higher \( \Phi_F \)) (Figure 1.4). The various NPQ mechanisms employed to reduce photooxidative damage under high irradiances, functionally increase \( \Phi_T \) at the expanse of \( \Phi_P \) and \( \Phi_F \) (Figure 1.4). Moreover, the activation of NPQ causes the predicted inverse linear relationship between \( \Phi_P \) and \( \Phi_F \) from Butler’s model to become highly non-linear (Lin et al. 2016). Lastly, it is important to note that the observed \( \tau \) does not follow a single exponential decay, but rather it comprises several decay rates that can be ascribed to various components of the photosynthetic unit and phycological state (Holzwarth 1986; Kuzminov and Gorbunov 2016).

\[ \frac{1}{\tau_0} = 8\pi n^2 c \ln 10 \int \varepsilon_m(2\nu_0 - \nu)^2 \frac{d\nu}{\nu} N 10^{-3} \]

Figure 1.4 Variable fluorescence and fluorescence lifetime representative traces. a) Variable fluorescence trace obtained by a fast repetition rate fluorescence type instrument (FIRe). The various parameters derived from the trace are marked. b) Picosecond fluorescence lifetimes of Chl \textit{a} at three different physiological states (in black); \( F_0 \), \( F_m \) and \( F_{NPQ} \). Measurements obtained with a PicoLiF instrument. From (Falkowski et al. 2017)
In order to holistically study phytoplankton physiology in the lab and across the global ocean, a pair of instruments were designed and constructed to simultaneously measure variable fluorescence (FIRe) and fluorescence lifetimes (Picosecond Lifetime Fluorescence, PicoLiF) in vivo, thereby obtaining $\Phi_P$ and $\Phi_F$ directly. As the sum of all three dissipative pathways is unity, $\Phi_T$ can be derived by subtraction, and the energy budget can be closed (Lin et al. 2016).

### 1.5 Phytoplankton physiology, and productivity from satellite observations

Traditional ship-board measurements of phytoplankton productivity and physiology are significantly limited to a small fraction of the ocean surface during infrequent expeditions. For this reason, satellite-based observations of ocean color have become an increasingly important tool to study phytoplankton dynamics across the global ocean surface, with particular interest in deriving primary productivity rates. The primary parameter related to phytoplankton that can be retrieved remotely is Chl $a$ concentration, which is calculated based on the atmospherically corrected ratio of green to blue water leaving radiances (O’Reilly et al. 1998; Bloudeau-Patissier et al. 2014). Chl $a$ concentration has been measured by satellites since the late 1970s with the lunch of the Coastal Zone Color Scanner Experiment (CZCS).

The Vertically Generalized Production Model (VGPM), the standard algorithm that derives primary production rates from satellite products, includes terms representing Chl $a$ concentration, the light field and the temperature dependent response of photophysiological parameters (Behrenfeld and Falkowski 1997). Over the years, several other algorithms have been developed such as the Eppley-VGPM, that incorporates an exponential function relating temperature and growth/physiology (Carr et al. 2006) or the Carbon based Production Model (CbPM) which relies on retrievals of carbon, not Chl $a$, for estimates of biomass (Behrenfeld et al. 2005; Westberry et al. 2008). In either case, these models inherently rely on an implicit understanding of the
physiological status and processes controlling it, lacking an explicit underlying mechanism. It is apparent then, that satellite primary productivity models need to incorporate more accurate, mechanistic physiological processes which can also be detected remotely.

With the launch of NASA’s Moderate-resolution Imaging Spectroradiometer (MODIS) sensor on board the Aqua satellite it became theoretically possible to derive $\Phi_F$ from satellite retrievals of solar induced fluorescence (SIF) ($\Phi_{F\text{sat}}$) (Huot et al. 2005; Behrenfeld et al. 2009; Lin et al. 2016). As the name suggests, SIF refers to the fraction of absorbed photons, originating from the sun, that are reemitted as fluorescence, in essence $\Phi_F$, which is highly related to phytoplankton physiology as discussed above. The ability to assess $\Phi_F$ from satellites provides a method to study dynamics in phytoplankton physiology on a global scale. By the 1980s it was recognized that measurements of upwelled radiance spectrum from aquatic systems displayed a distinct peak at 683nm, termed the fluorescence line height (FLH) (Neville and Gower 1977) (Figure 1.5). The FLH is the unambiguous result of SIF of phytoplankton Chl a (Abbott and Letelier 1999). The algorithm that calculates the FLH uses 3 spectral bands measured by the MODIS satellite (665.1 nm, 676.7 nm, and 746.3 nm) (Figure 1.5). These bands have a high signal to noise ratio, needed to capture the fluorescence signal which is very weak, and they are spectrally narrow to avoid atmospheric absorption features. It is important to note that while the fluorescence peak is located at 683 nm, operationally, 676.7 nm is used to avoid a nearby absorption band of oxygen at 687 nm. Prior to the FLH calculation the upwelled radiance undergoes calibration, scan geometry and atmospheric corrections.

In general, the FLH algorithm assesses the increase in water-leaving radiance at 676.7 nm versus a linear baseline of estimated water-leaving radiance between 665.1 nm and 746.3 nm (Figure 1.5).
A notable use of $\Phi_{\text{Fsat}}$ is in the assessment of nutrient stress, mainly of iron. For example, it was reported that high values of $\Phi_{\text{Fsat}}$ were observed in regions of the ocean where iron is known to be a limiting factor. However, the opposite was observed as well, i.e., in certain regions where iron limitation is well established low values of $\Phi_{\text{Fsat}}$ were observed (Behrenfeld et al. 2009). Hence the relationship between high $\Phi_{\text{Fsat}}$ and iron stress is not as straightforward as assumed, and more research is needed.

Figure 1.5 FLH algorithm schematic. Blue, green, and red dashed lines represent the three MODIS spectral bands used by the FLH algorithm. Blue and purple lines show the upwelled radiance at the surface of the ocean for two Chl $a$ concentrations: 0.01 and 10 mg/m$^3$. Note the use of two Y-axes for the two Chl $a$ concentrations. Black line represents the baseline (in this case for the case of 10 mg/m$^3$ Chl $a$ concentration) used to calculate the FLH. From (Abbott and Letelier 1999).
A main issue hindering the broad scale use of $\Phi_{F_{sat}}$ to study phytoplankton physiology and improve primary productivity models is a poor link between satellite derived $\Phi_{F_{sat}}$ and absolute $\Phi_F$ measured in situ, which was not possible to do until the development of the PicoLiF.

A global comparison of $\Phi_F$ from in situ and satellite retrievals has shown a weak correlation between the two measurements, but more notably for this dissertation, it showed that remotely sensed values far exceed the range of variability seen in in situ measurements (Lin et al. 2016). A few main sources of variability include iron stress, boundary regions (sea ice or cloud edges) effects on satellite spectral estimates and extremely low Chl a concentration in oligotrophic regions of the ocean, where FLH is very low. Underlying these sources are varying control mechanisms on phytoplankton photophysiology, which need to be understood.

A main goal of this dissertation is to provide a fundamental understanding of the mechanisms that control the way phytoplankton utilize and dissipate solar energy in the photosynthetic unit. This is crucial in order to better incorporate phytoplankton physiology in future algorithms of primary productivity resulting in accurate global estimates. This is even more true when considering the effects of climate driven changes to the physical and chemical setting of different ocean regions, which will cause changes in phytoplankton photophysiology. The ability to rapidly assess phytoplankton physiology from satellites on a global scale potentially exists. However, we still lack the mechanistic understanding of these dynamics. The results of this dissertation will contribute and push forward our knowledge of the mechanisms controlling energy harvest, transfer, and dissipation pathways across several levels of complexities.
Chapter 2: Lhcx proteins provide photoprotection via thermal dissipation of absorbed light in the diatom *Phaeodactylum tricornutum*

Diatoms possess an impressive capacity for rapidly inducible thermal dissipation of excess absorbed energy (qE), provided by the xanthophyll diatoxanthin and Lhcx proteins. By knocking out the *Lhcx1* and the *Lhcx2* genes individually in *Phaeodactylum tricornutum* strain 4 and complementing the knockout lines with different Lhcx proteins, multiple mutants with varying qE capacities are obtained, ranging from zero to high values. We demonstrate that qE is entirely dependent on the concerted action of diatoxanthin and Lhcx proteins, with Lhcx1, Lhcx2 and Lhcx3 having similar functions. Moreover, we establish a clear link between Lhcx1/2/3 mediated inducible thermal energy dissipation and the reduction in the functional absorption cross section of photosystem II. This regulation of the functional absorption cross section can be tuned by altered Lhcx protein expression in response to environmental conditions. Our results provide a holistic understanding of the rapidly inducible thermal energy dissipation process and its mechanistic implications in diatoms.

2.1 Introduction

Upon absorption of a photon, the singlet excited state of a chlorophyll *a* molecule has three major fates: photochemistry, heat dissipation, and fluorescence emission. The optimization of photochemistry is achieved when the rate of photon absorption is equal to the rate of electron transfer. While this requires a large absorption cross section of photosystem II under low light
conditions, the same absorption cross section would lead to an overflow of energy in the photosynthetic system under high light conditions, resulting in massive oxidative damage. Hence, in oxygenic photosynthetic organisms, photon capture can be regulated by adjusting the cross section of photosystem II on time scales of minutes. In practice, this phenomenon leads to changes in the balance between excitons directed to reaction centers and those that are dissipated as heat.

There are a number of photoprotective mechanisms that thermally dissipate excess absorbed energy as heat; collectively these are called Non-Photochemical Quenching (NPQ). This phenomenon is present in all photosynthetic eukaryotes and in many cyanobacteria (Niyogi and Truong 2013; Goss and Lepetit 2015; Derks et al. 2015), and is characterized by a downregulation of chlorophyll fluorescence at high irradiance. Mechanistically, NPQ comprises several processes (Goss and Lepetit 2015; Malnoë 2018). The most rapid NPQ component, called energy-dependent quenching (qE), is strongly dependent on light intensity, reflecting the balance between fluorescence quenching and an increase in thermal dissipation (Genty et al. 1990). qE has been shown to decrease the functional absorption cross section of PSII ($\sigma_{\text{PSII}}$), thus reducing the flux of absorbed energy into photochemistry (Genty et al. 1990; Gorbunov et al. 2001; Koblížek et al. 2001; Holzwarth et al. 2009; Xu et al. 2018; Tian et al. 2019). Consequently, light-enhanced thermal dissipation reduces the excitation pressure and hence the probability of photooxidative damage. In higher plants, however, the correlation between the onset of qE and the reduction of $\sigma_{\text{PSII}}$ has recently been challenged (Belgio et al. 2014).

qE has been found in several major algal lineages as well as in mosses and land plants (Goss and Lepetit 2015; Pinnola and Bassi 2018). The initiation of qE is driven by the establishment of a pH gradient between the thylakoid lumen and plastid stroma ($\Delta pH$) upon excess light exposure. It often also correlates with the conversion of xanthophyll pigments in the so-called xanthophyll cycle, which, for diatoms, is the conversion of diadinoxanthin (Dd) into diatoxanthin (Dt) (Olaizola
et al. 1994; Lavaud et al. 2002; Goss et al. 2006). In addition, in plants, qE requires the presence of PsbS, a specialized protein that belongs to the light harvesting complex protein (LHC) family (Li et al. 2000). In contrast, the green alga *Chlamydomonas reinhardtii* requires Lhcsr proteins for qE (Peers et al. 2009). Similarly, in diatoms the importance of the Lhcsr related Lhcx1 proteins for qE has been proven by silencing the *Lhcx1* genes in *Phaeodactylum tricornutum* (Bailleul et al. 2010) and *Cyclotella meneghiniana* (Ghazaryan et al. 2016).

Diatoms are unicellular microalgae with complex plastids, which were acquired during serial secondary endosymbiosis from a green and a red alga (Dorrell et al. 2017). They constitute one of the most important phytoplankton groups in terms of productivity (Geider et al. 2001) and biodiversity (Mann and Vanormelingen 2013). Their abundance is also based on their capacity to thrive in turbulent waters (e.g., coasts or upwelling regions), where they can exploit the huge amount of available nutrients (Tozzi et al. 2004). This goes in line with their variable qE capacity depending on the light characteristics of the respective habitats (Lavaud et al. 2007; Lavaud and Goss 2014; Barnett et al. 2015). In contrast to *C. reinhardtii*, which contains only two different Lhcsr proteins, many diatoms possess multiple Lhcx proteins, e.g., *Fragilariopsis cylindrus* contains 11 Lhcx (Mock et al. 2017). For several diatom species, qE capacity correlates with the expression of different Lhcx proteins, which indicates the involvement of various Lhcx proteins in triggering qE under different environmental conditions (Zhu and Green 2010; Zhu et al. 2010; Lepetit et al. 2013, 2017; Laviale et al. 2015; Taddei et al. 2016, 2018; Blommaert et al. 2017). *P. tricornutum* contains only four Lhcx proteins (Bowler et al. 2008) and therefore is a particularly good model to study the impact of individual Lhcx proteins on qE capacity. These four proteins are structurally similar, but are differentially expressed under varying environmental conditions (Serif et al. 2017).
Here, we used a model diatom, *P. tricornutum*, to examine two basic questions: First, besides Lhcx1, do other Lhcx proteins equally confer qE capacity and if so, is it correlated with the xanthophyll cycle. Second, is qE in diatoms correlated with a reduction of $\sigma_{PSII}$. Experimentally, we knocked out the *Lhcx1* gene in *P. tricornutum* that resulted in mutant lines devoid of qE. Then, we individually over-expressed all other Lhcx proteins in this knockout background. In addition, we knocked out the *Lhcx2* gene and expressed the Lhcx2 and Lhcx3 protein in this knockout background. This experimental design allowed us to quantify the influence of each Lhcx protein on qE and $\sigma_{PSII}$.

### 2.2 Materials & methods

#### 2.2.1 Cell culturing

Experiments were performed in *Phaeodactylum tricornutum* strain 4 (Pt4, UTEX 646). All strains were grown in batch cultures on a shaker at 20°C in a 16 h day/8 h night cycle exposed to white light with an intensity of 40 µmol photons m$^{-2}$ s$^{-1}$ (onset at 8:00 a.m.) defined as low light (LL). Cells were cultured in sterile Provasoli's enriched F/2 seawater using Tropic Marin Classic artificial sea salts (Dr. Biener, Germany) with 16.6 g sea salt per 1 L medium. For all experiments, only cultures in the logarithmic growth phase were used, i.e., a chlorophyll *a* concentration of 1.5 mg L$^{-1}$ or a cell concentration of 3.5 million cells mL$^{-1}$ at maximum. Therefore, cultures were regularly diluted with fresh F/2-medium in order to avoid any nutrient limitation, which would strongly influence Lhcx expression (Taddei et al. 2016). Chlorophyll *a* concentration was determined as described in ref. (Lepetit et al. 2013). Cell counts were obtained using a Multisizer 3 Coulter Counter (Beckman, USA). For long term light stress experiments, cells were exposed to white light with an intensity of ~400 µmol photons m$^{-2}$ s$^{-1}$ for 24 hours. Light intensities were measured with a spherical quantum sensor (US-SQS/L, Walz, Germany).
2.2.2 Generation of TALEN and complementation constructs

TALEN-knockout plasmids for Lhcx1 and Lhcx2 were generated following the procedure for creating TALEN-KO lines in *P. tricornutum* by (Serif et al. 2017). This method relies on Golden Gate reactions, where digestion with TypeIIIs restriction enzymes and ligation are carried out in one step (Sanjana et al. 2012). For constructing the specific TALENs, we assembled the respective TALEN monomers into the respective backbone vectors available at Addgene. For Lhcx1 we constructed two TALEN pairs, targeting two different sites in the *Lhcx1* gene. For Lhcx2 we constructed one TALEN pair. All genomic target sites contained a thymine before the actual TALE recognition site. For each TALEN we assembled 18 monomers, each with a repeat variable di-residue (RVD) which recognizes one specific DNA base. The 19th monomer is only a half monomer and is included in the respective TALEN backbones (Serif et al. 2017). Supplementary Table 2.1 indicates the RVDs of the respective TALEN constructs and the corresponding DNA target sequence as well as the backbone vector (available at Addgene) which contains the half monomer, the FokI nuclease coding region as well as the antibiotics resistance cassette.

In order to complement/supplement our KO strains, *Lhcx1, Lhcx2, Lhcx3* and *Lhcx4* were cloned inside a modified pPha-T1 vector pPTbsr, respectively, which instead of a Bleomycin resistance cassette contains a Blasticidin-S resistance cassette (Buck et al. 2018). The *FcpA* promoter of this vector was removed via deletion PCR and replaced by two different *Lhcx* promoters: The genes designated to complement the x1KO background were cloned in between the *Lhcx1* promoter (starting 721 bases upstream to the *Lhcx1* gene start codon) and terminator (617 bases downstream of the *Lhcx1* stop codon), while for the genes designated to complement the x2KO strain the *Lhcx2* promoter (605 bp upstream of translation start of *Lhcx2*) and terminator (422 bp downstream of *Lhcx2* stop codon) was used. When the KO lines were complemented with
their original gene (Lhcx1 for x1KO, Lhcx2 for x2KO), modified gene sequences using synonymous codons were used, in order to prevent the TALENs from cutting the newly introduced genes. Except for Lhcx1, all genes were cloned including their respective introns. Additionally, the modified Lhcx1 gene was also cloned in between the original FcpA promoter and terminator of the pPTbsr vector.

Sequences for the Lhcx genes of Pt4 are slightly different from those of the sequenced Pt1 strain (https://genome.jgi.doe.gov/Phatr2/Phatr2.home.html) (Lhcx1 JGI ID: 27278; Lhcx2 JGI ID: 44733; Lhcx3 JGI ID: 44733; Lhcx4 JGI ID: 38720). All gene, promoter and terminator sequences were obtained by amplifying genomic DNA with specific primers using Kapa Hifi DNA Polymerase (Roche, Switzerland) and purification of the PCR product from agarose gels using Geneclean Turbo Kit (MP Biomedicals, Germany) except for the modified Lhcx1 and Lhcx2 sequences, which were ordered from BioCat (Germany). Correct amplification and vector integration were verified by sequencing (Microsynth AG, Switzerland). All sequences for the genes, promoters and terminators used in this study are provided in Supplementary Table 2.2. As an example, the transformation vector maps for Lhcx3 complementing the x1KO and x2KO line, respectively, are depicted in Supplementary Figure 2.19.

2.2.3 Generation of mutated P. tricornutum cell lines

Wild-type Pt4 cells were biolistically transformed with the TALEN bearing vectors according to (Kroth 2007). Positive clones were selected on Zeocin™ (75 µg mL⁻¹; Invitrogen, USA) and Nourseothricin (150 µg mL⁻¹; ClonNAT, Werner Bioagents, Germany) containing solid medium plates containing 1.2% Bacto Agar (BD, USA) in F/2 medium (16.6 g L⁻¹ sea salt). Pre-screening of the colonies was performed using an Imaging PAM system (Walz, Germany), searching for clones with altered NPQ characteristic. Further characterization included Western
blot, PCR and DNA sequencing. Confirmed KO clones were spread on individual agar plates in a suitable dilution to obtain clones from single cells. Three out of these were reanalyzed for each knockout line and one of each KO line was used for all follow-up experiments. In addition, whole genome sequencing was done on the three created x1KO lines. All initial KO clones observed were homogeneous, i.e., clones obtained from single cells from the initial clones all showed the same knockout phenotype.

Two obtained x1KO lines were complemented with a modified Lhcx1 gene by a second biolistic transformation using Blasticidin-S (4 µg mL⁻¹) containing solid medium plates in low-salt F/2 medium (8.3 g L⁻¹ sea salt) for selection. Additionally, the native Lhcx2, Lhcx3 and Lhcx4 genes were transformed in the x1KO_1a line. The x2KO line was complemented with a modified Lhcx2 gene, and also transformed with the native Lhcx3 gene.

2.2.4 DNA isolation and allele specific PCR

Genomic DNA was isolated using the Nexttec™ 1step DNA isolation kit (Biozym, Germany) according to the manufacturer's instructions. A cell pellet corresponding to 10 mL of culture was used as starting material. The concentration of genomic DNA was measured with a Nanodrop 2000 (Thermo Fisher Scientific, Germany). In order to prove that both alleles were mutated, allele specific PCR was applied for the x2KO mutants only, as the Lhcx1 gene of Pt4 does not contain allele specific differences in the TALEN targeted region. The sequences of the two primer pairs for both alleles as well as of all following primers are specified in Supplementary Table 2.3. The gene sequence for Lhcx2 of Pt4 is depicted in Supplementary Figure 2.20. Allele specific PCR, however, provided no PCR product in the x2KO line (Supplementary Figure 2.6a). We additionally used the primer combination Lhcx2_prom-fw and Lhcx2_term-rev, which amplifies the Lhcx2 promoter, Lhcx2 gene and Lhcx2 terminator in a length of ~1970 bp in wild-
type. In the x2KO mutant, in one of the alleles a deletion of 918 bp occurred (Supplementary Figure 2.6). The sequence of this mutated allele is indicated in Supplementary Figure 2.21.

To screen the x1KO mutants and the complemented lines, we used different primer combinations. The primer pair Lhcx1_all-fw and Lhcx1_all-rev amplifies 102 bp at the 3’-end of the Lhcx1 gene in the Pt4 strain, the x1KO strains and the Lhcx1 complemented lines. It served as a control. The primer pair Lhcx1_wild-type-fw and Lhcx1_wild-type-rev amplifies a 347 bp sequence only in the wild-type strain, while the primer pair Lhcx1_comp-fw and Lhcx1_wild-type-rev amplifies 262 bp only in the complemented x1KO lines. Note that for the two latter primer combinations only the forward primer was different, binding either to the wild-type sequence of Lhcx1 or the modified sequence of the complemented Lhcx1 gene with synonymous codon usage.

To screen the x1KO_1a + FcpA_x1 complemented mutants, the primer pair FcpA_Lhcx1-fw and FcpA_Lhcx1-rev amplifies a 479 bp fragment including the FcpA promoter and the first base pairs of the modified Lhcx1 gene. This combination of FcpA promoter and modified Lhcx1 gene, as well as the modified TALEN-binding sites on which FcpA_Lhcx1-rev binds, are unique for the complementation construct and do not occur in wild type cells.

PCR was performed using HiDi polymerase according to the manufacturer’s instructions for 30 cycles (MyPols, Germany), with either the primer pairs Lhcx1_wild-type-fw/Lhcx1_wild-type-rev and Lhcx1_all-fw/Lhcx1-all_rev or with Lhcx1_comp-fw/Lhcx1_wild-type-rev and Lhcx1_all-fw/Lhcx1-all_rev. PCR products were separated on 1% agarose gels.

2.2.5 Whole Genome sequencing

In order to identify the correct DNA sequence for both alleles of Lhcx1 and Lhcx2 in Pt4 and in order to verify the biallelic knockout of the Lhcx1 gene in the x1KO lines, the genomic DNA of Pt4 and the three x1KO strains were isolated using the MasterPure DNA Purification Kit
(Epicentre, USA) according to the manufacturer’s instructions and sequenced by Illumina 125 bp paired end sequencing by GATC Eurofins (Germany). Quality control for raw reads was done using FastQC. Low quality reads (quality score ≤20) were trimmed by FASTQ/A Trimmer (http://hannonlab.cshl.edu/fastx_toolkit/index.html). As no reference genome for Pt4 existed so far, we first produced a new Pt4 reference genome, by assembling and aligning the trimmed reads of Pt4 with Bowtie2 (Langmead et al. 2009) against the available genome of Pt1 CCAP 1055/1 deposited in Ensembl, which is an update of the previously deposited genome in JGI (Bowler et al. 2008). Then, the SAM files created by Bowtie2 were converted into BAM files using SAM tools (Li et al. 2009) and a new consensus sequence for Pt4 was created by aid of the BCF tools pipeline (Narasimhan et al. 2016). Finally, the quality controlled and trimmed reads of the x1KO mutants were mapped against the reads of the new Pt4 reference genome by aid of Bowtie2.

2.2.6 Fluorescence analyses

Pre-screening of mutants was performed with an Aqua-Pen (PSI Instruments, Czech Republics) or an Imaging-PAM (Walz, Germany). Fine fluorescence kinetics were recorded with a Dual-PAM or a Fluorescence Induction and Relaxation instrument (a mini-FIRe). For the Dual-PAM measurements, cells were concentrated to 10 μg chlorophyll a mL⁻¹, and 4 mM NaHCO₃ was added. Before starting experiments, cells were acclimated to dim light for 30-45 min. Kinetic measurements were performed by exposing the cells to 10 min of actinic light with an intensity of 1700 μmol photons m⁻² s⁻¹, consisting of similar proportions of red and blue light photons, followed by 18 min of recovery conditions with 40 μmol photons m⁻² s⁻¹. Saturating flashes (8000 μmol photons m⁻² s⁻¹ red light, 800 ms) were applied every 30 s. For some experiment, DTT (500 mM final concentration) was added 5 min prior to fluorescence recording. To monitor acclimation under prolonged high light, cells were exposed for 130 min to 1700 μmol photons m⁻² s⁻¹, followed by 30
min recovery conditions with 40 µmol photons m$^{-2}$ s$^{-1}$. NPQ was calculated as $F_m/F_m' - 1$. Here, we set $F_m$ to the first light step $F_m'$, as this value is usually higher than the $F_m$ obtained for dark acclimated *P. tricornutum* cells.

The variable fluorescence signatures of minimal ($F_0$) and maximal ($F_m$) fluorescence, corresponding to states where all PSII reaction centers are open or closed, respectively, as well as the functional absorption cross sections of PSII, were measured using a mini-FIRE (Gorbunov and Falkowski 2005). Fluorescence induction was achieved with blue light emitting diodes (450 nm, 30 nm half bandwidth) and measured in the red light region (680 nm, 20 nm bandwidth). An 80 µs pulse, with peak optical power of 1 W/cm$^2$, ensured that all PSII reaction centers were reduced with a single turnover of PSII. From this single turnover protocol, parameters such as $\sigma_{\text{PSII}}$, electron transfer rates, NPQ and $Y(\text{NPQ})$ can be calculated (Kuzminov and Gorbunov 2016). For all strains, these parameters were measured in response to increasing actinic light in 15 steps with 1 min acclimation at each step (i.e., light curves, 0-800 µmol photons m$^{-2}$ s$^{-1}$ of blue light), where variable fluorescence (resulting in $F'$ and $F_m'$) was recorded at the end of each light step. In addition, after each light step, the actinic light was turned off for 1 s to allow for re-opening of reaction centers. This 1 s dark period was short enough to prevent NPQ relaxation (Goss et al. 2006; Xu et al. 2018) but did allow us to directly measure $F_0'$, which is needed for the calculation of 1-$q$L. Electron transfer rates were calculated from $E \times \sigma_{\text{PSII}}' \times (F_v'/F_m')/(F_v/F_m)$ (Gorbunov et al. 2001). $Y(\text{NPQ})$ and 1-$q$L were calculated following (Xu et al. 2018). $\Sigma_{\text{PSII}}'$ was derived from $F' \rightarrow F_m'$ transition induced by 80 µs single turnover flash (Kolber et al. 1998). Using the $F' \rightarrow F_m'$ transition allowed us to make more acquisitions, improving the signal-to-noise ratio, and reducing the error in the fitting procedure. A comparison of $\sigma_{\text{PSII}}'$ and $\sigma_{\text{PSII}}'_{1s}$ (i.e., obtained after 1 s darkness) showed little difference in the values or the interpretation of our results (cf. Figure 2.5 and Supplementary Figure 2.17).
Fluorescence lifetimes were measured using a custom-built picosecond lifetime fluorometer (PicoLiF) as described in (Kuzminov and Gorbunov 2016). We measured lifetimes for \( F_0 \) and \( F_m \) levels in the dark, followed by measurements of \( F_0' \) and \( F_m' \) lifetimes during exposure to 850 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \) (blue light) for 10 min to induce NPQ. The fluorescence acquisition interval was 20 s, short enough to avoid changes in the state of the photosynthetic units. Average fluorescence lifetimes were calculated by fitting the collected data to a sum of three exponential decays, which were deconvoluted from the instrument response function (Kuzminov and Gorbunov 2016).

### 2.2.7 Pigment analysis

Pigment extraction and HPLC separation followed the protocol established by (Jakob et al. 1999). In short, pigments of cells filtered on a 1.2 \( \mu \text{m} \) isopore filters (Millipore) were extracted with a mixture of 81% methanol/9% 0.2 M ammonium acetate/10% ethyl acetate (vol/vol/vol). After centrifugation, the supernatant was injected onto a calibrated Hitachi LaChrom Elite HPLC system equipped with a 10°C-cooled autosampler and a Nucleosil 120-5 C18 column (Macherey-Nagel, Germany). Pigments were separated using a linear gradient system consisting of eluent A (90% methanol/10% 0.5 M ammonium acetate, vol/vol) and eluent B (90% methanol/10% ethyl acetate, vol/vol).

### 2.2.8 Quantitative real-time PCR (qPCR)

RNA extraction was performed using a combination of Peqgold RNAPure and Peqgold Total RNA Kit S-Line with on column DNA digest using the Peqgold DNAsel I Digest kit (VWR, Germany). cDNA synthesis was performed by means of the Primescript kit (Takara Bio Europe,
RNA was extracted from LL grown cultures in the exponential growth phase. In order to assess Lhcx transcript level of light stressed cells, the different strains were concentrated to 2 µg mL⁻¹ chlorophyll a, treated with 0.8 mM NaHCO₃ and exposed for two hours to ~700 µmol photons m⁻² s⁻¹ white light in a glass cylinder under constant water-cooling and stirring at 20°C. For the qPCR analyses, the reference gene was 18S_rRNA (Ensembl Gene ID: EPrPhatr3G00000013183) as it is well-suited for both low and high light acclimated P. tricornutum cultures (Lepetit et al. 2013). Primer sequences for Lhc1, Lhcx3, Lhcx4 and 18S were the same as (Lepetit et al. 2013) and are indicated in Supplementary Table 2.3. For Lhcx2 we used Lhcx2_qPCR_fw and Lhcx2_qPCR_rev (Supplementary Table 2.3). This primer pair recognizes both alleles, in contrast to that used by (Lepetit et al. 2013). qPCR was run on a 7500 Fast RT-PCR system (Applied Biosystems, USA). Each strain was measured in biological triplicates, and on top each gene per sample was measured three times. Cycle threshold values and gene amplification efficiencies were obtained by utilizing PCR Miner 4.0 (Zhao and Fernald 2005). Relative transcript levels were calculated according to (Pfaffl 2001).

2.2.9 Western blot

Protein extraction and separation followed the protocol described by (Coesel et al. 2009), but using 14% lithium dodecyl sulfate-polyacrylamide gel electrophoresis for protein separation. Samples corresponding to an amount of 1 µg chlorophyll a were loaded on the gel. Proteins were blotted on a Bio-Trace PVDF (Pall Corporation, USA) or an Amersham Protran nitrocellulose membrane (GE Healthcare, GBR) using the semidry blotting technique by means of a Biorad Trans-Blot Turbo system (Hercules, USA). Previously, either an anti-Lhcsr, raised against a recombinant Chlamydomonas Lhcsr protein, had been used to quantify Lhcx protein expression in P. tricornutum (Bailleul et al. 2010; Lepetit et al. 2013; Taddei et al. 2016, 2018) or it had been the
anti-FCP6 raised against a specific C-terminal peptide of the FCP6 protein in *Cyclotella cryptica* (Laviale et al. 2015; Lepetit et al. 2017). We designed a new polyclonal anti-*P. tricornutum*-Lhcx (manufactured by Agrisera, Sweden) raised against the peptide MAQELVNGKGILEHL, because 14 (Lhcx1, Lhcx3), 13 (Lhcx2) and 12 (Lhcx4) out of these 15 amino acids are contained specifically in the C-terminus of the respective Lhcx proteins in *P. tricornutum*, thus making this antibody superior in detecting the different Lhcx proteins with a similar affinity. Anti-Lhcx was applied in 1:10,000 dilution overnight. Anti-Rubisco (AS03 037, Agrisera, Sweden, 1:10,000 dilution) served as a loading control. After binding of the secondary antibody for 1 h (goat anti-rabbit IgG, Sigma-Aldrich, USA; 1:10,000 dilution for Lhcx, 1:20,000 for Rubisco), signals were detected using Roti-Lumin Plus (Carl Roth, Germany) in an Odyssey FC Imaging System (LI-COR, USA).

2.2.10 Statistics

Significance with a *P* value ≤ 0.05 was determined with a two tailed unpaired Student’s *t*-test calculated with SigmaPlot on biological replicates as indicated in the respective legends. For gene expression we used the Pairwise Fixed Reallocation Randomization Test performed by REST according to (Pfaffl et al. 2002) with 2000 randomizations.

2.3 Results

2.3.1 The effect of different Lhcx proteins on qE

By using the TALEN method (Serif et al. 2017), we independently targeted two different sites of the *Lhcx1* gene in *P. tricornutum* strain Pt4 (UTEX 646). Three Lhcx1 knockout (x1KO) lines were obtained (cf. Supplementary Figure 2.1) for genetic characterization via PCR and 125
bp paired end whole genome sequencing), which lacked the Lhcx1 protein under low light (LL) growth conditions (Figure 2.1a). Two of these are based on TALEN pair 1 (x1KO_1a/1b), and the third on TALEN pair 2 (x1KO_2). Although the wild-type cells activated qE rapidly during three minutes of supra-optimal light exposure, the three x1KO clones lacked this qE induction (Figure 2.1b). Moreover, the very low NPQ values obtained after 3 min of supra-optimal light exposure in the x1KO lines even increased during the following dark phase, indicating it is not of qE origin. To prove that the observed phenotype is indeed related to the knockout of Lhcx1, we complemented the independent x1KO_1a and x1KO_2 lines with an Lhcx1 gene that was modified at the TALEN binding sites by synonymous codon usage, in order to prevent a re-cutting by the TALEN system. To express the gene, we used either the native Lhcx1 promoter/terminator or the FcpA (Lhcf1) promoter/terminator. Out of several lines created (Supplementary Figure 2.1A and B), some were further characterized regarding their NPQ characteristics. These lines showed a rescue of the qE phenotype to different extents (Figure 2.1b).
Figure 2.1 qE capacity in *P. tricornutum* Lhcx1 mutants. **a**) Western blot of low light grown strains, from left to right: wild type, three x1KO lines (x1KO_1a, 1b and 2), two complemented lines of the x1KO_1a with the *Lhcx1* gene cloned between the *Lhcx1* promoter and terminator (x1KO + x1a,b) and between the *FcpA* promoter (x1KO + Fcp_x1a,b), respectively, and one complemented line of the x1KO_2 line (x1KO_2 + x1). After blotting, the blot was cut and the upper part was incubated with a Rubisco antibody, whereas the lower part was incubated with the Lhcx antibody. Protein mass marker bands are indicated on the left. **b**) NPQ development during 3 min of actinic high light (white bar) followed by 6 min of recovery in darkness (black bar). Representative traces are depicted. **c**) Mean doubling time in the exponential growth phase in the wild type and three x1KO lines during low light growth. SE is given. Statistically significant differences between the x1KO lines and the wild-type are indicated by a * (unpaired t-test, eight degrees of freedom, $\rho < 0.05$, $n = 5$ biological replicates).
For closer examination, we used the x1KO_1a strain, as it showed no statistically significant difference in growth compared to the wild-type (Figure 2.1c), which is expected for cells cultivated under LL conditions where qE is not induced. We transformed this strain with each of the three other Lhcx genes to generate x1KO+x2/x3/x4 supplemented strains. To ensure similar regulation and expression of the other Lhcx genes as the original Lhcx1, we used the Lhcx1 promoter and terminator in all transformed strains. Our aim was to see the possible effects of each Lhcx protein on triggering qE. Normally, these effects are hidden or dampened by the presence of Lhcx1, which is the most expressed Lhcx protein under LL cultivation (Taddei et al. 2016) (Figure 2.1a). From the obtained clones we chose two for the Lhcx2 and Lhcx3, respectively, and three for the Lhcx4 gene, all of which showed strong expression of the respective gene (Supplementary Figure 2.3) as well as protein under LL growth conditions (Figure 2.2). We then investigated the qE pattern upon exposure to 10 min of supra-optimal actinic light and subsequent recovery under LL conditions known to relax qE in P. tricornutum better than darkness (Goss et al. 2006). Although the x1KO line showed only a slight linear increase in NPQ which did not relax under LL conditions and may rather be related to photoinhibition processes (qI), the x1KO+x2 and x1KO+x3 supplemented lines recovered qE capacity (Figure 2.3a; Supplementary Figure 2.4). The extent of qE varied in the two chosen strains supplemented with the same gene (i.e., x2a vs. x2b, and x3a vs. x3b; Figure 2.3a; Supplementary Figure 2.4), most likely caused by differential expression of the respective genes owning to positional effects on the inserted vector and the amount of inserted copies (Madhuri et al. 2019). Interestingly, Lhcx4 supplemented lines were unable to restore qE capacity (Figure 2.3a; Supplementary Figure 2.4).
Figure 2.2 Western blot of wild type, x1KO_1a and supplemented x1KO_1a strains. Cells were grown under low light. After blotting, the blot was cut and the upper part was incubated with a Rubisco antibody, whereas the lower part was incubated with the Lhcx antibody. Lhcx1 has the lowest molecular mass, Lhcx2 the highest, and Lhcx3 and Lhcx4 have a molecular mass in between. Protein mass marker bands are indicated on the left.
Figure 2.3 NPQ kinetics and xanthophyll cycle activity in wild type and mutant strains. Strains were concentrated to a chlorophyll $a$ amount of 10 mg L$^{-1}$. a) NPQ capacity of wild type (four biological replicates (BR)), x1KO_1a (four BR), and x1KO_1a supplemented lines x1KO + x2a (three BR), x1KO + x3a (four BR) and x1KO + x4a (three BR) during 10 min exposure to 1700 µmol photons m$^{-2}$ s$^{-1}$ (white bar), followed by 18 min of low light (gray bar). Red points indicate samples that had been incubated with DTT prior to high light exposure in order to prevent diatoxanthin formation; b) pool size of diadinoxanthin + diatoxanthin (Dd + Dt) per chlorophyll $a$ and de-epoxidation state (DES) following 10 min illumination with 1700 µmol photons m$^{-2}$ s$^{-1}$ without and with prior application of DTT. Three BR were measured.
Diatoms show a strong correlation between qE and the concentration of Dt, which is formed via de-epoxidation of Dd (Olaizola et al. 1994; Lavaud et al. 2002; Goss et al. 2006; Grouneva et al. 2008; Lavaud and Lepetit 2013; Blommaert et al. 2017). However, Dd de-epoxidation was similar in all our strains regardless of qE capacity (Figure 2.3b; Supplementary Figure 2.5). When the Dd to Dt conversion was inhibited by dithiothreitol (DTT) in the qE containing strains (wild-type, x1KO+x2, x1KO+x3), the qE capacity was lost (Figure 2.3; Supplementary Figure 2.4). Instead, those strains exhibited NPQ characteristics similar to the x1KO mutants without DTT treatment. Hence, Dt can only confer qE in the presence of Lhcx1/2/3 proteins, and vice versa, and the slower NPQ phase, observed under prolonged high light intensities, is independent of both compounds.

Under LL conditions, Lhcx1 is the highest expressed Lhcx gene, followed by Lhcx2, while Lhcx3 and Lhcx4 are hardly expressed (Taddei et al. 2016). We created an Lhcx2-KO line (x2KO, verified by allele specific PCR and sequencing, qPCR and Western Blot, Supplementary Figure 2.6, Supplementary Figure 2.7, and Supplementary Figure 2.8), which did not exhibit a reduction in qE capacity under LL cultivation (Inset in Supplementary Figure 2.19). However, Lhcx2 had been proposed to provide additional qE capacity under prolonged exposure to supra-optimal light (Lepetit et al. 2013, 2017; Taddei et al. 2018). Indeed, the x2KO strain, which lacks the high light induction of Lhcx2 (Supplementary Figure 2.7, and Supplementary Figure 2.8), showed a lower increase of qE capacity compared to the wild-type when exposed to two hours of supra-optimal light (1700 µmol photons m\(^{-2}\) s\(^{-1}\)), particularly during the second hour (Supplementary Figure 2.9). Still, the clearly visible increase of qE is likely caused by the up-regulation of Lhcx3 (Supplementary Figure 2.8) (Lepetit et al. 2013; Taddei et al. 2018). The x2KO+x2 complemented line rescued the capacity for qE increase during prolonged supra-optimal illumination and displayed the highest capacity for qE right from the beginning.
2.3.2 The relationship between qE and the $\sigma_{\text{PSII}}$

The different Lhcx mutated lines generated offered us a unique opportunity to investigate the extent to which Lhcx mediated qE can influence $\sigma_{\text{PSII}}$ in diatoms. $\sigma_{\text{PSII}}$, the functional absorption cross section of PSII, represents the probability of an absorbed photon of a given wavelength to drive a successful charge separation. To examine this phenomenon, we selected those strains that exhibited varying degrees of qE when grown under LL conditions. For reference, we ordered these lines from high to no qE capacity: $x2KO+x2 > \text{wild-type} = x2KO > x1KO+x3a > x1KO+x2a > x1KO = x1KO+x4a$. In addition, we included another strain, in which Lhcx3 is expressed under the control of the Lhcx2 promoter in the x2KO background, termed $x2KO+x3$. This strain had a similar qE capacity as the wild-type and the x2KO line under LL cultivation. For these strains we measured variable fluorescence as a function of light intensity at 15 light steps. After each light step, cells were exposed to a series of short flash leading to a single turnover of PSII, hence the transfer of one electron from the PSII reaction center to the acceptor Q$_A$, from which electron transport rates and $\sigma_{\text{PSII}}$ can be calculated, the latter by fitting the rise in fluorescence to a cumulative one-hit Poisson function (Ley and Mauzerall 1982). Electron transport rates started to saturate at $\sim 130$ $\mu$mol photons m$^{-2}$ s$^{-1}$ for all cultures (Supplementary Figure 2.10). qE became apparent at similar light intensities in wild-type, x1KO+x3a, x2KO, x2KO+x2 and x2KO+x3. Following the initiation, qE rapidly increased until light intensities of $\sim 350$ $\mu$mol photons m$^{-2}$ s$^{-1}$, after which NPQ rose slowly until reaching its maximum values of 0.6-0.7 at 800 $\mu$mol photons m$^{-2}$ s$^{-1}$; in the x2KO+x2 line NPQ reached even 0.8 (Supplementary Figure 2.11; Figure 2.4a). In contrast, in the x1KO and x1KO+x4a strains NPQ decreased to negative values at light intensities up to 250 $\mu$mol photons m$^{-2}$ s$^{-1}$ and reached maximum values of around 0.2 at 800 $\mu$mol photons m$^{-2}$ s$^{-1}$. In the x1KO+x2a strain, qE started at a higher light intensity in comparison to the wild-type, and maximum NPQ was in between the x1KO/x1KO+x4a and the other strains. When inhibiting Dt synthesis with DTT, the
rapid phase of NPQ development in the qE possessing strains was completely abolished, resulting in NPQ traces resembling those of the x1KO and x1KO+x4a strains without DTT (Supplementary Figure 2.11). In the two latter strains, DTT application did not change NPQ characteristics at all. We do not consider the slight linear increase of NPQ at higher light intensities as qE. We observed this increase in all strains and it was independent of the presence of Lhcx proteins or of a functional Dd de-epoxidation.
Figure 2.4 NPQ development and changes in $\sigma_{\text{PSII}}$ during increasing light intensities. Wild-type and mutant strains cultivated under low light (a) and 24 h of high light (~400 μmol photons m$^{-2}$ s$^{-1}$) (b) were exposed to 15 steps of increasing light intensity (1 min duration each), and NPQ (black trace) and $\sigma_{\text{PSII}}$ (red trace) were recorded. Values are the mean of six (low light cultures) or five (high light cultures) biological replicates. SD is given. Dashed black line denotes zero NPQ.
σ_{PSII} values of low light grown strains, measured in the dark, were between 500 and 550 Å² upon blue light (450 nm) exposure, typical for *P. tricornutum* (Levitan et al. 2015). We observed a slight decline in σ_{PSII} as light intensities increased to 70-100 µmol photons m⁻² s⁻¹ (Figure 2.4a; Supplementary Figure 2.12). At higher light intensities up to 350-400 µmol photons m⁻² s⁻¹, σ_{PSII} increased in the x1KO and the x1KO+x4a lines and remained at the same level in x1KO+x2a line. In contrast, σ_{PSII} decreased to a greater extent in the strains with high qE capacity (wild-type, x1KO+x3a, x2KO, x2KO+x2/x3), down to values of 400-450 Å² PSII⁻¹ (a reduction of 15-20%). Further increased light intensities, which induced a slight linear increase of NPQ capacity not related to qE (see above), did not further down-regulate σ_{PSII}. When inhibiting Dt synthesis by addition of DTT, qE possessing strains showed a σ_{PSII} development similar to the x1KO and the x1KO+x4a strains where σ_{PSII} was unaffected by the addition of DTT (Supplementary Figure 2.12).

Besides calculating the changes in σ_{PSII} based on single turnover saturating flashes leading to one QA- per PSII, σ_{PSII} is also often determined by applying prolonged weak flashes in the presence of DCMU, which yields to a full reduction of QA, but has some side effects due to the application of DCMU (reviewed in (Tian et al. 2019). In order to corroborate our results, we compared the effect of three minutes supra-optimal light exposure on NPQ establishment and σ_{PSII} behavior in wild-type and x1KO strains using the DCMU method and applying the calculation and correction procedure as described by (Tian et al. 2019). Using this method, we also observed a significant reduction in σ_{PSII} in the wild-type after supra-optimal light exposure, which was absent in the x1KO strain (Supplementary Figure 2.13, Supplementary Figure 2.14). Based on these results we conclude that there is a pronounced influence of qE on changes in σ_{PSII}.

Furthermore, we investigated the relationship between σ_{PSII} and NPQ after one day of high light growth. qE capacity increased in strains, which already possessed qE capacity under LL growth and was now present even in the x1KO and x1KO+x4a line (Figure 2.4b). This is owing to
the high amounts of Lhcx2 and Lhcx3 (cf. protein levels under LL and high light cultivation, Figure 2.2, and Supplementary Figure 2.8), which can partially rescue qE capacity in the absence of Lhcx1, as already demonstrated by the analysis of the x1KO supplemented lines (cf. Figure 2.3). As opposed to LL grown cells, qE initiated below 100 µmol photons m\(^{-2}\) s\(^{-1}\) and leveled off at 300 to 400 µmol photons m\(^{-2}\) s\(^{-1}\), thereafter, only slowly rising with higher light intensities. The highest NPQ values were obtained in the x2KO+x2/x3 and the x1KO+x3a lines, with NPQ values of 1.6-1.7 at 400 µmol photons m\(^{-2}\) s\(^{-1}\), i.e., when the rapid phase of qE induction was complete. Simultaneously, $\sigma_{\text{PSII}}$ decreased in all lines substantially, by up to 40-45% in wild-type, x1KO+x3a, x2KO and x2KO+x2/x3 and by up to 30% in x1KO and x1KO+x4a (Figure 2.4b Supplementary Figure 2.12). Strain x1KO+x2a displayed a decrease in between these two groups. As for LL grown cells, the rapid decline of $\sigma_{\text{PSII}}$ up to light intensities of 250-400 µmol photons m\(^{-2}\) s\(^{-1}\) well correlated with the rapid development of qE, with the exception of the decline of $\sigma_{\text{PSII}}$ under very weak light intensities in the beginning (Figure 2.4b). The weaker and linear increase of NPQ under further increased light intensities did not have any effect on $\sigma_{\text{PSII}}$.

We also investigated the excitation pressure on PSII (monitored as 1-qL, a proxy for the reduction state of the plastoquinone pool (Kramer et al. 2004)) required to reduce $\sigma_{\text{PSII}}$. Strains possessing a higher qE capacity exhibited a substantial decrease of $\sigma_{\text{PSII}}$ at much lower PSII excitation pressure. While for LL grown cultures 1-qL values of 0.6-0.8 were necessary to reduce $\sigma_{\text{PSII}}$ in qE possessing strains, high light acclimated cultures started to decrease $\sigma_{\text{PSII}}$ at 1-qL values of ~0.2-0.3 (Supplementary Figure 2.15). This correlates with the onset of qE at lower light intensities in high light vs. LL grown cultures (cf. Figure 2.4).

NPQ is the most widespread parameter used to characterize thermal dissipation. However, due to its derivation method, it may exaggerate the effect of thermal dissipation at higher values. To bound NPQ between 0 and 1, the parameter Y(NPQ) has been proposed to better visualize the
effect of thermal dissipation (Genty et al. 1996; Klughammer et al. 2008; Xu et al. 2018). By plotting NPQ versus Y(NPQ) we observed an almost linear correlation up to NPQ values of ~0.6 (Supplementary Figure 2.16). Above this threshold, the relative increase of NPQ vs. Y(NPQ) was enhanced. The maximum Y(NPQ) values were obtained for high light grown cultures and reached ~0.6, i.e., an induced thermal dissipation of 60% of absorbed photons. We plotted all $\sigma_{\text{PSII}}$ values against Y(NPQ) for both LL and high light grown strains together, excluding those points where Y(NPQ) was absent or did not further downregulate $\sigma_{\text{PSII}}$ (see details legend Figure 2.5). The latter points represent $\sigma_{\text{PSII}}$ values measured when NPQ shifted from the fast qE component to a slower component and had no additional effect on $\sigma_{\text{PSII}}$ (see discussion). Interestingly, we obtained a fairly good linear correlation between $\sigma_{\text{PSII}}$ and Y(NPQ). This indicates that no matter which Lhcx proteins are expressed, they all mediate qE in parallel to a downregulation of $\sigma_{\text{PSII}}$ (Figure 2.5, Supplementary Figure 2.17).
2.3.3 The relationship between qE and fluorescence lifetimes

At last, we investigated the relationship between qE capacity and the average fluorescence lifetimes when qE was induced by 10 min of continuous supra-optimal illumination. Fluorescence lifetime is a measure of the time span a molecule remains in the excited state before returning to the ground state through emitting fluorescence. With the induction of qE and the resultant fluorescence quenching, fluorescence lifetimes shorten (Kuzminov and Gorbunov 2016). In line

Figure 2.5 \(\sigma_{\text{PSII}}\) vs. Y(NPQ) from rapid light curves and the corresponding linear regression. Individual data points of all measured strains cultivated both under low light (six biological replicates each) and high light (five biological replicates each). In order to discern Lhcx and Dt-dependent qE processes from qI processes, data points where an increase of Y(NPQ) did not lead to a further downregulation of \(\sigma_{\text{PSII}}\) are not included in the regression calculation. There are indicated in light gray. This was determined by calculating the percent change of each \(\sigma_{\text{PSII}}\) from its previous light step. If \(\sigma_{\text{PSII}}\) decreased by < 5% of the total measured decrease for that curve while Y(NPQ) increased, it was omitted. Data points above 600 \(\mu\text{mol photons m}^{-2}\ \text{s}^{-1}\) and data points with a negative Y(NPQ) were also removed. A linear regression line, the 95% confidence interval, the regression equation and the \(r^2\) are indicated.
with the rapid onset of qE (cf Figure 2.3), we saw a pronounced decrease of fluorescence lifetimes in the LL grown qE possessing strains (wild-type, x1KO+x2a, x1KO+x3a, x2KO, x2KO+x2, x2KO+x3) during the first minute of illumination, which was absent in the x1KO and the x1KO+x4a strains (Supplementary Figure 2.18). Thereafter, there was a slight linear decline in all strains. In contrast, in high light grown cells a rapid decrease of fluorescence lifetimes was visible in all strains already after 1 min of illumination. Overall, the decreases in lifetime well reflected the capacity for qE in the different strains.

### 2.2 Discussion

By knocking out the Lhcx1 gene, we obtained the *P. tricornutum* strain (x1KO) that had no capacity for qE, i.e., the rapid component of NPQ (Figure 2.3; Figure 2.4). Our results support previous findings by Bailleul et al. (2010) regarding the involvement of Lhcx1 in qE. We suggest that Lhcx1 provides essentially all qE capacity in *P. tricornutum* grown under LL conditions. This is different to *C. reinhardtii*, where no qE is developed under LL growth conditions (Peers et al. 2009), but both existing Lhcsr proteins provide qE after prolonged exposure to high light (Girolomoni et al. 2019). Our results also revealed that Lhcx2 and Lhcx3 provide qE capacity in *P. tricornutum*, alongside Lhcx1, but cannot specify a role for Lhcx4 in this process, at least not for high light inducible, Dt dependent qE and independent of Lhcx1. This is in line with the strong upregulation of Lhcx4 under prolonged dark conditions (Nymark et al. 2013; Taddei et al. 2016) which requires future research to elucidate its function.

As a consequence of the lack of Lhcx1, the x1KO strain, as well as the x1KO+x4a strain, exhibit no decrease in $\sigma_{\text{PSII}}$ under high light exposure. Instead, they show a slight increase in $\sigma_{\text{PSII}}$, as expected when PSII reaction centers become progressively closed, causing excitons to jump from one PSII unit to another, in search for an open PSII center (Joliot et al. 1973; Xu et al. 2017).
On the other hand, the onset of qE in wild-type and the five other strains leads to a substantial decrease in both fluorescence lifetimes and $\sigma_{PSII}$. This decrease in $\sigma_{PSII}$ clearly indicates a reduction in the flux of energy to the PSII core, which provides photoprotection under supra-optimal light.

While previously it has been shown that the decrease in $\sigma_{PSII}$ correlates to a certain degree with the NPQ capacity in different algal species (Olaizola et al. 1994; Koblížek et al. 2001; Kuzminov and Gorbunov 2016; Giovagnetti and Ruban 2017; Xu et al. 2018; Perkins et al. 2018; Tian et al. 2019), here, we prove that in the diatom *P. tricornutum* this decrease is mediated in a concerted manner via Lhcx proteins (Lhcx1, Lhcx2 and Lhcx3) and the conversion of Dd to Dt. Lhcx proteins or the xanthophyll cycle alone - at least under LL growth conditions - do not activate qE or cause a reduction in $\sigma_{PSII}$ under supra-optimal irradiances.

As Dt binds to LHC antenna proteins (Lepetit et al. 2010; Wang et al. 2019) and Lhcx proteins are not part of the PSII core (Umema et al. 2011), the decrease in $\sigma_{PSII}$ is indicative of thermal dissipation of absorbed energy in the LHC antenna complexes, as originally proposed by Genty et al. (1990). Accordingly, analysis of picosecond lifetime kinetics revealed that the exposure to supra-optimal irradiances leads to functional modifications in both antennae and PSII reaction centers, but the thermal dissipation occurs only in the antennae (Kuzminov and Gorbunov 2016). Our results also showed that the slow phase of NPQ, which is activated under prolonged exposure to higher light levels, has no effect on $\sigma_{PSII}$. A similar slow NPQ phase with no reduction in $\sigma_{PSII}$ is observed in the mutants lacking qE (x1KO and x1KO+x4) as well as in all strains treated with DTT (which inhibits the formation of Dt). These results suggest that the slow phase of NPQ is related to photoinhibitory damage to the PSII reaction center (qI).

The combination of NPQ and $\sigma_{PSII}$ measurements in a variety of mutants with different NPQ extents allowed us to better characterize multiple NPQ processes that are otherwise hardly distinguishable (Goss and Lepetit 2015; Malnoë 2018). Accordingly, the most rapid NPQ process
(qE) can be easily identified by a pronounced reduction in $\sigma_{PSII}$ upon exposure to high light. Whether this scenario holds true also for other algal taxa remains to be tested, but recent results in the green alga *Ostreococcus taurii* suggest a similar correlation between qE and $\sigma_{PSII}$ (Xu et al. 2018). Our measurements of a significantly decreased $\sigma_{PSII}$ under qE conditions are fully consistent with the proposal that qE shortens the time an exciton can travel before being thermally dissipated, and thus decreases the effective excitation diffusion length for an exciton to reach an open PSII reaction center (Bennett et al. 2018).

Because the PSII reaction center is energetically a shallow trap (Schatz et al. 1987; Kuzminov and Gorbunov 2016), excitons that reach a PSII core may transfer back to the peripheral antennae where they can be thermally dissipated once all PSII reaction centers are closed. Such a mechanism has been identified in plants, termed ‘economic photoprotection’ (Belgio et al. 2014), and later was also postulated to exist in diatoms (Giovagnetti and Ruban 2017). This economic photoprotection, however, does not involve a decrease in $\sigma_{PSII}$ upon induction of qE (Belgio et al. 2014), which is in sharp contrast to our results.

As we obtained a linear correlation between the extent of Y(NPQ) and the decrease in $\sigma_{PSII}$ driven directly by qE (Figure 2.5, Supplementary Figure 2.17), we can use the regression equation to calculate $\sigma_{PSII}$ for a theoretical maximal Y(NPQ) of 1, i.e., where all absorbed energy is dissipated as heat. This leads to a value of $\sigma_{PSII} = 111 \text{ Å}^2$ at Y(NPQ) = 1 (and 115 Å$^2$ if both parameters are measured after 1 second darkness). Using a chlorophyll $a$ specific absorption coefficient of 9.8 m$^2$ (g chlorophyll $a$)$^{-1}$ for blue light (Schellenberger Costa et al. 2013) (as used here for the $\sigma_{PSII}$ measurements) and assuming a dimeric PSII core for diatoms in vivo (Nagao et al. 2013), containing 70 chlorophyll $a$ molecules (Umena et al. 2011), we calculated a functional PSII core cross section of 112 Å$^2$. This value is virtually identical to the one calculated at Y(NPQ) = 1 based on our $\sigma_{PSII}$ vs. Y(NPQ) regression. In a similar approach for the centric diatom *T. pseudonana,*
Campbell and co-workers calculated with a monomeric PSII and concluded that at $Y(\text{NPQ}) = 1$ the remaining $\sigma_{\text{PSII}}$ is provided by the PSII core and some LHCs that are not thermally down regulated (Xu et al. 2018). If one assumes that the existing dimeric PSII (Nagao et al. 2013) is excitonically coupled and shares a common peripheral LHC antenna, then the regression to a $Y(\text{NPQ})$ of 1 provides a residual $\sigma_{\text{PSII}}$, which also in *T. pseudonana* corresponds to the functional absorption cross section of the dimeric PSII core. In either cases, the results Xu et al. (2018) and this study indicate that regulation of $\sigma_{\text{PSII}}$ upon induction of qE does not involve the PSII core, otherwise the regression at $Y(\text{NPQ})$ of 1 would lead to $\sigma_{\text{PSII}}$ values close to 0. The most parsimonious explanation is that Lhcx mediated qE mechanistically leads to a disconnection of the peripheral LHC antennae - very much in line with recent results obtained by a different experimental approach (Giovagnetti and Ruban 2017) - and these functionally disconnected LHC antennae dissipate the excess absorbed light as heat. Such a mechanism has been identified as one compound of NPQ in *A. thaliana* using electron microscopy (Betterle et al. 2009).

It is not clear yet how this disconnection of the LHC antennae is achieved, but recent results indicate a strong role of Dt molecules in influencing thylakoid membrane rigidity (Bojko et al. 2019). Moreover, traces of Lhcx proteins have been detected in photosystem II preparations (Taddei et al. 2018), indicating that Lhcx proteins might be more strongly connected to the PSII core than the peripheral LHC proteins. Both aspects could influence peripheral LHC antennae connectivity with the PSII cores in such a way that, upon induction of qE, the peripheral antennae are moved away slightly, exceeding the maximal distance for functional Förster resonance energy transfer to the PSII core. In such functionally disconnected LHC antennae, internal heat dissipation and chlorophyll fluorescence emission - the remaining energy dissipation pathways - would compete and a further synthesis of Dt under prolonged supra-optimal light would decrease fluorescence yield even more by increasing thermal dissipation, but without further affecting $\sigma_{\text{PSII}}$. 
These processes, together with photoinhibited PSII reaction centers (Kuzminov and Gorbunov 2016), may explain the observation of very high NPQ values which do not affect $\sigma_{\text{PSII}}$ (Koblížek et al. 2001; Giovagnetti and Ruban 2017; Xu et al. 2018).

There had been several reports on two potential qE quenching loci in diatoms based on fluorescence lifetime kinetics. The first is associated with an uncoupling of antenna complexes from the PSII core, which is independent of Dt formation. The second is taking place close to the PSII reaction center and is directly dependent on the xanthophyll cycle supposedly (Miloslavina et al. 2009; Lavaud and Goss 2014; Chukhutsina et al. 2014; Goss and Lepetit 2015; Giovagnetti and Ruban 2017; Taddei et al. 2018). These reports are corroborated by classical PAM analyses in centric diatoms, where one component of qE is independent of the conversion of Dd to Dt (Grouneva et al. 2008) and clearly different antenna organizations exist (Gundermann et al. 2019). However, the two qE quenching sites concept is difficult to reconcile with the prime dependency of qE on the amount of Dt in the pennate *P. tricornutum* (Olaizola et al. 1994; Lavaud et al. 2002; Goss et al. 2006; Lavaud and Lepetit 2013) and with our results which point to only one mechanism of qE. One reason for this discrepancy may be that in both time resolved fluorescence studies revealing the two quenching sites in *P. tricornutum* (Miloslavina et al. 2009; Taddei et al. 2018) the time span of cells exposed to supra-optimal light conditions was much longer than necessary to induce full qE and hence also qI related processes likely had been recorded. Indeed, when Taddei et al. (2018) and colleagues measured LL grown *P. tricornutum* cells, they observed only one quenching site. Analyses of picosecond lifetime kinetics during the NPQ development in *P. tricornutum* revealed a functional modification in the PSII reaction center, in line with previous results (Eisenstadt et al. 2008), which, however, is independent of the xanthophyll cycle and does not lead to any thermal dissipation (Kuzminov and Gorbunov 2016). Instead, this modification in the energy transfer in PSII centers reduces the probability of producing triplet chlorophyll
Our results demonstrate that only Dt and Lhcx mediated qE provides thermal dissipation in the antennae which likely involves uncoupling of functional antennae. This is characterized by one quenching site which corroborates the conclusions obtained by Kuzminov and Gorbunov (2016).

We have to note that our analyses have been conducted in *P. tricornutum* strain 4, which had been originally isolated from the Baltic Sea and which has a lower NPQ capacity than other *P. tricornutum* strains (Bailleul et al. 2010). Some analyses regarding two different quenching sites and mechanisms had been performed in another *P. tricornutum* strain (Pt2) (Miloslavina et al. 2009; Giovagnetti and Ruban 2017), which has a higher NPQ capacity than Pt4. Thus, in principle there could exist an additional quenching mechanism in Pt2. However, the fact that also in Pt2 there is a 1:1 dependency of NPQ on the amount of Dt (Lavaud and Lepetit 2013), together with the explanations for the apparent occurrence of a second quenching site provided above, rather argue against this assumption.

Finally, it had been speculated that different Lhcx proteins have different capacities to provide NPQ, with Lhcx1 being the most effective (Taddei et al. 2018). We indeed observed no qE in the LL grown cells if Lhcx1 is knocked out, but this is rather owing to a much lower expression of Lhcx2 and Lhcx3 under these conditions (Taddei et al. 2016) (Figure 2.2). Although we observe different extents of qE in the x1KO supplemented lines as compared to the wild-type, it remains unclear whether this is owing to an altered efficiency of Lhcx2 and Lhcx3 in providing qE or due to differential protein expression. Also, whether qE provided by the different Lhcx proteins is activated at different light exposure conditions (either light intensity or exposure duration) remains to be elucidated. However, our results clearly demonstrate that the extent of qE always correlates with a reduction in $\sigma_{\text{PSII}}$ with the same linear relation in all lines (Figure 2.5 Supplementary Figure 2.17). This implies that Lhcx1/2/3 have the same capacity for decreasing the flux of absorbed light
energy to PSII via thermal dissipation in the LHC antennae. While some individual Lhcx proteins, such as Lhcx4, could possess other functions than qE, our results suggest that the primary role of Lhcx proteins is providing qE by decreasing the functional absorption cross section.

Then, why do diatoms have several different Lhcx genes, in the case of the polar strain *Fragilariopsis cylindrus* even eleven? As has been shown in (Coesel et al. 2009; Lepetit et al. 2013, 2017; Juhas et al. 2014; Taddei et al. 2016), all four Lhcx proteins of *P. tricornutum* are modulated in their expression by different environmental triggers, such as e.g., low light (Lhcx1), high light (Lhcx2, Lhcx3), blue light (Lhcx1-3), iron limitation (Lhcx2), or nitrogen starvation (Lhcx3, Lhcx4). We propose that one gene alone cannot contain all required regulatory cis-elements in order to respond to the multitude of environmental triggers. Instead, through gene duplications during evolution, several regulatory cis-elements could be integrated into several Lhcx gene promoters to modulate the expression of proteins with very similar functions and thus to tune the functional absorption cross section depending on the environmental conditions.

Overall, our study reveals the molecular mechanism of how diatoms fine tune qE, allowing them to thrive in continuously changing light environments, where NPQ is one of the most important physiological processes (Lin et al. 2016).
Chapter 3: Photosynthetic energy conversion efficiency in the West Antarctic Peninsula

The West Antarctic Peninsula (WAP) is a highly productive polar ecosystem where phytoplankton dynamics are regulated by intense bottom-up control from light and iron availability. Rapid climate change along the WAP is driving shifts in the mixed layer depth and iron availability. Elucidating the relative role of each of these controls and their interactions is crucial for understanding of how primary productivity will change in coming decades. Using a combination of ultra-high-resolution variable chlorophyll fluorescence together with fluorescence lifetime analyses on the 2017 Palmer Long Term Ecological Research cruise, we mapped the temporal and spatial variability in phytoplankton photophysiology across the WAP. Highest photosynthetic energy conversion efficiencies and lowest fluorescence quantum yields were observed in iron replete coastal regions. Photosynthetic energy conversion efficiencies decreased by ~ 60% with a proportional increase in quantum yields of thermal dissipation and fluorescence on the outer continental shelf and slope. The combined analysis of variable fluorescence and lifetimes revealed that, in addition to the decrease in the fraction of active reaction centers, up to 20% of light harvesting chlorophyll-protein antenna complexes were energetically uncoupled from photosystem II reaction centers in iron-limited phytoplankton. These biophysical signatures strongly suggest severe iron limitation of photosynthesis in the surface waters along the continental slope of the WAP.
3.1 Introduction

Iron availability limits phytoplankton growth and production across ~30% of the ocean’s surface (Moore et al. 2013). However, iron requirements vary dramatically among species (Ho et al. 2003) and phytoplankton communities may remain relatively iron replete even in regions with extremely low concentrations of iron, such as the South Pacific Gyre (Bonnet et al. 2008). Consequently, there is a need to develop sensitive diagnostic tools for iron limitation in phytoplankton (Hopkinson et al. 2007; Behrenfeld and Milligan 2013). The Southern Ocean has garnered particular interest as it is the world’s largest iron limited region (Boyd 2002; Strzepek et al. 2012).

Over several decades, variable fluorescence signals from photosystem II (PSII) have been used to measure photosynthetic conversion efficiencies. This efficiency, commonly denoted as $F_v/F_m$, is the quantum yield of photochemistry in PSII ($\Phi_{PSII}$); i.e., the ability of absorbed light to drive photosynthetic electron transport from water to a terminal electron acceptor (Kolber et al. 1998). The rate of induction of variable fluorescence on the microsecond time scale can also be used to calculate the effective absorption cross section of PSII ($\sigma_{PSII}$) (Kolber et al. 1998; Gorbunov and Falkowski 2005). This latter parameter is a product of the optical absorption cross section of PSII (i.e., the size of the PSII antennae) and the quantum yield of photochemistry in the reaction center (RC) (Ley and Mauzerall 1982; Kolber et al. 1998; Falkowski et al. 2004b; Falkowski and Raven 2007).

Fluorescence emission and non-radiative thermal dissipation (with the quantum yields of $\Phi_F$ and $\Phi_T$, respectively) compete with photochemistry to dissipate absorbed photons (Butler and Strasser 1977; Butler 1978; Falkowski et al. 2017). The three are complementary, meaning the sum of the three yields is 1.00 (Butler 1978). Moreover, they are remarkably sensitive to the effects of iron limitation on phytoplankton physiology (Lin et al. 2016). Extensive measurements of variable
fluorescence under iron limitation have revealed substantial decreases in maximal $\Phi_{\text{PSII}}$ and pronounced increases in $\sigma_{\text{PSII}}$. These responses were observed in cultures (Greene et al. 1991; Vassiliev et al. 1995; Strzepek et al. 2012, 2019) and in situ (Greene et al. 1994; Gervais et al. 2002; Suzuki et al. 2002; Behrenfeld and Milligan 2013). Furthermore, shipboard and in situ iron enrichment experiments, revealed rapid increases in $F_v/F_m$ and decreases in $\sigma_{\text{PSII}}$ following iron amendment (Hutchins et al. 2002; Gervais et al. 2002; Coale et al. 2004; Hopkinson et al. 2007; Moore et al. 2007; Ryan-Keogh et al. 2017).

Low $\Phi_{\text{PSII}}$ reflects a downregulation in functional RCs, complemented by an increased pool of light harvesting complexes (LHC), some energetically uncoupled from the RCs (Greene et al. 1991; Schrader et al. 2011; Macey et al. 2014). The LHCs that are still coupled energetically serve fewer functional RCs, resulting in an increased $\sigma_{\text{PSII}}$. With this, phytoplankton economize the high iron quota of RCs (Strzepek et al. 2012). At low light, large LHCs increase excitation energy loss through thermal dissipation and fluorescent emission before being trapped in an active RC (Wientjes et al. 2013). In the Southern Ocean, Strzepek et al. (2019) proposed the low temperatures mitigate this loss. Conversely, at saturating light, the few active RCs are subjected to overexcitation and damage (Greene et al. 1992). To cope, iron limited phytoplankton increase rapid non-photochemical quenching (NPQ) components (Petrou et al. 2011; Alderkamp et al. 2013). NPQ represents a suite of photoprotective mechanisms activated at high light, effectively increasing $\Phi_T$ and simultaneously decreasing $\sigma_{\text{PSII}}$ (Goss and Lepetit 2015; Kuzminov and Gorbunov 2016; Buck et al. 2019). Further work is needed to rapidly assess the occurrence and function of these physiological responses to iron limitation in natural assemblages (Behrenfeld and Milligan 2013). However, for a truly comprehensive evaluation an additional yield needs to be measured alongside $\Phi_{\text{PSII}}$. Previous studies have suggested methods to derive additional yields from variable
fluorescence (Hendrickson et al. 2004; Kramer et al. 2004). However, these methods critically depend on a priori assumptions regarding the antenna-RC organization.

To that end, we developed an extremely sensitive, sea-going instrument, PicoLiF (Picosecond Lifetime Fluorescence), which continuously measures in situ chlorophyll fluorescence lifetimes in the picosecond time domain. When the measured lifetimes are normalized to the natural lifetime (15,000 ps, or 15 ns in the case of chlorophyll \(a\); Brody and Rabinowitch 1957) the result is the quantum yield of fluorescence, \(\Phi_F\). As all three quantum yields sum to unity, direct measurements of \(\Phi_{PSII}\) and \(\Phi_F\) allow quantification of \(\Phi_F\) by difference (Lin et al. 2016) and the fraction of energetically uncoupled LHC-RC complexes (Park et al. 2017). In addition, they provide insight into regulation of energy transfer (e.g., Buck et al. 2019) and photoprotection (e.g., Kuzminov and Gorbunov 2016) in PSII. In the oceans, \(\Phi_F\) varies about fivefold in response to light and nutrients (Lin et al. 2016). Indeed, the direct measurement of \(\Phi_F\) from lifetimes in the picosecond time domain is the only way to calibrate or verify remotely sensed \(\Phi_F\), which is a highly derived product (Huot et al. 2005; Behrenfeld et al. 2009; Lin et al. 2016).

Here, we evaluated surface phytoplankton photophysiology in the West Antarctic Peninsula (WAP). In this region a cross shelf iron gradient exists, hypothesized to control phytoplankton abundance and productivity (Annett et al. 2017). Custom-built fluorometers were deployed during the 2017 annual WAP Long Term Ecological Research cruise in the austral summer. A F1Re (Fluorescence Induction and Relaxation) instrument measured \(F_v/F_m\), \(\sigma_{PSII}\). Simultaneously, the PicoLiF instrument measured fluorescence lifetimes. We hypothesized our combined measurements would reveal a distinct iron-limited physiology, with significantly higher \(\Phi_T\), and an increased pool of uncoupled LHC-RC in the WAP offshore waters.
3.2 Materials & methods

3.2.1 Study area

Data were collected on board the ASRV Laurence M. Gould. Sampling was carried out along perpendicular cross shelf transects spaced 100 km apart. The study region corresponds to the LTER project grid lines 100-600 (Waters and Smith 1992) (Figure 3.1). Following (Steinberg et al. 2015) we differentiate between three sub regions across the WAP; the shallow coastal region, the continental shelf and the deep continental slope roughly 200 km offshore.

Figure 3.1 WAP LTER site and sampling locations. Insert at top left shows the WAP in relation to South America. Black dots on main figure represent the sampling stations along the 100 line in the south to the 600 line in the north. Red dashed lines denote the three subregions along the WAP: the coast, the continental shelf and the continental slope. Red star is the location of the U.S Palmer station on Anverse Island. Bathymetry in figure and insert is from ETOPO1 dataset.
3.2.2 Sample collection and analysis

Sampling stations were at 20 km intervals along each grid line. Samples were collected for chlorophyll \( a \) and dissolved inorganic nutrients (nitrate, phosphate and silicate) following (Carvalho et al. 2019). Variable fluorescence and fluorescence lifetime data were collected continuously from surface waters (~5 m) while underway with FIRe and PicoLiF fluorometers respectively, as described by (Lin et al. 2016). The instruments used flow through cuvettes connected to the ship’s surface water intake pump. The water passed through two de-bubblers prior to entering the cuvette.

Mixed layer depth (MLD) was defined as the depth at which the maximum buoyancy frequency was observed in CTD profiles, following (Carvalho et al. 2017). The critical depth was calculated from surface daily integrated photosynthetically available radiation (PAR), collected from the mast of the ship (QSR-240P, Biospherical Instruments, San Diego, CA) and the light attenuation coefficient \( (K_{\text{PAR}}) \) calculated from the empirical relationship of \( K_{\text{PAR}} \) and chlorophyll \( a \) concentration, as proposed by (Sverdrup 1953; Nelson and Smith 1991). Night and day were differentiated using NOAA’s solar calculator (https://www.esrl.noaa.gov/gmd/grad/solcalc/).

3.2.3 Photophysiology

We recorded variable fluorescence using a mini-FIRe instrument as previously described (Gorbunov and Falkowski 2005; Kuzminov and Gorbunov 2016). Variable fluorescence was induced by a saturating single turnover flash (STF) from blue light-emitting diodes (450 nm with 30 nm half bandwidth), which cumulatively reduce all PSII RCs within ca. 100 \( \mu \)s. This excitation protocol results in minimum and maximum fluorescence yields \( (F_0 \ and \ F_m) \). The quantum yield of photochemistry in PSII was then calculated as \( (F_m-F_0)/F_m = F_v/F_m \) (Butler 1978; Kolber et al. 1998).
The effective absorption cross section of PSII, $\sigma_{\text{PSII}}$ (at 450 nm), is calculated by fitting the fluorescence rise to a cumulative one-hit Poisson function (Ley and Mauzerall 1982).

Every ~30 min. the flow-through on the mini-FIRE was automatically paused in order to conduct slow fluorescence irradiance (FE) curves. These were used to retrieve electron transport rates (ETR) as a function of irradiance and to characterize the state of phytoplankton photoacclimation to their short- and long-term light history (Falkowski 1994; Ralph and Gademann 2005). During FE curves, the water sample was trapped in the cuvette for ca. 10 min and then exposed to increasing PAR levels (0-800 μmol photons m$^{-2}$ s$^{-1}$) with an actinic blue light source (450 nm). Every light step lasted 30-40 seconds to promote short-term acclimation to each new PAR level, followed with standard measurements. FE curves in this study are termed slow as light steps were longer than other comparable studies, where light steps lasted 10-20 seconds (Serôdio et al. 2006; Suggett et al. 2015). This was done, as the acclimation is slower at lower water temperatures. From FE curves, we calculated the rate of photosynthetic electron transport normalized per PSII RC (ETR$_{\text{PSII}}$, with units of e$^{-}$ s$^{-1}$ RC$^{-1}$), as a function of PAR (Gorbunov et al. 2000, 2001) from

$$E_{\text{TR}_{\text{PSII}}} = E \times \sigma_{\text{PSII}} \times \left[ (\Delta F'/F'_m) / (F'/F_m) \right]$$

Equation 3.1

Here, $E$ is irradiance, $F'/F_m$ and $\sigma_{\text{PSII}}$ are measurements in the dark (PAR=0), respectively. $\Delta F'/F'_m$ is the quantum yield of photochemistry at a given PAR level, with the prime notation indicating measurement under ambient light ($\Delta F' = F'_m - F'$, where $F'$ is a steady-state fluorescence at a given light step). FE curves were than fitted to a hyperbolic tangent function to derive maximal ETR through PSII (ETR$_{\text{PSII}}^{\text{max}}$), and the $E_K$ value (saturating light level) following (Jassby and Platt 1976) as

$$E_{\text{TR}_{\text{PSII}}} = E_{\text{TR}_{\text{PSII}}^{\text{max}}} \times \tanh(E/E_K)$$

Equation 3.2
Picosecond fluorescence decays, measured with the PicoLiF, were deconvoluted from the instrument response function and fitted to a sum of three exponentials with a custom TCSPFIT Matlab package utilizing a Nelder-Mead simplex algorithm (Enderlein and Erdmann 1997). $\Phi_F$ was then calculated from

$$\Phi_F = \tau / \tau_0$$

Equation 3.3

Where $\tau$ is the measured lifetime and $\tau_0$ is the natural lifetime of chlorophyll $a$ (Brody and Rabinowitch 1957; Brody 2002). The natural lifetime is the time that would be required for a molecule to return to the ground state from an excited state if fluorescence were the sole dissipation pathway. For chlorophyll $a$, $\tau_0$ is 15 ns and is constant, independent of solvent, organism or environmental condition (Brody and Rabinowitch 1957; Brody 2002; Lakowicz 2006). We then calculated the quantum yield for thermal dissipation ($\Phi_T$) as

$$\Phi_T = 1 - \left[ \frac{F_v}{F_m} + \frac{\tau}{\tau_0} \right]$$

Equation 3.4

All fluorescence measurements were corrected for the blank signal measured routinely from filtered seawater (0.2 µm) (Bibby et al. 2008). To minimize changes in temperature, the flow through system relies on thick-walled tubing for insolation.

In the current setup, phytoplankton experienced ~10 minutes of low-light acclimation from the time they entered the ship’s underway system to when the F1Re and PicoLiF measurements were conducted. In this timeframe, most of the rapid NPQ mechanisms (e.g., xanthophyll cycling) relax, leading to a recovery in $F_v/F_m$. However, this time is not sufficient to alleviate the effects of photoinhibition, which requires >10 min to recover (Alderkamp et al. 2013). Considering this, our standard measurements represent a state in which phytoplankton are not in an entirely low-light
acclimated state. In effect, this means that $F_v/F_m$ may be slightly underestimated when the measurements are conducted during the day. This issue is more pertinent for the calculation of ETR, as the underlying assumption relies on a truly low-light acclimated baseline (Equation 3.1). As there is an additional low-light acclimation period before a FE curve is measured the deviations mentioned above would decrease. In any case, from Equation 3.1 it can be seen that underestimating $F_v/F_m$ and $\sigma_{PSII}$ results in an underestimation of ETR. To minimize any under- or overestimations, preferential weight is given to data collected during the night. Although data collected during the day may cause some issues in interpretation, we argue that the low-light acclimation periods used here are sufficient nonetheless to provide distinct differences in the photophysiological status of phytoplankton in response to iron and light availability in this region.

### 3.2.4 Statistical analyses

The average of each photophysiological variable reported in this paper represents the median value. The median was chosen because each individual variable was not normally distributed and included statistical outliers. To describe the deviation from the median, we calculated the median absolute deviation, a robust measure of dispersion around the median (Leys et al. 2013). For improved spatial comparison of variables, surface maps were produced using a Locally Weighted Scatter-plot Smoother (LOESS). Significance with a $\rho \leq 0.05$ was determined from Pearson’s linear correlation.

### 3.3 Results

#### 3.3.1 WAP physical and chemical setting
Surface concentrations of chlorophyll $a$ ranged from $0.06 \text{ mg m}^{-3}$ to $16.9 \text{ mg m}^{-3}$. Surface chlorophyll $a$ concentration significantly correlated with distance to shore (Table 3.1), with higher concentrations along the coast, decreasing offshore over the continental shelf and slope (Figure 3.2a). Macronutrients were replete along the entire grid, with increasing nitrogen (as nitrate) and phosphate over the slope (Figure 3.2b & c). Furthermore, N/P ratio tracked closely with the canonical 16/1 Redfield ratio, indicating that nitrogen and phosphate were not limiting factors in this region. Silicate, a crucial nutrient for diatoms, also decreased but was still abundant nonetheless (Figure 3.2d). Overall, our data supports the notion that along the WAP, oceanic conditions transition over a short distance (~200 km), from a coastal- to an HNLC-ecosystem over the continental slope, where previous studies indicate iron depletion (Annett et al. 2017).

Table 3.1 Pearson’s linear correlation coefficients matrix. Correlation assessed between independent variables (surface PAR and distance to shore) and dependent variables ($F_{v}/F_{m}$, fluorescence lifetime, $\Phi_T$, $\sigma_{PSII}$, surface Chl $a$ concentration, and $ETR_{PSII}^{max}$). Linear relationship strength between each set of pairs, is proportional to the cell color, with warmer colors indicating a positive relationship and colder colors a negative relationship. All coefficients are significant ($\rho \ll 0.05$).

<table>
<thead>
<tr>
<th></th>
<th>Surface PAR</th>
<th>Distance to shore</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{v}/F_{m}$</td>
<td>-0.444</td>
<td>-0.614</td>
</tr>
<tr>
<td>Fluorescence lifetime</td>
<td>-0.481</td>
<td>0.644</td>
</tr>
<tr>
<td>$\Phi_T$</td>
<td>0.435</td>
<td>0.653</td>
</tr>
<tr>
<td>$\sigma_{PSII}$</td>
<td>-0.237</td>
<td>0.624</td>
</tr>
<tr>
<td>Surface Chl $a$ conc.</td>
<td>0.089</td>
<td>-0.406</td>
</tr>
<tr>
<td>$ETR_{PSII}^{max}$</td>
<td>0.522</td>
<td>0.319</td>
</tr>
</tbody>
</table>
Figure 3.2 Surface distribution maps of biochemical parameters. a) Chl $a$, b) nitrate and nitrite c) phosphate, and d) silicate. Data collected from CTD rosette cast or underway water collection, denoted by black dots. Red dashed lines highlight the borders between the WAP coast, shelf, and slope regions. Note that Chl $a$ is presented on a logarithmic scale to accommodate a range in orders of magnitude.
3.3.2 Spatial variability in photophysiological parameters

Surface maps of \( F_v/F_m \), fluorescence lifetimes, \( \sigma_{\text{PSII}} \) and \( \Phi_T \) show clear gradients across the continental shelf (Figure 3.3, Table 3.2). The differences are significant, showing the highest degree of correlation with distance from shore (Table 3.1). As seen in Table 3.2, throughout a diel cycle, \( F_v/F_m \) values along the coast were relatively high (0.42±0.06), and progressively decreased by up to 50\% offshore along the shelf (0.3±0.08) and slope (0.22±0.04). Fluorescence lifetimes, in contrast, increased offshore. Along the coast fluorescence lifetimes were relatively low, 0.77±0.07 ns, increasing to 1.03±0.17 ns along the shelf and 1.29±0.23 ns along the slope. Consequently, \( \Phi_T \) increased from 0.5±0.05 along the coast, to 0.62±0.06 and 0.69±0.04 at the shelf and slope. Similarly, \( \sigma_{\text{PSII}} \) increased from 484±54 Å\(^2\) along the coast to 694±132 Å\(^2\) over the shelf, reaching 760±168 Å\(^2\) at the slope. Likewise, diel averaged \( ETR_{\text{max}}^{\text{PSII}} \) rates progressively increased from 58±107 e\(^-\) s\(^{-1}\) RC\(^{-1}\) along the coast to 137±55 and 230±105 e\(^-\) s\(^{-1}\) RC\(^{-1}\) out over the shelf and slope (Table 3.2).
Figure 3.3 Surface distribution maps of photophysiological parameters. **a)** Fv/Fm, **b)** $\sigma_{\text{PSII}}$, **c)** fluorescence lifetime, and **d)** thermal dissipation quantum yield ($\Phi_T$). Fv/Fm and $\sigma_{\text{PSII}}$ collected from underway FIRe measurements. Fluorescence lifetime collected from underway PicoLiF measurements. $\Phi_T$ calculated from Equation 3.4. Black dots denote measurement locations. Red dashed lines highlight the borders between the WAP coast, shelf, and slope regions.
3.3.3 Diel variability in photophysiological parameters

Both $F_v/F_m$ and fluorescence lifetimes displayed a pronounced diel cycle throughout the WAP (Figure 3.4), negatively correlated with surface PAR (Table 3.1). Median $F_v/F_m$ values during the night across the whole grid were $0.38 \pm 0.1$, concurrent with an average fluorescence lifetime of $1.03 \pm 0.21$ ns. During the day, as light intensity increased, $F_v/F_m$ decreased by $\sim 20\%$ to $0.29 \pm 0.1$, while fluorescence lifetimes decreased to $0.84 \pm 0.15$ ns. Conversely, $\Phi_T$ increased from $0.53 \pm 0.08$ to $0.61 \pm 0.09$ between night and day (Table 3.2). A weak negative correlation between $\sigma_{\text{PSII}}$ and

### Table 3.2 Median and median absolute deviation of photophysiological parameters. These include $F_v/F_m$, $\sigma_{\text{PSII}}$, fluorescence lifetime, $\Phi_T$, and $ETR_{\text{PSII}}^{\text{max}}$. Data were parsed by location; coast, shelf, and slope, and by time; night or day.

<table>
<thead>
<tr>
<th></th>
<th>Night</th>
<th>Day</th>
<th>Full diel cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_v/F_m$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coast</td>
<td>0.48 ± 0.03</td>
<td>0.39 ± 0.07</td>
<td>0.42 ± 0.06</td>
</tr>
<tr>
<td>Shelf</td>
<td>0.35 ± 0.07</td>
<td>0.27 ± 0.08</td>
<td>0.3 ± 0.08</td>
</tr>
<tr>
<td>Slope</td>
<td>0.24 ± 0.03</td>
<td>0.19 ± 0.04</td>
<td>0.22 ± 0.04</td>
</tr>
<tr>
<td>Full grid</td>
<td>0.38 ± 0.1</td>
<td>0.29 ± 0.1</td>
<td>0.31 ± 0.1</td>
</tr>
<tr>
<td>$\sigma_{\text{PSII}}$ [Å²]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coast</td>
<td>476 ± 42</td>
<td>492 ± 58</td>
<td>484 ± 54</td>
</tr>
<tr>
<td>Shelf</td>
<td>710 ± 142</td>
<td>678 ± 125</td>
<td>694 ± 132</td>
</tr>
<tr>
<td>Slope</td>
<td>792 ± 174</td>
<td>744 ± 162</td>
<td>760 ± 168</td>
</tr>
<tr>
<td>Full grid</td>
<td>588 ± 126</td>
<td>564 ± 120</td>
<td>568 ± 124</td>
</tr>
<tr>
<td>Fluorescence lifetime [ns]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coast</td>
<td>0.81 ± 0.08</td>
<td>0.76 ± 0.1</td>
<td>0.77 ± 0.09</td>
</tr>
<tr>
<td>Shelf</td>
<td>1.11 ± 0.13</td>
<td>0.97 ± 0.18</td>
<td>1.03 ± 0.17</td>
</tr>
<tr>
<td>Slope</td>
<td>1.5 ± 0.06</td>
<td>1.07 ± 0.21</td>
<td>1.29 ± 0.23</td>
</tr>
<tr>
<td>Full grid</td>
<td>1.03 ± 0.21</td>
<td>0.84 ± 0.15</td>
<td>0.9 ± 0.17</td>
</tr>
<tr>
<td>$\Phi_T$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coast</td>
<td>0.46 ± 0.02</td>
<td>0.53 ± 0.05</td>
<td>0.5 ± 0.05</td>
</tr>
<tr>
<td>Shelf</td>
<td>0.58 ± 0.06</td>
<td>0.65 ± 0.07</td>
<td>0.62 ± 0.06</td>
</tr>
<tr>
<td>Slope</td>
<td>0.66 ± 0.02</td>
<td>0.72 ± 0.09</td>
<td>0.69 ± 0.04</td>
</tr>
<tr>
<td>Full grid</td>
<td>0.53 ± 0.08</td>
<td>0.61 ± 0.09</td>
<td>0.59 ± 0.09</td>
</tr>
<tr>
<td>$ETR_{\text{PSII}}^{\text{max}}$ $[e^{-1} s^{-1} RC^{-1}]$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coast</td>
<td>58 ± 15</td>
<td>119 ± 37</td>
<td>107 ± 39</td>
</tr>
<tr>
<td>Shelf</td>
<td>59 ± 17</td>
<td>176 ± 53</td>
<td>137 ± 55</td>
</tr>
<tr>
<td>Slope</td>
<td>208 ± 118</td>
<td>263 ± 100</td>
<td>230 ± 105</td>
</tr>
<tr>
<td>Full grid</td>
<td>64 ± 21</td>
<td>149 ± 53</td>
<td>128 ± 55</td>
</tr>
</tbody>
</table>
PAR intensities was seen (Table 3.1). However, despite this, a diel cycle was not a prominent feature, particularly along the coast (Figure 3.5d, Table 3.2), and σ_{PSII} values during the night and day averaged 588±126 Å² and 564±120 Å² respectively (Table 3.2). On the other hand, ETR_{PSII}^{max} correlated positively with PAR (Table 3.1). During the night ETR_{PSII}^{max} was low (64±21 e⁻ s⁻¹ RC⁻¹), while during the day ETR_{PSII}^{max} more than doubled (149±53 e⁻ s⁻¹ RC⁻¹) (Table 3.2).

Figure 3.4 Diel cycles in photophysiology. a) F'/F_m (blue) and b) fluorescence lifetime [ns] (blue) on the left Y axis from underway FLRe and PicoLiF measurements. Right Y-axis shows atmospheric PAR intensity (gray dots) as measured from the ships mast. Data represent the first week of the cruise.
3.4 Discussion

Our results reveal a clear gradient in photophysiological characteristics across the continental margin of the WAP (Figure 3.3, Table 3.2). This gradient is consistent with bottom-up control by iron availability in surface waters and supports the hypothesis that iron strongly limits phytoplankton photochemical energy conversion offshore (Annett et al. 2017; Schofield et al. 2018). The combination of variable fluorescence and lifetimes revealed an increased amount of
uncoupled LHC complexes under iron limitation (Figure 3.6) as well as a clear tradeoff between photochemistry and thermal dissipation (Figure 3.7), resulting from the spatial gradient in iron stress across the WAP. To support this conclusion, we discuss a number of physiological responses to iron limitation across the WAP. These include spatial variabilities in nighttime values and diel cycles of photophysiological parameters, LHC-RC uncoupling, and \( \Phi_T \). In addition, we examine the variability in \( \text{ETR}^{\text{PSII}}_{\text{max}} \).

**Figure 3.6 Relationship between \( \frac{F_v}{F_m} \), fluorescence lifetime and \( \sigma_{\text{PSII}} \).** Circles represent data collected from the coast and triangles data from the slope. Symbol color represents \( \sigma_{\text{PSII}} \). In both locations only data collected during the night is shown in order to remove the non-linear effect of NPQ on the otherwise linear relationship between \( \frac{F_v}{F_m} \) and Fluorescence lifetime (Lin et al. 2016). Dashed lines indicate the fraction of uncoupled reaction centers. 0% dashed lines represents the modeled linear dependency of \( \frac{F_v}{F_m} \) and fluorescence lifetime (Butler 1978). 10%-30% dashed lines represent the dependency of these parameters in detached antenna PSII reaction center complexes.
At night, when NPQ is nil (Lin et al. 2016), $F_v/F_m$ over the continental slope decreased by 50% in comparison to the iron richer regions closer to the coast (Table 3.2). At the same time, fluorescence lifetimes and $\sigma_{PSII}$ increased by 85% and 65%, respectively. These trends are diagnostic of iron stressed photosynthesis along the continental slope. The extremely high values of $\sigma_{PSII}$ offshore corroborate previous laboratory measurements on iron-limited Southern Ocean species (Strzepek et al. 2019). Diel cycles of photophysiological parameters were similar across the WAP, with a nighttime maxima and midday minimum (Figure 3.5). However, the magnitude of diel variations was much larger in iron limited regions (Figure 3.5 and Table 3.2). The diel cycles observed in the iron-limited WAP continental slope contrast with previously established signatures

![Figure 3.7 Relationship between $F_v/F_m$ and the quantum yield of thermal dissipation ($\Phi_T$).](image)

The quantum yield of thermal dissipation is calculated from underway measurements of variable fluorescence with a FIRe instrument and fluorescence lifetime with a PicoLiF instrument as $\Phi_T = 1 - \left[ \frac{F_v}{F_{m(\Phi_T)}} + \frac{\tau}{\tau_0} \right]$, where $\tau$ is the measured lifetime in ns and $\tau_0$ is the natural lifetime of Chl $a$, 15 ns (See main text). A linear regression line, the 95% confidence interval, the regression equation and the $r^2$ are indicated ($n = 7590$).
of iron limitation observed in the Equatorial Pacific (Behrenfeld and Kolber 1999). In that region, dominated by cyanobacteria, $F_v/F_m$ decreased by 35%-60% following the sunset and recovered at sunrise, resulting in a pillared nighttime feature. Behrenfeld and Kolber (1999) concluded this diel fluorescent pattern was due to state transitions in iron-limited plankton. Such diel patterns were not observed in iron-limited regions in the WAP (Figure 3.4 and Figure 3.5). This is because phytoplankton assemblages in the WAP are dominated by diatoms (Schofield et al. 2017), in which state transitions are absent (Owens 1986).

We next consider the occurrence of energetically uncoupled LHC complexes. The basic biophysical model for energy distribution in the photosynthetic unit predicts an inverse relationship between low-light acclimated $\Phi_{PSII}$ and $\Phi_F$ (Butler 1978; Lin et al. 2016; Falkowski et al. 2017). The correlation between $F_v/F_m$ and fluorescence lifetime collected at night validates our assumption of linearity in these two yields (Pearson’s linear correlation coefficient of -0.91, $\rho < 0.05$). However, the data are not consistent with Butler’s model (Figure 3.6). To calculate the fraction of uncoupled LHC complexes, the relationship between $F_v/F_m$ and fluorescence lifetime was modeled for three physiological states with different lifetimes (Park et al. 2017). Two states represent cases in which LHC complexes are coupled to RCs and RCs are fully open or fully closed with lifetimes of 0.5 ns and 1.5 ns, respectively. The third represents uncoupled LHCII complexes with a very long lifetime of 4 ns (Palacios et al. 2002). The presence of such energetically detached antenna complexes would ultimately lead to longer measured lifetimes, and these lifetimes may exceed the values observed for fully closed reaction centers (~1.5 ns).

In Figure 3.6, nighttime $F_v/F_m$ values are plotted against lifetimes. A distinct deviation from the classical inverse relationship predicted by Butler’s model is seen at the low $F_v/F_m$. Moreover, two clusters are clearly seen. The first cluster represents coastal data, with high $F_v/F_m$, low fluorescence lifetimes and small $\sigma_{PSII}$. This coastal cluster aligns fairly well with the modeled case.
for open RC with nearly fully coupled antenna complexes, as expected for iron-replete conditions. The second cluster represents data from the continental slope, with low $F_v/F_m$ and long fluorescence lifetimes. This analysis suggests that 20%-30% of antenna complexes are detached in iron-limited waters offshore.

Over the WAP slope, $\Phi_T$ significantly increased (Figure 3.3d, Table 3.2), confirming our hypothesis that phytoplankton increase $\Phi_T$ as iron limitation intensifies. At night, in the absence of NPQ, $\Phi_T$ along the slope was $\sim$45% higher than in coastal waters (Table 3.2). This increase in $\Phi_T$ is driven by a reduction in the photosynthetic use efficiency. With few active RC, and a significantly large and uncoupled LHC, excitons are more likely to dissipate as heat (Strzepek et al. 2019). In addition to the positive correlation with distance to shore, $\Phi_T$ also positively correlated with increasing light availability (Table 3.1). During the day, $\Phi_T$ increased by an additional 15% across the WAP (Table 3.2), yet offshore values were still $\sim$40% higher than onshore values. This daytime increase in $\Phi_T$ indicates NPQ activation that effectively increases thermal dissipation. As iron-stressed phytoplankton are more prone to photooxidative damage at the active RCs (Greene et al. 1992; Strzepek et al. 2012), increasing $\Phi_T$ acts to further decrease excitation pressure on the RC in favor of thermal dissipation in the uncoupled antenna complex. To examine this hypothesis, we plotted the relationship between $F_v/F_m$ and thermal dissipation from the full dataset (Figure 3.7). A linear regression revealed a slope of -0.94 ($r^2=0.98$); the deviation from a -1.00 slope is attributed to ca. 6% dissipation by fluorescence (Lin et al. 2016). The increased $\Phi_T$ observed in the WAP supports recent studies showing higher photoprotective capacities in iron limited phytoplankton assemblages (Alderkamp et al. 2013; Schallenberg et al. 2020).

Moreover, the increase in uncoupled complexes combined with exceptionally high $\Phi_T$ offshore, strongly agrees with the proposed mechanism for efficient NPQ in diatoms. This mechanism attributes the rapid NPQ capacity to thermal dissipation in the LHC, driven by
xanthophyll pigment cycling and the presence of Lhcx proteins (Lepetit et al. 2017; Buck et al. 2019). These presumably cause a conformational change that distances the antenna complex from the RC, increasing the Förster resonance energy transfer distance, functionally mediating the energetic uncoupling of the LHC and RC.

Lastly, we turn to evaluate possible light limitation in the WAP surface waters, a second, potentially important bottom-up control in this region (Moline 1998). Increased photoprotective activity during the day across the WAP, inferred from $\Phi_T$ (Table 3.2), suggests that phytoplankton are exposed to saturating light intensities in the near-surface layer. Combined with the high $E_{\text{TR}}^{\text{PSII}}$ values measured during the day (Table 3.2) it is highly unlikely that surface phytoplankton were light limited.

Although $E_{\text{TR}}^{\text{PSII}}$ was initially assessed in regard to light limitation, it was surprising and perhaps counterintuitive to observe significantly higher (~120%) $E_{\text{TR}}^{\text{PSII}}$ in the iron-limited waters offshore (Table 3.2). A similar response in $E_{\text{TR}}^{\text{PSII}}$ to iron limitation has been reported in phytoplankton assemblages from the Northeast subarctic Pacific, where iron amendment experiments resulted in decreased $E_{\text{TR}}^{\text{PSII}}$ (Schuback et al. 2015). Similar results were seen in laboratory experiments with the diatom *Thalassiosira oceanica*, the haptophyte *Chrysochromulina polylepis* (Schuback et al. 2015) and the cyanobacterium *Synechococcus sp.* (Blanco-Ameijeiras et al. 2018). The iron limited increase in $E_{\text{TR}}^{\text{PSII}}$ is assumed to be an additional effect of the iron economizing physiology. In this manner, more excitons are funneled from the large antenna to fewer functional RC, leading to increased ETR per active PSII RC, each associated with a larger $\sigma_{\text{PSII}}$. While we argue that our data provides little evidence for light limitation in the surface waters we measured, and is supported by previous studies (Moline et al. 1996), the effect of the MLD on light availability in the water column can’t be overlooked. Indeed, along the coast, MLD (13.3±5.1) nearly reached the critical depth (14.9±8.4 m), while along the slope, the MLD exceeded by up to
~30% the critical depth (30±6 m 22.9±10.9 m, respectively). Sverdrup’s critical depth hypothesis (Sverdrup 1953) appears to imply that light limitation is particularly severe in the water column along the continental slope. Why then, is there little photophysiological evidence for light limitation in the surface waters offshore? We speculate that our data agrees with the hypothesis that in Southern Ocean phytoplankton, the photophysiological response to iron limitation eliminates the antagonistic co-limitation of iron and light (Strzepek et al. 2012). Accordingly, the high capacity for light harvesting in the iron limited slope community alleviates light limitation. On the other hand, in the iron replete coastal community, light limitation is more probable and agrees with a recent study along the coast (Carvalho et al. 2019). Still, during the day coastal phytoplankton in the surface waters themselves experience light saturating conditions. This may result from a long-term acclimation to limiting light conditions in the water column, subjecting phytoplankton to overexcitation at saturating light, likely only met at the surface.

Our analysis assumes a uniform taxonomic composition across the WAP, which can potentially influence fluorescence measurements (Suggett et al. 2009a). This is a fairly safe assumption as HNLC regions are anomalous in this respect, yet with relatively consistent fluorescence signatures across taxa (Suggett et al. 2009a). This is further supported in Southern Ocean species (e.g. Strzepek et al. 2019), in particular diatoms, the dominant species in the WAP (Schofield et al. 2017).

Data presented here provides strong evidence for a distinct gradient in the degree of iron limitation across the WAP during the summer. Iron limitation was shown to be minimal at the coast and severe further offshore. As we hypothesized, combined measurements of $\Phi_{\text{PSII}}$ and $\Phi_T$ showed increased fractions of uncoupled LHC-RC complexes as well as clear increases in $\Phi_T$ resulting from iron stress. The deep MLD across the WAP may have caused light limitation in the water column. Nonetheless the clear acclimation to iron stress in the surface waters along the slope
effectively reduced potential light limitation to a degree that phytoplankton were more susceptible to light saturation. Our in-depth analysis of strictly biophysical mechanisms in response to iron stress is highly supported by a large number of studies, further strengthening our conclusions.

The WAPs case study, presented here, highlights the potential of our \( \Phi_{\text{PSII}} \) and \( \Phi_{\text{F}} \) coupled measurements as a rapid diagnostic tool for \textit{in situ} assessments of iron limitation at high spatial and temporal resolution. More critically, this diagnostic tool provides a unique new avenue to assess \textit{in situ} the role of uncoupled complexes in natural assemblages, their effect on satellite retrieved chlorophyll fluorescence and primary productivity models.
Chapter 4: The photophysiological response of nitrogen-limited phytoplankton to tropical instability waves across the Equatorial Atlantic

In the Equatorial Atlantic, a low-nutrient low-chlorophyll region, nitrogen is assumed to limit phytoplankton dynamics. While the Equatorial Atlantic is a major contributor to global carbon fixation, *in situ* measurements of phytoplankton physiology and productivity are sparse in comparison with the North Atlantic. Following the formation of the Equatorial cold tongue in the summer, tropical instability waves (TIW) occur, causing localized upwelling. Here, we assessed changes in phytoplankton photophysiology in response to TIW driven upwelling as well as short-term nutrient addition experiments using a pair of custom-built fluorometers that measure underway chlorophyll *a* variable fluorescence and fluorescence lifetimes. The fluorometers were deployed during a transatlantic cruise along the Equator in the Fall of 2019. We hypothesized that the Equatorial Atlantic is nitrogen-limited, with an increasing degree of limitation to the west, and that infrequent nitrate injection by TIW is the primary source alleviating this limitation. We further hypothesized phytoplankton are well acclimated to the low levels of nitrogen, and once nitrogen is supplied they can rapidly utilize it to stimulate growth and productivity. Across three upwelling events encountered, we observed increased productivity and chlorophyll *a* concentration concurrent with a decreased photochemical conversion efficiency and overall photophysiological competency. Moreover, an observed decrease in dawn-time photosynthetic turnover rates toward the western section reflected a relative 70% decrease in growth rates in the western sections compared to the eastern section. This decrease aligned with the increased growth rates observed following 24h incubation with added nitrate in the western section. These results support our
hypotheses that nitrogen is the limiting factor in the region and that phytoplankton are in a state of balanced growth, waiting to “surf” waves of nutrients which fuel growth and productivity.

4.1 Introduction

The Atlantic Ocean is the best studied ocean basin. However, the vast majority of research efforts have been focused on the North Atlantic. On the other hand, the tropical Atlantic, and in particular the Equatorial Atlantic (EA), is greatly under-sampled, specifically with regard to phytoplankton dynamics. Moreover, in situ data is sporadic, and is based mostly on data collected during Equatorial crossings of the Atlantic Meridional Transect (AMT) program. These cruises mostly occur between April-June and September-November, and as such, generally miss the most productive summer months when, based on satellite observations, phytoplankton bloom (Longhurst 1993; Pérez et al. 2005). As a result, phytoplankton dynamics in this region are poorly understood.

This sampling disparity is interesting, since satellite primary productivity assessments estimate that the EA (between 10°N-10°S) contributes more to global carbon fixation than the entire Spring bloom region of the North Atlantic (Longhurst 1993). Nonetheless, studies have shown that most of the EA is a low-nutrient low-chlorophyll (LNLC) region in which nitrogen is the limiting factor for phytoplankton growth and photosynthetic yields (Mills et al. 2004; Davey et al. 2008; Moore et al. 2008, 2013).

Phytoplankton in the EA rely on infrequent mesoscale turbulent events that upwell nutrient-rich waters from below the thermocline. Such upwelling events introduce nitrate into the surface waters that increases new production, i.e., primary production fueled by nitrate, rather than regenerated ammonium (Dugdale and Goering 1967). Under steady-state conditions, new production is balanced by export production (in order to maintain a mass balance) (Laws et al. 2011). In order to successfully utilize these nutrient pulses, phytoplankton need to rapidly adjust
and synthesize key photosynthetic components that will allow bursts of growth and productivity (Falkowski et al. 2017). In layman’s terms, phytoplankton in LNLC regions need to optimize their physiological state in a manner that will allow them to rapidly “surf” upwelling waves.

It has been suggested that one critical and potential source of localized upwelling along the EA is Tropical Instability Waves (TIW). TIW are westward propagating, cusp-shaped, oscillations in the Equatorial cold tongue (de Decco et al. 2018). TIW are seasonally variable, appearing during the summer months, several weeks following the formation of the Equatorial cold tongue (Emery et al. 2006; de Decco et al. 2018). TIW are clearly seen in sea surface temperature (SST) data (Menkes et al. 2002). TIW form due to baroclinic instabilities and velocity shear between the South Equatorial current and North Equatorial countercurrent, as well as the Equatorial undercurrent (Philander 1978; de Decco et al. 2018). The waves develop in the Eastern Equatorial and propagate West with a wavelength of ~1000 km and a velocity of 30-50 cm/s, resulting in periods of ~30 d (Cox 1980; Emery et al. 2006). As TIW propagate intense upwelling is observed in the warm troughs, where current velocities diverge, while downwelling is seen in the cold crests, where current velocities converge (Philander et al. 1986). It is thus apparent that the role TIW play in nutrient supply for phytoplankton is of great interest, however direct measurements linking the physical processes and phytoplankton dynamics have lacked so far (Menkes et al. 2002).

Variable fluorescence measurements of the maximum quantum yield of photochemistry ($F_v/F_m$) in photosystem II (PSII) are widely used to rapidly assess the extent of nutrient limitation in situ (Falkowski 1994; Greene et al. 1994; Parkhill et al. 2001; Bonnet et al. 2008; Ko et al. 2020). Rapid (<48h) increases in $F_v/F_m$ following the addition of the limiting nutrient have been used to further support the existence of limitation (Falkowski et al. 1992; Suggett et al. 2009b; Ko et al. 2020). Variable fluorescence techniques, such as FRR (fast repetition rate) or FIRe (fluorescence induction and relaxation), provide additional parameters and information regarding the
photophysiological state of PSII, which can be used to assess and quantify the extent of nutrient limitation. These parameters include the effective absorption cross section of PSII ($\sigma_{\text{PSII}}$), kinetics of electron transport on the acceptor side of PSII, and the maximum electron transfer rate through PSII ($\text{ETR}_{\text{PSII}}^{\text{max}}$) (Gorbunov et al. 2000, 2001).

Variable fluorescence measurements can be combined with simultaneous picosecond fluorescence lifetime measurements in order to resolve the three pathways in which phytoplankton can utilize or dissipate the absorbed solar energy; photochemistry, fluorescence emission, and non-radiative thermal dissipation (Butler and Strasser 1977; Butler 1978; Lin et al. 2016; Gorbunov and Falkowski 2022). Chlorophyll $a$ (Chl $a$) fluorescence lifetimes measure the time it takes an excited state Chl $a$ molecule to return to the ground state, which is directly proportional to the quantum yield of fluorescence emission in the photosynthetic unit (Brody and Rabinowitch 1957; Brody 2002; Lakowicz 2006). The simultaneous measurements of variable fluorescence and fluorescence lifetime provide further insight into the photophysiological response of phytoplankton to nutrient limitation and relief.

Even though $F_v/F_m$ is commonly used to assess nutrient limitation this parameter alone is insufficient to indicate nitrogen limitation, as its relationship with growth rate is highly non-linear (Parkhill et al. 2001; Gorbunov and Falkowski 2020). Several ship-board nutrient addition experiments conducted in the oligotrophic sub-tropical Atlantic and tropical Pacific showed that while alleviation from nitrogen limitation results in increased chlorophyll synthesis, a lack of response, or even a slight decline, was observed in $F_v/F_m$ (Behrenfeld et al. 2006; Moore et al. 2008). A key contributor to such response was the predominance of cyanobacteria across the LNLC regions. In cyanobacteria the primary light harvesting complex (LHC) for PSII (and PSI) is the phycobilisome. The phycobilisome is a large, water-soluble, peripheral structure on the thylakoid membrane, which consists of phycobilins, a group of photosynthetic pigments. The phycobilins
transfer excited state energy to the RC via an energy gradient between the three main pigments, phycoerythrin, phycocyanin and allophycocyanin (Sidler 1994). The energy transfer is not 100% and the two latter pigments overlap spectrally with the fluorescence bands of Chl a, resulting in an increased background fluorescence when measuring \( F_v/F_m \) (Campbell et al. 1998). This further complicates the use of \( F_v/F_m \) alone to assess the extent of nitrogen limitation in LNLC tropical regions. On the other hand, the maximum electron transport rate (\( \text{ETR}^{\text{max}}_{\text{PSII}} \)) at saturating irradiances, conventionally derived from kinetic-based variable fluorescence (Gorbunov et al. 2000) provides a better quantitative indicator for nitrogen limitation. Recently, Gorbunov and Falkowski (2020) developed a method to calculate electron transport rates from the photosynthetic turnover rate measured at saturating irradiances, referred to as ETR\( \tau \), using a kinetic fluorescence analysis. Using this method improves the accuracy of ETR measurements substantially, in addition to being very sensitive to nitrogen limitation. Moreover, Gorbunov and Falkowski showed that the photosynthetic turnover rate at saturating irradiances can be used to quantify the reduction in growth rates and electron yield of carbon fixation (i.e., the number of electrons required to accumulate one carbon molecule) due to nitrogen limitation. This allows to potentially model net primary production from variable fluorescence, and in the case of new production fueled by upwelled nutrients, even export production.

In this study, we examined the response of phytoplankton photophysiology across the EA to upwelling events driven by TIW. A pair of custom-built fluorometers were deployed on-board the R/V Meteor during a trans-Equatorial cruise (Fig. 1). The first, a FIRe (Fluorescence Induction and Relaxation) instrument measured \( F_v/F_m \), \( \sigma_{\text{PSII}} \) and ETR\( \tau \). The second instrument, the PicoLiF measured fluorescence lifetimes.

Our main hypothesis was that the paucity of nitrogen is the primary factor limiting productivity in the EA, and TIW are the most important mechanism supplying nitrogen to the
surface via upwelling. Moreover, we hypothesized that since the Equatorial cold tongue is prevalent East of 23°W, the degree of nitrogen limitation increases to the west. Interestingly, a natural system like this, where nitrogen limitation is hypothesized to be disrupted infrequently by waves of nutrients, allowed us to test a third hypothesis. Here, we hypothesized phytoplankton in the EA surface waters can rapidly “body surf” these upwelled nitrogen waves, quickly fine-tuning their photophysiology and optimizing energy harvesting and transfer to fuel growth and primary productivity.

4.2 Materials and Methods

4.2.1 Study area and sample collection and analysis

Data were collected on board the R/V Meteor as part of the Transatlantic Equatorial Cruise I (TRATLEQ I) between 5°E to 44°45’W during September-October 2019 (Figure 4.1). Across the Equator CTD sampling stations were conducted at each one degree of longitude. At each station water samples were collected for Chl a and dissolved macro inorganic nutrients (nitrate, nitrite, phosphate, and silicate). Depths sampled included at the least the surface as well as above, in and below the deep chlorophyll maximum (DCM). Variable fluorescence and fluorescence lifetime data were collected continuously from surface waters (~5 m depth) while underway with FIRE and PicoLiF fluorometers respectively, as described by (Sherman et al. 2020). The two instruments utilized flow through cuvettes connected to the ship’s surface water intake pump. Prior to entering the cuvette, the water passed through two de-bubblers.
4.2.2 Photophysiology

A mini-FIRE instrument measured fluorescence before and after a saturating single turnover flash (STF) from blue light-emitting diodes (450 nm +/- 30 nm half bandwidth), which cumulatively reduces all PSII RCs within ca. 80 μs. This STF protocol results in minimum and maximum fluorescence yields (F₀ and Fₘ). The quantum yield of photochemistry in PSII was then calculated as (Fₘ - F₀)/Fₘ = Fᵥ/Fₘ (Butler 1978; Kolber et al. 1998). The effective absorption cross section of PSII, σₚₛᵢᵢ (at 450 nm), is calculated by fitting the fluorescence rise to a cumulative one-hit Poisson function (Ley and Mauzerall 1982). The rate of fluorescence relaxation after the STF recorded under saturating irradiance is then used to calculate the photosynthetic turnover rate (Gorbunov and Falkowski 2020).

Every ~30 min. the water flow into the mini-FIRE instrument was automatically paused to conduct Slow Light Curves, i.e., fluorescence-versus-irradiance (FE) curves. These were used to calculate electron transport rates (ETR) as a function of irradiance and to depict the state of
phytoplankton photoacclimation to their light history (Falkowski 1994; Ralph and Gademann 2005). During FE curves, water was trapped in the cuvette for ca. 10 min to allow for low-light acclimation, and then exposed to increasing PAR levels (0-900 μmol photons m⁻² s⁻¹) with an actinic blue light source (450 nm). From FE curves, we calculated ETRτ per PSII RC (units of e⁻ s⁻¹ RC⁻¹) achieved at saturation following (Gorbunov and Falkowski 2020)

\[
ETR_\tau = \frac{1}{\tau} \left( \frac{E}{F_m'} \Delta F'/F'_m \right) \div \left( \frac{E_{\text{max}}}{F_m'} \Delta F'/F'_m \right)
\]  

Equation 4.1

Here, E is irradiance and ΔF'/F'_m is the quantum yield of photochemistry at a given PAR level. Their multiplication represents the relative ETR. The prime notation indicates that the measurement was conducted under ambient light (ΔF' = F'_m - F', where F' is the steady state fluorescence at a given light step). The relative ETR is then divided by the relative ETR under saturating irradiance (E_{max}) to normalize to unity and multiplied by the photosynthetic turnover rate (1/τ in Equation 4.1) to calculate the absolute ETR (ETRτ) per PSII RC. E_{max} is defined as ~3 times E_K, the saturating light level (Falkowski and Raven 2007)

The PicoLiF measured picosecond fluorescence decays which were deconvoluted from the instrument response function and then fitted to a sum of three exponentials with a custom TCSPFIT Matlab package utilizing a Nelder-Meade simplex algorithm (Enderlein and Erdmann 1997). Φ_F was then calculated from

\[
Φ_F = \frac{τ}{τ_0}
\]  

Equation 4.2
Where \( \tau \) is the measured lifetime and \( \tau_0 \) is the natural lifetime of chlorophyll \( a \) (Brody and Rabinowitch 1957; Brody 2002). The natural lifetime is the time that would be required for a molecule to return to the ground state from an excited state if fluorescence were the sole dissipation pathway. For Chl \( a \), \( \tau_0 \) is 15 ns and is constant, independent of solvent, organism or environmental condition (Brody and Rabinowitch 1957; Brody 2002; Lakowicz 2006). We then calculated the quantum yield for thermal dissipation (\( \Phi_T \)) as

\[
\Phi_T = 1 - \left[ \frac{F_v}{F_m} + \frac{\tau}{\tau_0} \right]
\]

Equation 4.3

All fluorescence measurements were corrected for the blank signal measured routinely from filtered seawater (0.2 \( \mu \)m) (Bibby et al. 2008).

4.2.3 Nutrient Amendment experiments

At five CTD stations across the Equator additional surface water was collected to initiate short term nutrient amendment experiments (NAE). The NAEs were conducted at 4°W, 12°W, 28°W, 35°W and 40°W and consisted of an unamended control and experimental groups in which nitrate (5\( \mu \)M), phosphate (1\( \mu \)M), nitrate and phosphate combined, or iron (2nM) were added. The collected water was distributed into 250 mL polycarbonate bottles, spiked with nutrients, and placed in a deck-board incubator with flowing water to maintain in situ temperatures. Bottles were also screened to approximate the light levels in the surface waters. Each treatment included triplicates. At the time of collection standard FIRe measurement were conducted both in the dark and at a saturating light level. Following this, measurements were conducted after 24h and after 48h. To minimize photophysiological changes due to the diel cycle, we attempted to keep all measurements around
noon. Measurements included 5 technical replicates for each biological replicate (i.e., each bottle). The saturation level was chosen from an FE curve conducted on a control sample at $t_0$. To account for photophysiological variability in the control group due to changes in the light conditions and various other bottle effects, all measurements were normalized to the control group. We decided to focus on data collected after 24h to characterize the rapid response in phytoplankton physiology to nutrient amendments. In the warm Equatorial waters this time frame is sufficient. Longer term incubations would introduce changes that can be due to a change in the community composition. Lastly, we calculated the reduction in growth rates ($\mu/\mu_{\text{replete}}$) and electron yield of net primary production ($\Phi_{\text{NPC}}$) under nutrient limitation (particularly by nitrogen) following (Gorbunov and Falkowski 2020) as

$$\frac{\mu}{\mu_{\text{replete}}} = 1.85 \times \frac{\tau_{\text{replete}}}{\tau} - 0.85$$  \hspace{1cm} \text{Equation 4.4}$$

$$\Phi_{\text{NPC}} = \Phi_{\text{NPC}}^{\text{max}} \times \left(1.78 \times \frac{\tau_{\text{replete}}}{\tau} - 0.78\right)/\frac{\tau_{\text{replete}}}{\tau}$$  \hspace{1cm} \text{Equation 4.5}$$

In Equations 4.4 and 4.5 $\frac{\tau_{\text{replete}}}{\tau}$ represents the relative decrease in photosynthetic turnover rate by nitrogen stress, where $\tau$ is the photosynthetic turnover time and $\tau_{\text{replete}}$ is the photosynthetic turnover time in nutrient replete conditions. We understand the double use of $\tau$ here and for the average fluorescence lifetime may be confusing. This stems from the fact both measurements are measuring relaxation kinetics commonly denoted by $\tau$, however the two measure different processes operating at vastly different time scales. Aside for the Equations above we refrain from using the term $\tau$ alone in order to avoid confusion. In Equation 4.5 $\Phi_{\text{NPC}}^{\text{max}}$ represents the maximum electron yield of net carbon production for nutrient-replete conditions (0.076 e'/C) (Gorbunov and Falkowski 2020).
Note that at 12°W there was no NP group, and that iron addition were only done at 12°W, 28°W and 35°W.

4.3 Results

4.3.1 The Equatorial Atlantic system

Oceanographic conditions across the EA reflected the tropical ocean’s stratified, oligotrophic mean state. The stratified surface layer extended down to the 25°C isotherm, denoting the top of the thermocline (dashed black line in all Figure 4.2 panels). As expected, the thermocline was deep in the west (125-150 m) and shoaled towards the east (~60 m) (Figure 4.2a). Similarly, the pycnocline followed the shoaling trend (24 kg m$^{-3}$ isopycnal in Figure 4.2b). Macronutrient (nitrate and phosphate) distributions along the EA were strongly controlled by the water column structure. In the stratified surface layer, nutrient concentrations were vanishingly low and significantly increased below the thermocline by ~2-3 orders of magnitude (Figure 4.2c, phosphate not shown). The nitrate/phosphate ratio across the EA, calculated from the first 120 m averaged ~7, a value below the canonical 16/1 Redfield ratio. In the stratified surface sections the nitrate/phosphate ratio was significantly lower, averaging 1.7.

Chl $\alpha$ concentration in the stratified surface was remarkably low (Figure 4.2d). Generally, the DCM (white dash-dot line in all Figure 4.2 panels) formed on top of the thermocline and similarly sloped down to the west. Moreover, the DCM correlated well with the nutricline (denoted by the 1 μmol L$^{-1}$ isoline, Figure 4.2c). Lastly, Chl $\alpha$ concentration in the DCM decreased significantly (by an order of magnitude) in the west (Figure 4.2d). Below, we will refer to the stratified surface as the steady-state sections.

Along the EA we came across three distinct upwelling events associated with TIW fronts. The first, termed uw1, occurred between 6°W-10°W and was the most pronounced event
encountered in which the thermocline (25°C isotherm) upwelled to the surface resulting in a ~2°C drop in SST (Figure 4.2a). In this upwelling core, surface nitrate and Chl $a$ increased by an order of magnitude (Figure 4.2c, d). In addition, while the core of uw1 was somewhat narrow, the elevated Chl $a$ was observed as far west as 18°W (Figure 4.2d). A second event, uw2, occurred between 26°W-30°W. While higher nitrate concentrations were measured in the surface waters along uw2, surface Chl $a$ only slightly increased. This is highlighted by a slight shoaling of the 0.5 mg m$^{-3}$ Chl $a$ isoline at 40 m (which reached the surface in uw1). The third event, uw3, occurred between 35°W-40°W, represented a TIW front we came across twice, evident by a narrow double peak most clearly seen in the density and Chl $a$ data (Figure 4.2b, d). In uw3, both nitrate and Chl $a$ concentration increased. As upwelled waters along uw2 and uw3 originated from above the thermocline SST didn’t drop drastically, nonetheless these sections diverged from the steady-state conditions. Overall, these upwelling events increased surface nitrate and Chl $a$ concentration by roughly 5- and 8-fold, as well as increasing the average nitrate/phosphate ratio to 3.5.
4.3.2 Surface photophysiology

Figure 4.3 presents a general overview of the photophysiological parameters measured by the FIRe and PicoLiF instruments while underway alongside sea surface temperature. Table 4.1 summarizes the dawn-time means of the main photophysiological parameters across each section. Under steady-state conditions, photophysiology was characterized by extremely low chlorophyll...
biomass (as evident from low \( F_m \) and \( F_0 \)), relatively high \( F_v/F_m \) and photosynthetic turnover rates, average fluorescence lifetimes of ~1 ns and small \( \sigma_{PSII} \) (Figure 4.3, Table 4.1). Across uw1 significant increases in \( F_m \) (and \( F_0 \), to an even larger degree), fluorescence lifetimes and \( \sigma_{PSII} \) were observed. Concurrently, \( F_v/F_m \) and photosynthetic turnover rates significantly decreased (Figure 4.3, Table 4.1) Across uw2 we observed similar trends, however, these were far more moderate. \( F_m \) and \( \sigma_{PSII} \) across uw3 were on par in magnitude with uw1, while \( F_v/F_m \), photosynthetic turnover rates and fluorescence lifetimes displayed a moderate response, as seen in uw2 (Figure 4.3, Table 4.1).

Maximum ETR\( \tau \) under saturating irradiance, calculated from the underway FE curves, revealed a ~40% decrease in dawn ETR\( \tau \) from the east to west steady-state sections. Between 5°E-4°W dawn ETR\( \tau \) averaged 242±66 e\( \cdot \)s\(^{-1}\)RC\(^{-1}\). Further west, between 19°W-25°W and 30°W-34°W ETR\( \tau \) decreased significantly to 151±29 e\( \cdot \)s\(^{-1}\)RC\(^{-1}\) and 149±38 e\( \cdot \)s\(^{-1}\)RC\(^{-1}\), respectively (Table 4.1). This east-west gradient was statistically significant (two sample t-test, \( \rho \leq 0.05 \)). Across uw1 dawn ETR\( \tau \) averaged 223±81 e\( \cdot \)s\(^{-1}\)RC\(^{-1}\) (Table 4.1) and was not statistically different from the eastern steady-state ETR\( \tau \) mean. On the other hand, ETR\( \tau \) values across uw2 and uw3 were similar to the values seen in the western steady-state sections surrounding them (Table 4.1).

To better contrast the photophysiology of the steady-state and the upwelling sections, we compared the mean trend in diel cycles between the two. Since uw1 had the most pronounced response and it was the only event that we tracked for several days, providing statistical significance, we will focus on this event. Both in steady-state and uw1, the diel cycles of \( F_0 \) and \( F_m \) were characterized by a night-time maximum and a daytime minimum (Figure 4.4a, \( F_0 \) not shown). However, we did observe a difference in the dusk to dawn dynamics. Across the steady-state sections, after reaching the night-time maximum at dusk, \( F_0 \) and \( F_m \) plateaued until dawn when they began to decrease. On the other hand, across uw1, \( F_0 \) and \( F_m \) continually increased from the midday
minima until a midnight maximum followed by an abrupt decrease into the next day (Figure 4.4a). In both sections, the diel cycle in $F_v/F_m$ was characterized by double maxima, one at dusk, and a second, higher, dawn peak. The two peaks were flanked with midday and midnight minima (Figure 4.4b). While the diel trend in $F_v/F_m$ was consistent throughout the EA, the magnitude of the nighttime decrease was ~3 times higher along uw1 in comparison to the steady-state (35% vs. >10% decrease) (Figure 4.4b).

Fluorescence lifetimes were characterized by two distinct patterns (Figure 4.4c). Along the steady-state sections fluorescence lifetimes ranged between 0.8-1.2 ns. Despite this range, fluorescence lifetimes in these sections lacked a diel periodicity with similar day and night mean values (~1.1 ns). On the other hand, while daytime fluorescence lifetimes across uw1 were comparable to the steady-state sections (~1 ns), we observed dramatic increases in night-time values (as high as 2.2 ns) producing a distinct diel cycle. The diel cycle in $\sigma_{\text{PSII}}$ was relatively similar between the sections, with dawn/dusk maxima and daytime minima. In uw1 the daytime minima were reached earlier in the day in comparison to the steady-state section minima. Moreover, along uw1, $\sigma_{\text{PSII}}$ exhibited a night-time decrease (~20%), not observed along the steady-state sections (Figure 4.4d).

The unique diel cycle along uw1 is further characterized by a remarkable night to dawn transition in the fraction of energetically uncoupled PSII LHC-RC complexes (Figure 4.5). In uw1, we observed a very high fraction of uncoupled complexes during the night (ranging from 25% to over 40% uncoupling), however, at dawn, these complex recoupled and the fraction of uncoupled complexes drastically reduced and resembled the values along the steady-state (10%-20%). This night to dawn shift is absent in the steady state. Based on these results (Figures 4.3-4.5), data collected during the dawn represented the optimal state for PSII photophysiology (i.e., no evidence of NPQ, photoinhibition or state transitions).
Figure 4.3 Time series of near-surface temperature and photophysiology across the equatorial surface waters. Photophysiological data from underway FIRe and PicoLiF, representing low-light acclimated phytoplankton. Temperature from the ship thermosalinograph. From top to bottom; sea surface temperature (red), $F_m$ (green), $F_v/F_m$ (blue), fluorescence lifetime (black), $\sigma_{PSII}$ (dark blue), and photosynthetic turnover rate (cyan). Note the top and bottom x-axes ticks reflect the longitude in order to easily locate the steady-state sections and the upwelling events. The black and white bars at top and bottom denote night and day, highlighting the temporal variability of the data. Each series was smoothed using a rolling mean (30-minute window).

Table 4.1 Mean values and standard deviation of photophysiology at dawn. Parameters include $F_0$, $F_m$, $F_v/F_m$, fluorescence lifetime, $\Phi_T$, $\sigma_{PSII}$ and photosynthetic turnover rate at dawn. Data binned by sampling section. Each upwelling section means are statistically different from the steady state ($\rho<0.05$).

<table>
<thead>
<tr>
<th>Section name</th>
<th>$F_0$ (a.u.)</th>
<th>$F_m$ (a.u.)</th>
<th>$F_v/F_m$ (a.u.)</th>
<th>Fluorescence lifetime (ns)</th>
<th>$\Phi_T$ (a.u.)</th>
<th>$\sigma_{PSII}$ ($\AA^2$)</th>
<th>$1/\tau$ ($s^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steady state</td>
<td>87±15</td>
<td>162±25</td>
<td>0.46±0.04</td>
<td>1.08±0.17</td>
<td>0.47±0.04</td>
<td>489±29</td>
<td>893±175</td>
</tr>
<tr>
<td>uw1</td>
<td>402±75</td>
<td>597±96</td>
<td>0.33±0.03</td>
<td>1.27±0.18</td>
<td>0.59±0.02</td>
<td>719±56</td>
<td>492±64</td>
</tr>
<tr>
<td>uw2</td>
<td>189±8</td>
<td>308±18</td>
<td>0.39±0.01</td>
<td>1.13±0.05</td>
<td>0.54±0.01</td>
<td>639±12</td>
<td>621±83</td>
</tr>
<tr>
<td>uw3</td>
<td>316±96</td>
<td>511±145</td>
<td>0.39±0.04</td>
<td>1.18±0.08</td>
<td>0.53±0.03</td>
<td>786±72</td>
<td>580±69</td>
</tr>
</tbody>
</table>
Figure 4.4 Mean diel cycles in photophysiology. a) $F_{m}$, b) $F_v/F_m$, c) Fluorescence lifetime, and d) $\sigma_{PSII}$. Red dots represent the mean diel cycle averaged across the steady state section ($n=17$ days) and blue dots represent the mean diel cycle averaged across uw1 ($n=5$ days). Red and blue translucent lines denote the standard deviation. A loess smoother was applied to each section’s mean diel cycle (red and blue lines).
3.3 Nutrient amendment experiments

Within 24h of nutrient amendment we observed significant changes in the photophysiological parameters measured in the stations further to the west (28°W, 35°W and 40°W) (Figure 4.6). The addition of nitrate alone, or in combination with phosphate, resulted in a 100%-200% increase in Fₘ at these stations relative to the control (Figure 4.6a). On the other hand, at 4°W and 12°W Fₘ did not change, as compared to the control. Similarly, the addition of phosphate alone or iron had no effect on Fₘ relative to control (Figure 4.6a). While not as pronounced as the changes in Fₘ, we observed similar trends in Fᵥ/Fₘ in response to the nutrient amendments. In the three western NAEs the addition of nitrate alone increased Fᵥ/Fₘ relative to control by ~30%-40%. Moreover, amendment with nitrate and phosphate together further increased Fᵥ/Fₘ relative to control (40%-50%) (Figure 4.6b). The eastern NAEs on the other hand had either no response or only a very slight one in response to the addition of nitrate alone or with phosphate.
(Figure 4.6b). Overall, changes in ETR\(\tau\), measured under saturating irradiances, aligned with the trends observed in \(F_m\) and \(F_v/F_m\) (Figure 4.6c). At 4°W regardless of the treatment we observed no change relative to control in ETR\(\tau\). At 12°W the addition of nitrate or iron resulted in a 20% increase in ETR\(\tau\) relative to control, while phosphate had no change. We observed the most significant changes in ETR\(\tau\) at 28°W and 40°W. At 28°W ETR\(\tau\) increased by 40% relative to control in response to the addition of either nitrate or phosphate. In response to the combination of nitrate and phosphate ETR\(\tau\) further increased by 60% relative to control. In contrast, the addition of iron here had no effect. Similarly, at 40°W we observed increased ETR\(\tau\) when nitrate was added alone, and a higher increase when added in combination with phosphate (35% and 45% relative to control, respectively). In contrast, at 40°W, the addition of phosphate alone had little effect.
Figure 4.6 Relative change in photophysiology 24h after nutrient amendment. a) $F_m$, b) $F_v/F_m$, and c) ETR$_{\tau}$. Treatments included amendment with, +N, +P, +NP and +Fe. Each bar color indicates location where NAE was initiated. Change was calculated as $\frac{\text{Treatment}^{24\text{th}} - \text{Control}^{24\text{th}}}{\text{Control}^{24\text{th}}}$ for each parameter. Note the NAE at 4°W (gray bars) didn’t include a +Fe treatment, and the NAE 12°W (blue bars) didn’t include a +NP treatment.
4.4 Discussion

In this study we hypothesized that across the EA dissolved fixed nitrogen is the primary factor limiting phytoplankton productivity and that the degree of limitation intensifies to the west where the cold tongue is absent, and the sloping thermocline means that deep upwelling is needed to bring the cold, nutrient-rich water to the surface. We further hypothesized that TIW are the primary mechanism alleviating this limitation. In effect, we propose that phytoplankton in the EA surface waters “body surf” the infrequent waves of upwelled new nutrients driven by TIW. In this manner nutrient-limited phytoplankton adjust their photophysiology to maintain some degree of steady state growth while waiting for pulses of new nutrients to utilize rapidly, resulting in increased new production.

Overall, we observed significant increases in Chl $a$ and nitrate concentrations across the three upwelling events encountered along the EA compared to the steady-state, non-upwelling, regions (Figure 4.3, Figure 4.4). The doubling in the average nitrate/ phosphate ratio from 1.7 to 3.5 across the upwelling sections further showed that nitrate was upwelled preferentially more than phosphate. Likewise, across the upwelling sections primary production rates increased significantly. The 40% decrease in dawn ETR$_{\tau}$, recorded at saturating irradiances, observed from East to West further supports our hypotheses that nitrogen limits phytoplankton dynamics and that the degree of limitation increases to the west.

Interestingly however, in the LNLC steady-state sections, we observed photophysiologicaly competent phytoplankton with high $F_v/F_m$ and photosynthetic turnover rates, low $\sigma_{PSII}$ and $\Phi_T$, as well as relatively short fluorescence lifetimes (Figure 4.3, Table 4.1). This apparent disconnection between PSII photophysiology and environmental conditions may suggests that phytoplankton across the Equator are well acclimated to low nitrogen availability and have maintained a state of balanced growth (Parkhill et al. 2001). Likewise, as suggested by Gorbunov
& Falkowski (2020) the high Fv/Fm across the steady-state EA sections could reflect the non-linearity between Fv/Fm and growth rates. Moreover, Gorbunov & Falkowski (2020) showed that ETRτ at saturating irradiances is better suited to infer the degree of nitrogen limitation and can be used to quantify the reduction in growth rates and net primary production in nitrogen limited phytoplankton. Following their methodology, we used the average photosynthetic turnover time at dawn measured under saturating irradiance in the eastern steady-state section (~4130 s) as our nutrient replete term and the corresponding average value from the western steady-state section (~6620 s) as the nitrogen limited term in Equation 4.4. From this, we calculated a 70% decrease in growth rates in the western steady-state section as compared to the eastern section. Moreover, this decrease equates to a ~50% decrease in ΦNPc (Equation 4.5), indicating that, as hypothesized, the degree of nitrogen limitation intensifies to the west of the EA.

Additional evidence supporting our hypotheses was the rapid (24 h) and significant changes in phytoplankton physiology in response to nutrient amendment. The substantial increases in Fm, and modest increases of Fv/Fm and ETRτ following nitrate amendment (with or without phosphate) corroborates the existence of nitrogen limitation and its intensification to the west (Figure 4.6). Furthermore, the 40%-60% increase in maximum ETRτ values following the addition of nitrate (Figure 4.6c) reflected a 60%-70% reduction in growth rates in the control treatment, which is in good agreement with the 70% reduction in growth rates due to nitrogen stress estimated from the underway ETRτ measurements above. Likewise, the 100%-200% increases in Fm in the western NAEs (Figure 4.6a) corresponds as well with this postulation of a reduction in growth. The exception to this general pattern is the NAE at 35°W in which the responses in Fm and ETRτ were either small or lacking altogether (Figure 4.6). This NAE was conducted in uw3, where nitrate levels were much higher than in the surrounding waters. In essence, this NAE further highlights the importance of TIW in relieving nitrogen limitation.
While nitrogen is the proximal limiting factor as we show, in certain conditions a degree of co-limitation may develop. At 28°W the addition of phosphate resulted in a similar increase in ETRτ as the addition of nitrate and the combination of the two resulted in an even greater increase (Figure 4.6c). However, this is not reflected in the response of Fm or in Fv/Fm to phosphate addition (Figure 4.6a, b). This NAE occurred in uw2 where surface nitrate concentrations increased slightly (Figure 4.2c). As such we suggest that the relative relief from nitrogen limitation and increased growth disrupted the balanced growth. Phosphate was then needed to promote physiological adjustments to the rapid growth and nutrient utilization, resulting in a co-limitation in this section.

The changes we observed in response to nitrate amendment align well with previous nutrient addition experiments conducted in the North and South Atlantic gyres where nitrogen was the limiting factor (Moore et al. 2008, 2013).

Counterintuitively however, along the upwelling sections, where Chl a and primary production increased, PSII photosynthetic competency was significantly reduced, i.e., very low Fv/Fm, high σPSII and ΦT and long fluorescence lifetimes (Figure 4.3, Table 4.1). What then causes this discrepancy between the photophysiological status and the Chl a concentration and productivity in the upwelling sections? In addressing this issue, we will focus on uw1 specifically.

At first, the photophysiological data alone would suggest that phytoplankton are limited by iron. The effects of iron limitation on phytoplankton photophysiology, growth and production are well documented in the literature (e.g., Behrenfeld and Kolber 1999; Schuback et al. 2015; Strzepek et al. 2019; Sherman et al. 2020), and align with our underway data along the upwelling sections. In addition, the pillared dial cycle of Fv/Fm (Fig. 4b), with significant night-time decreases along uw1 (35%), resembles the diel cycle observed by Behrenfeld and Kolber (1999) in the LNLC iron limited South Pacific gyre dominated by cyanobacteria. In contrast, the diel cycle along the steady-state sections, with minor nighttime Fv/Fm decreases (<10%) resembles Behrenfeld and Kolber’s
data collected along the Central Atlantic gyres. Behrenfeld and Kolber argued that the unique diel cycle seen in the Southern Pacific, but not in the Atlantic Ocean, was the result of iron limitation effecting the fluorescence signature of state transitions.

This current study didn’t measure iron concentrations, nor maintain trace metals clean methodology, preventing us from fully ruling out iron-limitation across the upwelling sections. Nonetheless, the argument for iron limitation in the EA is difficult to defend. The Atlantic Ocean is not considered iron limited, including in its lower latitudes (Moore et al. 2013). Aeolian deposition of iron along the Equator ranges between 10 and 100 mg m\(^{-2}\) yr\(^{-1}\), an order of magnitude or two higher than the Pacific (Duce and Tindale 1991). While in situ iron concentration measurements are sparse at the Equator, data from AMT cruises have shown that iron levels in the region are ~3-6 times higher than the surface global dissolved iron mean concentration (Bowie et al. 2002). Moreover values along the Equator are 6-10 times higher than in situ iron concentrations measured in the South Pacific gyre (Bonnet et al. 2008). Here, we acknowledge the fact that during the 12°W NAE, we observed a 20% increase in ETR\(_\tau\) in response to iron amendment, which was on par with the response to nitrate amendment (Figure 4.6c). While ETR\(_\tau\) did increase, F\(_m\) and F\(_v\)/F\(_m\) decreased (Figure 4.6a, b). This NAE was conducted at the outskirts of the uw1 core where Chl \(_a\) was high, while ambient nitrate levels were low and photophysiology was very impaired (Figure 4.2, Figure 4.3). These trends resemble the response to the addition of phosphate in uw2 discussed above and could indicate that although iron is considered replete in this region a shown, a shift towards iron co-limiting phytoplankton could occur following the utilization of upwelled nitrogen which disrupts the balanced growth.

While iron co-limitation might occur when nitrogen is upwelled and rapidly utilized, it still does not fully explain the overall discrepancy between the impaired photophysiology and growth/
production. We therefore hypothesize that the seemingly impaired photophysiology reflects both an imprint of the underlying dominant cyanobacterial community and their light-history.

First, let us consider the decline in $F_v/F_m$ across the upwelling sections. For this, it is critical we highlight an inherent issue in variable fluorescence measurements in cyanobacteria dominated communities. Due to a spectral overlap between the fluorescence bands of Chl $a$ and phycobilins, in particular phycocyanin and allophycocyanin, the contribution of phycobilisome fluorescence to $F_0$ is nontrivial. Campbell et al. (1996) showed that $F_0$ increases as the ratio of phycocyanin/Chl $a$ increases. This overestimation of $F_0$ causes a significant underestimation of $F_v/F_m$, particularly in healthy, nutrient-replete cells (Campbell et al. 1998). In our current study, $F_0$ along uw1 increased disproportionately more than $F_m$, resulting in the apparent decline of $F_v/F_m$ (Table 4.1).

More critically, at uw1 the stratified steady-state structure of the water column broke down and the DCM reached the surface (Figure 4.2d). Such a mixing event exposed low-light acclimated, nitrogen-replete cells from the DCM to high surface irradiances. Across uw1, dawn $E_K$, the light saturation parameter, averaged 159±71 μmol photons m$^{-2}$ s$^{-1}$, 25% lower than the steady-state $E_K$ average of 214±55 μmol photons m$^{-2}$ s$^{-1}$ (statistically significant difference, two sample T-test $\rho <0.05$). In addition, high $\sigma_{PSII}$ and low photosynthetic turnover rates across the upwelling sections (Figure 4.3, Table 4.1) are typically associated with low-light acclimated phytoplankton (Gorbunov and Falkowski 2020). This low-light acclimation also aligns with the stronger midday activation of photoprotective mechanisms (increased $\Phi_T$, decreased $F_v/F_m$ and $\sigma_{PSII}$) observed across uw1 in comparison to the steady-state (Figure 4.4, Table 4.1). This activation also occurred earlier in the day (~1-2 h) along uw1 (Figure 4.4), further supporting the notion that these low-light acclimated communities need to respond rapidly to the high irradiance they are exposed to upon upwelling. Additionally, the 35% decrease in $F_v/F_m$ during the night across uw1 compared to a minor decrease across the steady-state (<10%) (Figure 4.4b) indicates a greater degree of state-transitions in uw1.
State transitions, which redistribute absorbed energy between PSII and PSI (Van Thor et al. 1998) have been suggested to play an important role in cyanobacterial acclimation to low-light (Mullineaux and Emlyn-Jones 2005).

Combined, our results strongly suggest that dissolved nitrogen is the primary limiting nutrient for phytoplankton across the EA and that TIW are critical to it supply and ensuing new production. Phytoplankton are acclimated to this limitation by balancing their low growth rate and photophysiology while they rapidly assimilate pulses of new nitrogen to fuel increased growth and primary production (i.e., “body surf”). The apparent uncoupling of photophysiology and growth/production across the upwelling sections highlights the notion that as phytoplankton rapidly “body surf” the nitrogen waves, they must also employ efficient mechanisms to cope with the inherently more variable and saturating light levels seen in turbulent water columns. This biological, “body surfing” strategy is, almost certainly ubiquitous across the low nutrient, low chlorophyll regions of the tropical and sub-tropical oceans.
Appendix A: Supplemental Material for Chapter 2
Supplementary Figure 2.1 Screening of *P. tricornutum* x1KO and complemented lines via PCR. A) Strains are characterized where the x1KO_1a/2 was complemented with an *Lhcx1* gene under the control of the *Lhcx1* promoter and terminator. B) Strains are characterized where x1KO was complemented with an *Lhcx1* gene under the control of an *FcpA* promoter and terminator. The primer combination *Lhcx1_all*-fw and *Lhcx1_all*-rev amplifies 102 bp at the 3’-end of the *Lhcx1* gene which is still intact in the x1KO lines. The primer combination *Lhcx1_Wt*-fw and *Lhcx1_Wt*-rev amplifies a 347 bp fragment only in the Wt *Lhcx1* gene but not in the x1KO lines, despite spanning the target sites for both TALEN pairs used to produce the x1KO lines. Using the primer combination *Lhcx1_comp*-fw/*Lhcx1_Wt*-rev yields a product of 262 bp, which is only present in the complemented x1KO+x1 lines, where an *Lhcx1* gene without introns and with synonymous codon exchanges of the TALEN binding sites had been introduced under the control of the *Lhcx1* promoter and terminator. The primer combination *FcpA_Lhcx1*-fw and *FcpA_Lhcx1*-rev amplifies a 479 bp fragment, which includes the whole *FcpA* promoter and the first part of the modified *Lhcx1*. *FcpA_Lhcx1*-rev primer is only able to bind to the first modified TALEN site of the complementation construct, while it cannot bind to the natural *Lhcx1* gene. The size of the corresponding marker bands is indicated in red.
Supplementary Figure 2.2 Graphical illustration of DNA sequencing results of the three *P. tricornutum* x1KO lines. DNA sequence preformed in the region of the *Lhcx1* gene using CLC Genomics (Qiagen, Germany). Illumina 125 bp paired end sequencing was performed. We obtained 23.4, 30.9, 20.1, and 12.6 million reads for the Wt4, x1KO_1a, x1KO_1b and x2KO strain, respectively. The blue/cyan bars indicate paired reads, with thicker lines depicting the sequenced reads and the thinner lines in between representing the region of the Wt4 (UTEX646) genome to which this part fits. Green bars indicate forward reads where the paired reverse read could not fit to the reference genome of Wt4. Red bars indicate reverse reads, where the paired forward read could not be mapped to the reference genome. For all three x1KO lines, the region around the TALEN cutting site (~997900 on chromosome 7 for the TALEN pair 1, ~998050 for TALEN pair 2) was only covered be single reads and never any paired read spanning this region could be observed, likely due to large insertions. This is also supported by the orange labelled non-perfect matches chart, reaching 100% in this region in three x1KO lines.
Supplementary Figure 2. Lhc expression of the modified *P. tricornutum* strains when cultivated under low light conditions. Relative expression was normalized to expression of the 18s gene, and then gene expression of the mutated strains was normalized to that of the wild type. Three biological replicates, each measured in technical triplicates, were analyzed and the mean + SE is indicated. Mean values of the biological replicates were tested for statistical significance using a randomization test performed by the REST algorithm. *** indicates p < 0.001 compared to wild type expression. n.d., not detected.
Supplementary Figure 2.4 NPQ capacity of wild type *P. tricornutum* and modified strains. NPQ capacity in wild type (4 biological replicates (BR)), x1KO_1a (4 BR), and x1KO_1a supplemented lines x1KO+x2b (3 BR), x1KO+x3b (3 BR), x1KO+x4b (3 BR) and x1KO+x4c (3 BR) during exposure of 10 min to 1700 µmol photons m$^{-2}$ s$^{-1}$ (white bar), followed by 18 min of low light (grey bar). Strains were concentrated to a chlorophyll *a* amount of 10 mg L$^{-1}$. Red points indicate samples which had been incubated with DTT prior to high light exposure in order to prevent diatoxanthin formation.
Supplementary Figure 2.5 Pool size of diadinoxanthin + diatoxanthin (Dd+Dt) per chlorophyll a and de-epoxidation state (DES). Data collected after 10 min illumination with 1700 µmol photons m\(^{-2}\) s\(^{-1}\) without and with prior application of DTT in wild type, x1KO_1a and x1KO_1a supplemented lines x1KO+x2b, x1KO+x3b, x1KO+x4b and x1KO+x4c. Three biological replicates each were measured.
Supplementary Figure 2.6 Characterization of *P. tricornutum* x2KO and complemented lines via PCR and subsequent agarose gel electrophoresis. a) The two Lhcx2 alleles were amplified via allele specific primers (primer combinations Lhcx2_allele1-fw/Lhcx2_allele1-rev and Lhcx2_allele2-fw/ Lhcx2_allele2-rev, respectively). While in the wild type both alleles could be successfully amplified, they could not be detected in the x2KO strain. In the complemented x2KO+x2 line, the introduced allele 1 was detected. The primer combination Lhcx1_all-fw and Lhcx1_all-rev amplifies 102 bp at the 3’-end of the Lhcx1 gene, serving as a PCR positive control. b) PCR using the primer combination Lhcx2_prom-fw and Lhcx2_term-rev which spans the entire coding region of the Lhcx2 gene. A band could be amplified in the x2KO strain which, after Sanger sequencing, was identified as a truncated allele 2 of Lhcx2, with a 750 bp deletion of the gene sequence and additional ~170 bp deletion of the terminator. In red, the size of the corresponding marker bands is indicated.
Supplementary Figure 2. Lhcx expression of wild type, x2KO and x2KO complemented with Lhcx2 (x2KO+x2) upon exposure to 2 hours of \(~700\, \text{µmol photons m}^{-2} \text{s}^{-1}\). Relative expression was normalized to expression of 18s gene. Three biological replicates, each measured in technical triplicates, were analyzed and tested for statistical significance using the REST algorithm. *** indicates \(p<0.001\) compared to wild type expression. SE is indicated. A different light intensity compared to Suppl. Fig. 9 had been used, because we needed to use another sample setup, as the cuvette in the Dual-PAM did not allow harvesting enough cells for RNA isolation.
Supplementary Figure 2.8 Western blots of *P. tricornutum* strains grown in low light or in 24h high light (~400 µmol photons m\(^{-2}\) s\(^{-1}\)). Three different blots (left, right and bottom) are shown. Upper blots - wild type, x1KO_1a, the supplemented x1 KO lines, the x2KO line. Lower blot - The x2KO line complemented with Lhcx2 and supplemented with Lhcx3. After blotting, the blots were cut and the upper half was incubated with a Rubisco antibody, while the lower half was incubated with the Lhcx antibody. Lhcx1 has the lowest, Lhcx2 the highest, and Lhcx3 and Lhcx4 have a molecular weight in between. Lhcx2 protein is not detectable under low light cultivation in the wild type, but only after prolonged high light cultivation. The x2KO line does not express Lhcx2 even after high light cultivation (upper right blot) as the x2KO+x3 line does not, too (lower blot).
Supplementary Figure 2.9 NPQ capacity in wild type, x2KO and x2KO complemented with Lhcx2 (x2KO+x2). NPQ was measured upon exposure to 130 min of 1700 µmol photons m\(^{-2}\) s\(^{-1}\) (white bar), followed by 30 min of low light recovery (grey bar). Three biological replicates are indicated. The inset shows the first 10 min of NPQ development in the three strains. Note that there are no major differences in NPQ capacity between the Wt and the x2KO line during the first hour of illumination, while during the second hour the Wt develops a higher NPQ capacity. The x2KO+x2 has a somewhat higher NPQ capacity right from the beginning.
Supplementary Figure 2.10 Electron transport rates of *P. tricornutum* wild type and mutants cultivated under low light. Values are the mean of six biological replicates. SE is given.
Supplementary Figure 2.11 NPQ development during rapid light curves with increasing light intensities of *P. tricornutum* wild type and mutants cultivated under low light. Samples were exposed to increasing light intensities without or with prior incubation with DTT. Values are the mean of six (-DTT) or three (+DTT) biological replicates. SE is given. Dashed black line denotes zero NPQ.
Supplementary Figure 2.12 Changes in σPSII during rapid light curves of wild type and mutants cultivated under low light and 24 h of high light (~ 400 μmol photons m\(^{-2}\) s\(^{-1}\)). Low light grown samples were additionally incubated with DTT. Values are the mean of six (low light cultures), five (high light cultures) or three (low light + DTT) biological replicates. SD is given.
Supplementary Figure 2.13 Changes in NPQ and $\sigma_{\text{PSII}}$ of *P. tricornutum* wild type and x1KO cells upon three minutes of supra optimum light exposure. The calculation of the change in $\sigma_{\text{PSII}}$ here followed the approach of (Tian et al. 2019). In order to induce qE, cells were exposed for 3 min to 1700 $\mu$mol photons m$^{-2}$ s$^{-1}$ (red light), after which light was switched off and DCMU was added 5 s later, followed by exposure to a weak red-light flash. To record $\sigma_{\text{PSII}}$ of unquenched cells, DCMU was added to dark acclimated cells, after which they were also exposed to a weak red-light flash. The resulting fluorescence rises were normalized according to (Tian et al. 2019), the reciprocal of the areas above the normalized fluorescence rise curve were determined as the relative functional absorption cross sections and the changes in functional absorption cross sections were determined - in analogy to the Stern Volmer equation of NPQ - as $(\sigma_{\text{PSII unquenched}}/\sigma_{\text{PSII quenched}}) - 1$. More methodological details can be found in Supplementary Figure 2.14. Values are the mean of five independent biological replicates and SE is given. Statistical significance between wild type and x1KO cells was tested using a two-tailed unpaired Student’s t-test with 8 degrees of freedom. ** p<0.01; *** p<0.001.
Supplementary Figure 2.14 Exemplary fluorescence induction traces of DCMU poisoned wild type and x1KO cells before and after supra-optimal light exposure. Cells were concentrated to 10 mg L⁻¹ chlorophyll a and 10 μM DCMU was added in the dark, in order to record the fluorescence induction curve of unquenched cells (black trace). In order to induce qE, cells were exposed to 1700 μmol photons m⁻² s⁻¹ (blue + red light) for 3 min, then shifted to darkness for 5 s after which DCMU was added (red trace). This time span was sufficient to allow a substantial re-oxidation of QA⁻ without a pronounced relaxation of qE. Fluorescence induction curves were recorded in the fast acquisition mode with a Dual-PAM (Walz, Germany) by applying a 300 ms red light flash with an intensity of 41 μmol photons m⁻² s⁻¹, 15 s after DCMU application. Fm was reached roughly 60 – 100 ms after flash onset. Note that DCMU treatment strongly affects the F₀ values and therefore has to be corrected, as described in (Tian et al. 2019). For the unquenched cells, F₀ values were directly determined before treatment with DCMU, while for the quenched cells F₀ was calculated as F₀’ = F₀/[1+(F₀*NPQ/Fm)]. Fm and Fm’ were obtained from averaging the fluorescence values recorded during the 90-100 ms time span of the red-light flash after DCMU application. The first 500 measured points (first 15 ms) of each fluorescence induction trace were linearly fitted and extrapolated to the corresponding F₀/F₀’ value using Origin (the straight line of the fluorescence trace). The fluorescence values were then normalized to values between 0 (F₀ and F₀’) and 1 (Fm and Fm’) and to the positive time range. The area above the fluorescence induction curve is inversely proportional to the functional absorption cross section of PSII, thus the reciprocal of this area is taken as the functional absorption cross section. Consequently, the functional absorption cross section in Wt is lower after 3 min exposure to supra-optimal light, while it is unchanged in the x1KO strain.
Supplementary Figure 2.15 Changes in $\sigma_{\text{PSII}}$ versus $1-q_L$ during rapid light curves in *P. tricornutum* wild type and mutants grown under low light and 24 h of high light (~ 400 µmol photons m$^{-2}$ s$^{-1}$). $1-q_L$ is a proxy for the reduction state of the plastoquinone pool and thus a proxy for excitation pressure on PSII. Values are the mean of three (low light cultures) or five (high light cultures) biological replicates. SE is given. $1-q_L$ was calculated as $1-\{(F_m' - F')/(F_m' - F_0') * (F_0'/F')\}$. 
Supplementary Figure 2.16 NPQ vs Y(NPQ) during rapid light curves in *P. tricornutum* wild type and mutants cultivated under low light and 24 h of high light (~ 400 µmol photons m⁻² s⁻¹). Plotted points represent the mean of six (low light cultures) or five (high light cultures) biological replicates. NPQ is calculated as \( F_{m}/F_{m}' - 1 \), and Y(NPQ) is calculated as \( F/F_{m}' - F/F_{m} \).
Supplementary Figure 2.17 $\sigma_{\text{PSII}}$ vs. $Y(\text{NPQ})_{1s}$ from a second measurement at each light step, following 1 second of darkness and the corresponding linear regression. Individual data points of all measured strains cultivated both under low light (three biological replicates each), indicated by closed symbols, and high light (five biological replicates each), indicated by open symbols. Data points where an increase of $Y(\text{NPQ})_{1s}$ did not lead to a further down-regulation of $\sigma_{\text{PSII}}$ are not included in the regression calculation, but indicated in light grey. This was determined by calculating the percent change of each $\sigma_{\text{PSII}}$ from its previous light step. If $\sigma_{\text{PSII}}$ decreased by less than 5% of the total measured decrease for that curve while $Y(\text{NPQ})_{1s}$ increased, it was omitted. Data points above 600 µmol photons m$^{-2}$ s$^{-1}$ and data points with a negative $Y(\text{NPQ})_{1s}$ were also removed. A linear regression line, the 95% confidence interval, the regression equation and the $r^2$ are indicated.
Supplementary Figure 2.18 Average fluorescence lifetimes during 10 min of supra-optimum light exposure in low and high light cultivated *P. tricornutum* wild type and mutant strains. Open symbols in the dark represent measurements under $F_0$ conditions. Subsequently, filled symbols represent data collected under $F_m$ conditions.
Supplementary Figure 2.19 Exemplary transformation vector maps. a) *P. tricornutum* x1KO_1a strain, and b) x2KO strain with *Lhcx3*. The vectors contain a Blasticidin-S resistance cassette. In a) the *Lhcx3* gene is under control of the *Lhcx1* promoter and terminator, while in b) it is controlled via the *Lhcx2* promoter and terminator.
Supplementary Figure 2.20 Lhcx2 gene sequence of P. tricornutum strain. 4 Bases before and after “/”, underlined and in bold, indicate alternating bases in the two alleles (in total 16, the first base always referring to allele 1), and the untranslated region is indicated in italics. Green letters indicate introns, while blue letters indicate the TALEN binding site.
Supplementary Figure 2.21 Alignment of \textit{Lhcx2} allele 2 amplified from wild type and the x2KO strain.

In the x2KO strain, the \textit{Lhcx2} gene is deleted from base pair 247 up to the end of the gene. The same deletion can also be found in the x2KO+x2 line. The binding sites for the forward and reverse TALEN construct used to induce the knockout in the \textit{Lhcx2} gene are indicated in blue and bold.
Supplementary Table 2.1 TALENs used to target *Lhcx1* and *Lhcx2*. Indicated are the repeat variable di-residues (single letter amino acid code) characteristic for the respective TALE monomers, the used backbone vectors (available at Addgene with the indicated vector code) and the targeted DNA sequence.

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<th>Backbone vector reverse TALEN</th>
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Lhcx1 terminator

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Lhcx1 promoter for modified Lhcx1 gene

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Lhcx2 promoter

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| | CAGTTCTCCCCTTAGCTCTCA | ATCAATTTCATGATCATTCACTAGTGATGACACATGTCATGGAATCATTGAATCAAGTATCGTGAAGTTTCCAACGAGGAACTTGCGGTCACACTATTCTTCGTACCAACCAGGGAAGCTCCTGGTCCATCATCAAGTACGCTGGTAG |
| **Lhcx1 gene modified without introns** | ATGAAGTTTGCGCCGAACCTATATTGCGCCCTTATCGGCTCTGCCGC | TGGTTTGCCTGTGTCACGGGCAGGTGCTGACCGTCAATTCGACGTCGTCAGGGCTGGCCTACCTGACTCATGCATTCCGCGTCCGCACAAAACTCACAGTCAGAGAGCCACTCCGAGAATCCTCCAGAATTTTTCGTGGAAGATTTTTTCGTCATCTTTTTC | GGAATATAACGTTCCAGTGTTTTTGAAACACTAGGCGGAGATGGACAAACAGAAGGATAGGCTGCAAGGAGACATTTCTTTCAGGTGAAGTTTTCGTTTGCGTTCTTCGTAAACACACTGCAAAGAGATTTACGTCCATTCCAACCCACTACGTACCC |
| | TGGTTTGCCTGGAACCTATATTGCGCCCTTATCGGCTCTGCCGC | ATCAATTTCATGATCATTCACTAGTGATGACACATGTCATGGAATCATTGAATCAAGTATCGTGAAGTTTCCAACGAGGAACTTGCGGTCACACTATTCTTCGTACCAACCAGGGAAGCTCCTGGTCCATCATCAAGTACGCTGGTAG |
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TTGGTCTCTCAA

Lhcx2 gene modified

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Lhcx3 gene

ATGAAGCGCATCGCCGCTATCGCCCTTCGCGGCACTACGCGGCT
CCGCCTTTAAGCATCTCCGTCGCGCAAGAAGGCGTCGCCCAAAAGGC
CGGTAGTCATGCGGAACCTTTTCTCCAAAGTACCCGCGTGGTG
GTGGATACGCTAGTGTTGTCGCGATTTGATTTTCTCACGAAACCACACTC
GTCCGCGTCTTCTGCCGCGACCAGTATTTCTGATCAAGATATCACTACCGGCT
GCTCGCGCTCCCTGGTGATCTTTTATCCCTCCGATTCTTTGCGGGCGCAAGCGC
ACGAGTGTCACCCGTCAGCGTACTACGAGGCAGCGGTACCAACGCAGACG
GGGTGGCCATTGTCGACACCTGGGTTTCTGTCGCCGAAGGGCTGG
AGGATACCCCTATCTTTTTGTATCCGATTTACCAAGACCAGCTTCTTCTC
ATCTCGCCAAATGGCGGCATCCGCTGTTGCTGCTGACATTTCATC
GGGGCGCGCCAGAAGACCGGTGCGATCGGCTGCGGGATCTTCC
### Supplementary Table 2.3 Primers used in this study.

Primers use for qPCR analyses are indicated as “gene name_qPCR_fw/rev”.

<table>
<thead>
<tr>
<th>Name</th>
<th>Forward primer sequence</th>
<th>Reverse primer sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lhx4_1_all-fw</td>
<td>5’-CTCTCCAGACGAAAGGAC-3’</td>
<td>5’-GATTCTCAGGATTCCC-3’</td>
</tr>
<tr>
<td>Lhx4_1_all-rev</td>
<td>5’-CTGCCACACATCTTG-3’</td>
<td>5’-GACGAACCACCTTGACG-3’</td>
</tr>
<tr>
<td>Lhx4_1_Wt-fw</td>
<td>5’-CTGCCACACATCTTG-3’</td>
<td>5’-GACGAACCACCTTGACG-3’</td>
</tr>
<tr>
<td>Lhx4_1_Wt-rev</td>
<td>5’-CTGCCACACATCTTG-3’</td>
<td>5’-GACGAACCACCTTGACG-3’</td>
</tr>
<tr>
<td>Lhx4_1_com-fw</td>
<td>5’-GCCGCAATATTTGACG-3’</td>
<td>5’-AAGAGATATATCAGCCAATCC-3’</td>
</tr>
<tr>
<td>Lhx4_2_allele1-fw</td>
<td>5’-AAGAGATATATCAGCCAATCC-3’</td>
<td>5’-AAGGACGACAGTAAACGAA-3’</td>
</tr>
<tr>
<td>Lhx4_2_allele1-rev</td>
<td>5’-AAGGACGACAGTAAACGAA-3’</td>
<td>5’-AAGGACGACAGTAAACGAA-3’</td>
</tr>
<tr>
<td>Lhx4_2_allele2-fw</td>
<td>5’-CGTAAACACACCCGAA-3’</td>
<td>5’-AATGACTTACTCTGAGG-3’</td>
</tr>
<tr>
<td>Lhx4_2_allele2-rev</td>
<td>5’-CGTAAACACACCCGAA-3’</td>
<td>5’-AATGACTTACTCTGAGG-3’</td>
</tr>
<tr>
<td>Lhx4_2_prom-fw</td>
<td>5’-CTCACAGTAAACATAGC-3’</td>
<td>5’-CTCACAGTAAACATAGC-3’</td>
</tr>
<tr>
<td>Lhx4_2_term-rev</td>
<td>5’-CTCACAGTAAACATAGC-3’</td>
<td>5’-CTCACAGTAAACATAGC-3’</td>
</tr>
<tr>
<td>FcpA_Lhx4_1-fw</td>
<td>5’-GGCTGACGAGGAGAATG-3’</td>
<td>5’-AGGCAATAGTGGGCCTG-3’</td>
</tr>
<tr>
<td>FcpA_Lhx4_1-rev</td>
<td>5’-GGCTGACGAGGAGAATG-3’</td>
<td>5’-AGGCAATAGTGGGCCTG-3’</td>
</tr>
<tr>
<td>Lhx4_2_qPCR_fw</td>
<td>5’-CGCCATTACTCACTCAACCAG-3’</td>
<td>5’-TCAACCCACCAATTTGAGC-3’</td>
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<tr>
<td>Lhx4_2_qPCR_rew</td>
<td>5’-CGCCATTACTCACTCAACCAG-3’</td>
<td>5’-TCAACCCACCAATTTGAGC-3’</td>
</tr>
</tbody>
</table>
References


Mock, T., R. P. Otillar, J. Strauss, and others. 2017. Evolutionary genomics of the cold-


Thieme, J., D. Zigmantas, R. Augulis, V. I. Novoderezhkin, M. Ferretti, E. Romero, and


Xu, K., J. L. Grant-Burt, N. Donaher, and D. A. Campbell. 2017. Connectivity among


Data availability

Chapter 2 Data availability

Whole-genome sequencing reads for Pt4, x1KO_1a, x1KO_1b, and x1KO_2 are deposited at the European Nucleotide archive (ENA) under the following accession code: “PRJEB33825”

Chapter 3 Data availability


Chapter 4 Data availability

Underway Fire and PicoLiF data deposited at https://seabass.gsfc.nasa.gov/.