Variations in phytoplankton photo-physiology and productivity in a dynamic eutrophic ecosystem: a fast repetition rate fluorometer-based study

YOSHIHISA MINO1*, SATSUKI MATSUMURA2, THAITHAWORN LIRDWITAYAPRASIT1, TETSUICHI FUJIKI3, TETSUO YANAGI4 AND TOSHIRO SAINO3

1HYDROSPHERIC ATMOSPHERIC RESEARCH CENTER, NAGOYA UNIVERSITY, FURO-CHO, CHIKUSA-KU, NAGOYA 464-8601, JAPAN, 2DEPARTMENT OF MARINE SCIENCE, CHULALONGKORN UNIVERSITY 254 PHAWATI RD, BANGKOK 10330, THAILAND, 3RESEARCH INSTITUTE FOR GLOBAL CHANGE, JAPAN AGENCY FOR MARINE-EARTH SCIENCE AND TECHNOLOGY, 2-15 NATSUMIWA-CHO, YOKOSUKA, KANAGAWA 237-0061, JAPAN AND 4RESEARCH INSTITUTE FOR APPLIED MECHANICS, KYUSU UNIVERSITY, 6-1 KASUGA-KOEN, KASUGA, FUKUOKA 816-8580, JAPAN

*CORRESPONDING AUTHOR: kuro@hyarc.nagoya-u.ac.jp

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We investigated the photoacclimation state and photosynthetic rate of cells in the highly eutrophic upper Gulf of Thailand (UGOT), using fast repetition rate (FRR) fluorometry. Observations revealed differences in photosystem II (PSII) parameters: the maximum photochemical quantum efficiency of PSII \((F_v/F_m)\), the functional absorption cross-section of PSII \((\alpha_{PSII})\) and the rate of reoxidation of the primary PSII electron acceptor \((1/\tau_{Qa})\), which were dependent on the hydrographic structure of the water column. FRR-derived parameters were used to estimate the chlorophyll \(a\)-normalized, photosynthetic rate \((P_B)\); the photosynthesis vs. irradiance \((P-E)\) curves were derived from in situ \(P_B\) profiles. The \(P-E\) parameters differed markedly between the upper stratified waters and the mixed water column; that is, the maximum photosynthetic rate \(P_{Bmax}\) and the light saturation parameter \((E_k)\) were higher in the upper stratified waters, while the initial slope of the \(P-E\) curve \((\alpha)\) was higher in the mixed waters. This indicates that cells acclimated to relatively bright light dominated the upper stratified waters, while cells acclimated to lower light dominated the mixed waters. A significant, positive relationship was found between the average \(P_B\) in the euphotic zone and surface photosynthetically available
radiation, from all profiles at both sampling sites, which suggests that phytoplankton photosynthesis in the UGOT was controlled primarily by irradiance. Furthermore, as stress from nutrient-limitation is unlikely in the UGOT, cells might realize their photosynthetic potential by means of photoacclimation, even under the different light regimes present in the UGOT.

**KEYWORDS:** phytoplankton; photoacclimation; FRR fluorometry; P–E curve; eutrophic coastal region

**INTRODUCTION**

Environmental loads, disturbances in ecological systems caused by human activities, have led to profound changes in both the structure and function of coastal ecosystems, including outbreaks of toxic algal blooms and the production of hypoxic waters, as well as shifts from coral- to algal-dominated communities (Pearl, 1997; McManus et al., 2000; Naqvi et al., 2000). As a result, fisheries and aquaculture operations have sustained significant damage, and these impacts have recently become a social issue in the Southeast Asia. As human population growth is increasing rapidly, it is imperative to develop quantitative predictions about the impacts of these disturbances on coastal systems (Talaue-McManus et al., 2003). However, it is difficult to simulate accurately the highly variable coastal productivity, which is typically the first sign of environmental loads, because multiple physical factors (e.g. tides, rivers, intrusion of oceanic water etc.) impact the light and nutrient availability to phytoplankton, in addition to the effect of competition among phytoplankton with different photosynthetic strategies. In anthropogenically generated eutrophic conditions, where there is typically a thin optical layer due to the strong attenuation of light by high concentrations of phytoplankton and suspended solids, cells can experience large, short-term changes in irradiance due to turbulent vertical transport. Therefore, the strategy for acclimation to a given light regime would be critical for their growth and success. Photoacclimation, which is defined as the physiological response to changes in light at the organism level, involves alterations in the molecular structure of the photosynthetic apparatus, which occurs through two strategies (reviewed by Falkowski and LaRoche, 1991). The first consists of an alteration in the size of light-harvesting antenna associated with each reaction centre of the photosystem, and the second is an alteration in the number of cellular reaction centres, which drive the differences in the functional relationship between photosynthesis and irradiance (P–E curve) among taxa (Falkowski and Owens, 1980; Perry et al., 1981). Thus, it is necessary to determine how cells respond to a changing light field to improve the estimates and simulations of near-shore productivity derived from the assessment of photosynthetic properties at high resolution, both spatially and temporally.

Fast repetition rate (FRR) fluorometry (Falkowski and Kolber, 1995) enables in situ, real-time, non-destructive measurements of physiological parameters of natural phytoplankton assemblages, with high resolution (Moore et al., 2003, 2005). A FRR fluorometer (FRRF) measures a single turnover fluorescence curve in photosystem II (PSII), induced by a series of subsaturating flashlets (Kolber et al., 1998). PSII-related parameters provided by the FRRF include the maximum photochemical quantum efficiency of PSII ($F_{v}/F_{m}$), the functional absorption cross-section of PSII ($\sigma_{\text{PSII}}$) and the turnover time of the primary PSII electron acceptor $Q_{a}$ ($\tau_{Q_{a}}$), which can be used to estimate the chlorophyll a (Chl a)-specific gross primary productivity ($P_{b}$) by phytoplankton in a model (Kolber and Falkowski, 1993):

$$
P_{b} = E \times \sigma_{\text{PSII}} \times q_{v} \times f \times n_{\text{PSII}} \times \varphi \times S,
$$

$E$ is the irradiance, $n_{\text{PSII}}$ the ratio of PSII reaction centres (RCII) to Chl a, $f$ the fraction of RCII that are capable of evolving oxygen ($O_{2}$), also known as the “functional” state and assumed to be $(F_{v}/F_{m})/0.65$, $q_{v}$ the photochemical quenching coefficient, which is a measure of the fraction of open RCII, $\varphi$, the quantum yield for oxygen evolution and $S$ a scaling factor (Table I).

Many research groups have applied FRR fluorometry to the above model, albeit with modifications, to study natural phytoplankton assemblages and have suggested its utility in measuring productivity (Suggett et al., 2001; Moore et al., 2003; Sarma et al., 2006; Fujiki et al., 2008, 2011). However, overestimations of photosynthetic rate by FRR fluorometry have been reported, especially under strong light, compared with other techniques (Suggett et al., 2001; Raateoja et al., 2004; Smyth et al., 2004; Corno et al., 2006). Possible causes for this are spectral differences between FRRF saturation pulses and ambient light (Suggett et al., 2001; Raateoja et al., 2004), as well as taxonomic differences in $n_{\text{PSII}}$ (Falkowski et al., 1981; Barlow and Alberte, 1983; Suggett et al., 2004). Kromkamp et al.
(Kromkamp et al., 2008) suggested that an overestimation of the quantum yield for O₂ evolution (φₑ), which is assumed to be constant (=1/4) in many studies, was the main cause for the overestimation of photosynthetic rate. Certainly, higher electron requirements (1/φₑ), relative to the theoretical minimum of four, have been reported for algal cultures under high actinic irradiance, due to electron recycling around PSII, induced by the reduction in the plastoquinone pool (Prasil et al., 1996; Feikema et al., 2006). Furthermore, chlororespiration via a plastid terminal oxidase (PTOX) and Mehler–ascorbate-peroxidase (MAP) activity have been identified as alternative electron flows to O₂ (Mehler, 1951; Peltier and Cournac, 2002; Mackey et al., 2008). These results imply that the whole-chain electron transfer rate has an upper limit (1/τPSII, which corresponds to the maximum rate of O₂ evolution), and a portion of the electrons transferred to Qₑ would be wasted, which might prevent oxidative damage of RCII when charge separation occurs at a rate faster than 1/τPSII.

To model such irradiance-dependence of φₑ, Kolber and Falkowski (Kolber and Falkowski, 1993) used 1/τPSII as the threshold rate:

$$\phi_e = 0.25 \quad \text{when} \quad E \times \sigma_{PSII} \times \phi_P < 1/\tau_{PSII}$$

$$\phi_e = \frac{0.25}{E \times \sigma_{PSII} \times \phi_P \times \tau_{PSII}} \quad \text{when} \quad E \times \sigma_{PSII} \times \phi_P > 1/\tau_{PSII}$$

(2)

This means that, at rates of charge separation exceeding 1/τPSII, the φₑ falls below the maximum value of 0.25; 1/τPSII has been known to vary in response to the photoacclimation state of cells (Sukinen et al., 1987), implying that equation (2) requires measured values of 1/τPSII, not constants, for natural populations. FRRF can derive the achievable rate of Qₑ reoxidation (1/τQₑ), with a somewhat low accuracy, using an in situ profiling protocol (Suggett et al., 2006), which would consist of 1/τPSII when downstream processes of the plastoquinone pool (i.e., carbon-fixation capacity) limit Qₑ reoxidation (Kolber and Falkowski, 1993; Kana et al., 2002). Moore et al. (Moore et al., 2006) reported that FRRF-measured 1/τQₑ was comparable to other independent estimates of 1/τPSII for cells and suggested that the changes were the main factor controlling both the maximum rate of carbon fixation and the saturating irradiance. Thus, we need to develop a method that uses FRRF for estimating productivity in natural environments, especially those with highly variable light regimes, taking into account the effect of variations in 1/τPSII (thereby φₑ) associated with the photoacclimation strategy of cells.

We used FRRF to investigate the photoacclimation state and productivity of phytoplankton assemblages in the upper Gulf of Thailand (UGOT), to which rivers supply extensive loads of freshwater and nutrients (Fig. 1). Our approach was to (i) examine the FRRF-derived maximum rate of whole-chain electron transport (1/τPSII), from the photosynthetic model [equations (1) and (2)], by comparing calculated P₈ with the measured gross primary productivity (GPP) from bottle incubations, and (ii) derive P-E curves from vertical profiles of instantaneous FRRF-based P₈. This revealed a marked, hydrography-dependent difference in the P-E parameters. Here, we discuss the relationship between physical forcing and the photoacclimation state of cells to water column productivity in a dynamic, eutrophic ecosystem.

### Table I: Definitions of photosynthetic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
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<tbody>
<tr>
<td>E</td>
<td>Irradiance (µmol quanta m⁻² s⁻¹)</td>
</tr>
<tr>
<td>Fₚ</td>
<td>Minimum fluorescence yield in darkness (arbitrary units: a.u.)</td>
</tr>
<tr>
<td>Fₚn</td>
<td>Maximum fluorescence yield in darkness (a.u.)</td>
</tr>
<tr>
<td>Fₚ/Fₚn</td>
<td>Potential photochemical efficiency of open reaction centers</td>
</tr>
<tr>
<td>f</td>
<td>Proportion of functional PSII reaction centers (=[(Fₚ - Fₚ0)/Fₚn] (dimensionless))</td>
</tr>
<tr>
<td>Fₚ₂</td>
<td>Minimum fluorescence yield under actinic light (=[(Fₚ/ Fₚ₂) + (Fₚ/Fₚn)] (a.u.))</td>
</tr>
<tr>
<td>Fₚ₄</td>
<td>Maximum fluorescence yield under actinic light (a.u.)</td>
</tr>
<tr>
<td>Fₚ₄’</td>
<td>Steady-state fluorescence yields under ambient irradiance</td>
</tr>
<tr>
<td>m</td>
<td>Effective absorption cross-section of PSII at 440 nm (Å² quanta⁻¹)</td>
</tr>
<tr>
<td>E₂</td>
<td>Euphotic zone-averaged Chl a-specific gross productivity (mg C mg Chl⁻¹ h⁻¹)</td>
</tr>
<tr>
<td>P</td>
<td>Photosynthetic quotient (ratio of evolved O₂ to fixed CO₂) (mol O₂ mol C⁻¹)</td>
</tr>
<tr>
<td>φₑ</td>
<td>Quantum yield for O₂ evolution (mol O₂ mol electron⁻¹)</td>
</tr>
<tr>
<td>σ₉</td>
<td>Photophysical efficiency of open reaction centers</td>
</tr>
<tr>
<td>σ₉₀</td>
<td>Effective absorption cross-section of PSII in darkness (Å² quanta⁻¹)</td>
</tr>
<tr>
<td>σ₉₀’</td>
<td>Effective absorption cross-section of PSII under actinic light (Å² quanta⁻¹)</td>
</tr>
<tr>
<td>1/τQₑ</td>
<td>Achievable rate of reoxidation of the primary PSII electron acceptor Qₑ (ms⁻¹)</td>
</tr>
<tr>
<td>1/τPSII</td>
<td>Maximum photosynthetic turnover rate (ms⁻¹)</td>
</tr>
<tr>
<td>φₑ</td>
<td>Quantum yield for O₂ evolution (mol O₂ mol electron⁻¹)</td>
</tr>
<tr>
<td>P₈, EZ</td>
<td>Index of hourly light utilization efficiency of EZ (mg C mg Chl⁻¹ h⁻¹)</td>
</tr>
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</table>

*Referred to Oxborough and Baker (Oxborough and Baker, 1997)*

### METHOD

Observations were made in July 2006, in the UGOT, aboard the R/V KASETSART-I (Fig. 1a). Water column structures within the UGOT, ~100 × 100 km² in size and <50 m in depth (Table II), undergo drastic changes, both temporally and spatially, due to changes in river discharge, tidal currents and seasonally distinct circulation.
Fig. 1. (a) Location of the study sites in the UGOT where four major rivers discharge their waters. Open circles indicate the positions of the two observation stations (Stns 1 and 2) with FRRF casts. A solid line indicates a transection line for the following figure. (b) Potential density ($\sigma_0$) section along a line from Stn. 1 to Stn. 2. This shows a distinct east–west difference in the water column structure, which was controlled mainly by salinity distributions (Fig. 2). Symbols indicate data positions.
Table II. Station positions and characteristics

<table>
<thead>
<tr>
<th></th>
<th>Stn. 1</th>
<th>Stn. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dates</td>
<td>26 Jul 2006</td>
<td>25 Jul 2006</td>
</tr>
<tr>
<td>Location</td>
<td>13°20’ N 100°50’ E</td>
<td>13°10’ N 100°10’ E</td>
</tr>
<tr>
<td>Depth (m)</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Water column structure</td>
<td>Well stratified</td>
<td>Mixed</td>
</tr>
<tr>
<td>Depth of EZ (m)</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>Chl a (mg m$^{-2}$) at 0 m</td>
<td>8.0 (5.5/10.6)</td>
<td>2.3 (2.1/2.6)</td>
</tr>
<tr>
<td>Chl a (mg m$^{-2}$) at 5 m</td>
<td>4.4 (3.1/6.7)</td>
<td>3.0 (2.4/3.4)</td>
</tr>
<tr>
<td>DIN (μM)</td>
<td>20.3 (10.6/31.9)</td>
<td>6.3 (2.0/10.1)</td>
</tr>
</tbody>
</table>

Depth of the EZ was determined as the depth at which PAR was 1% of the surface value. Concentrations of Chl a and DIN varied among the daytime casts of water sampling ($n = 3–5$). Chl a data are the mean (min/max) of all casts. DIN data are the mean (min/max) for the both the 0 and 5 m depths.

patterns in the outer Gulf, as a result of monsoons. At a strongly stratified site (Stn. 1: 13°20’N, 100°50’E) and a mixed site (Stn. 2: 13°10’N, 100°10’E; Fig. 1b), intensive FRRF observations were made every 2 h from early morning (06:00 or 08:00 h) to 18:00 h. Observation points were selected on the basis of tracking the same water mass with a GPS drifter (Michida et al., 2006).

FRRF measurements

Fluorescence of active Chl a was measured using a Diving Flash FRRF (Kimoto Electric). The FRRF, with photosynthetically available radiation (PAR) and depth sensors, was deployed from the sunward deck using a stand-alone frame, and lowered to near the bottom at a speed of ∼0.2 m s$^{-1}$ to obtain fine vertical resolution data. The FRRF has two optical chambers for measurements under dark and ambient light conditions; the “dark” chamber is fully shaded and the “light” chamber is open under strong ambient light, and is equipped with a dichroic cyan filter to prevent the red portion of ambient light from penetrating the chamber. This design allows for accurate measurements of red fluorescence signals, even under strong ambient light in near-surface waters.

Chl a fluorescence transients were obtained using a single-turnover protocol, which provides a flash sequence consisting of a series of 50 saturation and 20 relaxation flashes (wavelength of 470 nm, 25 nm bandwidth, 2 μs long flashes, with 4 μs intervals for saturation and 102 μs intervals for relaxation; Fujiki et al., 2008). Each acquisition is the average of 16 alternative sequences conducted in the light and dark chambers. Averaged fluorescence data were processed using the software provided by the instrument manufacturer. The fluorescence response data were fitted to the model of Kolber et al. (Kolber et al., 1998), from which we calculated the following photosynthetic parameters: $F_{m}$, $F_{m}'$, $F_{s}$/($F_{m} + F_{v}$), $P_{PSII}$, and $τ_{Q}$. For each parameter, values were derived for both dark and ambient light conditions, and the latter corresponds to $F'$, $F_{m}'$, $F_{v}'$, $F_{s}'/F_{m}'$, and $τ_{Q}'$ (Table I); the parameters were used to calculate the hourly $P_{B}$, using equations (1) and (2). Additionally, we used the empirical relationship between $F_{m}$ and measurements of Chl a concentrations in discrete water samples to derive the Chl a profiles ($r^{2} = 0.88$, $P < 0.01$, RMSE = 0.49 mg m$^{-3}$, $n = 29$).

Bottle incubation experiments

GPP was determined at each site, from in vitro changes in dissolved oxygen (DO), following light and dark bottle incubations. At dawn (06:00 h), seawater was collected from the 0, 5 and 10 m depths, and stored in 100 mL (gravimetrically calibrated) glass bottles. Three dark and light bottles were incubated, from each depth, for 12 h in the primary production array with the GPS drifter. After incubation, DO concentrations in the bottles were measured using the Winkler method. The Chl a-specific productivity ($P_{B}$) was calculated by dividing GPP by the Chl a concentration at each depth, from the 06:00 h cast.

Temperature, salinity, Chl a and nutrients

Temperature and salinity were measured with a direct-reading type CTD sensor (YSI Inc.). Water samples were filtered through 25 mm GF/F filters (Whatman), and filters were stored frozen until onshore analysis for Chl a. The pigment was extracted overnight with 90% acetone, and Chl a concentration was determined by fluorometric analysis. Subsamples for nutrients were kept frozen until analysis, and dissolved inorganic nitrogen (DIN: nitrate + nitrite + ammonium), phosphate and silicate were measured colourimetrically using an automated nutrient analyser (Skalar Analytical).

RESULTS AND DISCUSSION

Hydrographic conditions and water properties

As depicted in Fig. 1b, the water column of Stn. 2 was mixed, showing a relatively uniform profile of FRRF-derived Chl a and salinity (Fig. 2f and g). On the other hand, the eastern shallow of Stn. 1 showed a strong halocline around a depth of 2 m, with relatively higher and lower concentrations of Chl a, in the surface and deeper layers, respectively (Figs 1b and 2a and b). The average water column concentration of Chl a was higher at Stn. 1 (4.8 mg m$^{-3}$) than Stn. 2 (3.4 mg m$^{-3}$), which was consistent with a shallower euphotic zone (EZ) at Stn. 1 (∼7 m) than Stn. 2 (∼12 m; Table II).
(11–32 μM) were found at the stratified Stn. 1, which may have been due to high riverine inputs (Menasveta and Hongskul, 1988; Matsumura et al., 2006). Even at the mixed Stn. 2, average DIN concentrations were >6 μM, which indicated that algal growth was not limited by N, given the threshold value of ~1 μM reported by Dortch and Whitlege (Dortch and Whitlege, 1992). Chl a profiles, from the observations at Stns 1 and 2, showed marked fluctuations among daytime casts (Fig. 2b and g), which probably resulted from the advection of different water masses, even though we tracked the surface drifter with a drogue (drag centre set at ~5 m depth). However, in the following, photo-physiological parameters obtained from each FRRF cast are referred to as representatives of the site-distinctive populations of phytoplankton.

Variations in FRRF parameters and estimation of $P^B$

The typical profiles of $\sigma_{PSII}'$, $F_o/F_m$ and $1/\tau_{Qa}$ were dependent on both water column structure and diurnal variation (Fig. 2). The photochemical target size of PSII, $\sigma_{PSII}'$, ranged from 300 to 820 and 480 to 820 Å² photon⁻¹, at Stns 1 and 2, respectively (Fig. 2c and h). At both sites, $\sigma_{PSII}'$ in the upper layer showed a significant decline during the daytime and recovered towards the evening. Declines in $\sigma_{PSII}'$ around noon have been reported for various conditions (Kolber et al., 1988; Suggett et al., 2004, 2009; Moore et al., 2006) and are presumably the result of ambient light-dependent processes, non-photochemical quenching in the PSII (dissipation of excess energy as heat; Krause and Weis, 1991; Falkowski, 1992; Olazola et al., 1994; Gorbunov et al., 2011), as well as the state II transition (redistribution of absorbed energy to PSI; Falkowski and Raven, 1997) and alteration in the size of light-harvesting antenna associated with PSII, as the relative short-term photoacclimation process.

When $\sigma_{PSII}'$ values, with minimal effect from the processes described above (i.e. close to the dark-adapted $\sigma_{PSII}'$), were compared from morning and sunset casts, the profiles clearly differed between sites. The $\sigma_{PSII}'$ for Stn. 1 exhibited a vertical gradient, with lower and higher values in upper and deeper layers of water, respectively, which was consistent with the depth of the halocline; the $\sigma_{PSII}'$ for the mixed layer at Stn. 2 was distributed uniformly (Fig. 2c and h). The variations in $\sigma_{PSII}'$ reflect the taxonomic differences in antenna pigmentation, and the photoacclimation responses that alter the size of the PSII antenna (Suggett et al., 2004; Moore et al., 2006). Therefore, the gradient of $\sigma_{PSII}'$ at Stn. 1 implies that species with smaller PSII antenna dominate the bright, upper stratified waters, while species with larger antenna dominate the low-lit, deeper layers of water. Furthermore, the lower $\sigma_{PSII}'$ in the surface waters of Stn. 1, relative to that in mixed Stn. 2, indicates that the cells acclimated or adapted to strong light would be more abundant at Stn. 1. Such photoacclimation has been regarded as the strategy that adjusts the size of the PSII antenna (Falkowski and Owens, 1980). Our data for photochemical efficiency, $F_o/F_m$, ranged from 0.1 to 0.5 at both sites, showing a significant decrease in the upper layers of the water column during the daytime (Fig. 2d and i), as seen in estimations of $\sigma_{PSII}'$. This could, in part, be attributed to photodamaged PSII reaction centres (Prasil et al., 1992; Osmond, 1994; Falkowski and Kolber, 1995; Oliver et al., 2003).

Measurements of $1/\tau_{Qa}$ displayed different vertical profiles between sites, characterized by no diurnal variation (Fig. 2e and j). However, we noted that most of our data for $1/\tau_{Qa}$ exceeded the theoretical maximum rate of $Q_a$ reoxidation of 1–1.6 m s⁻¹ (Crofts and Wraith, 1983; Falkowski and Raven, 1997). Such invalid measurements might occur because in situ profiling, by means of FRRF, is too short to detect the fluorescence decay for a given parcel of water (Suggett et al., 2006). Additionally, we found gaps between the fluorescence transients and the modelled theoretical curve for estimating $\tau_{Qa}$, for some of the FRRF measurements (see Supplementary data, Appendix). Such deviations were mainly the result of a small increase in the fluorescence yield during the relaxation protocol, which could derive a lower $1/\tau_{Qa}$. However, the distribution of our $1/\tau_{Qa}$ data reflects the difference in water column structure among observation sites. At Stn. 1, relatively high $1/\tau_{Qa}$ values were found in the less saline, upper waters, while lower rates were found in the deeper part of the water column. At Stn. 2, a medium value was typical for the upper 7 m of water, and lower values for deeper waters. Such distributions could be explained by a photo-physiological response of cells, by which the number of cellular RCII is altered, and is one of the two photoacclimation strategies suggested by Falkowski and Owens (Falkowski and Owens, 1980). The higher ratio of RCII to carbon-fixation capacity results in decreases in the maximum rates of both $Q_a$ reoxidation ($1/\tau_{Qa}$) and whole-chain electron transport ($1/\tau_{PSII}$; Sukenik et al., 1987); that is, an increase in the number of cellular RCII, as an acclimation response to lower light, causes $1/\tau_{Qa}$ to decrease. Conversely, the acclimation to high irradiance leads to an increase in $1/\tau_{Qa}$. At Stn. 1, cells that were acclimated to bright light (>10% of surface PAR) in the stratified upper waters had a higher $1/\tau_{Qa}$. On the other hand, cells in the deep layer of water at Stn. 1 were acclimated to lower light, and had a lower $1/\tau_{Qa}$. In the water column at Stn. 2, cells that were exposed to a wide range of irradiance (from <1 to 100% of surface PAR), by deep mixing,
Fig. 2. Profiles of parameters at Stn. 1 (upper) and Stn. 2 (lower). Up and down arrows indicate that salinity and PAR data are related to the top and bottom X-axes, respectively. (a and f) Salinity (closed circle) and averaged relative PAR (open circle). (b and g) FRRF-based Chl a. Squares represent the mean Chl a for all casts at 1 m. (c and h) $\sigma_{PSI}'$ (Å² quanta⁻¹). (d and i) $F_v/F_m$ and (e and j) $1/\tau_{Q_a}$. Open and closed circles represent data from the midday casts (10:00–14:00 h) and data from the morning (06:00–08:00 h) or sunset casts (18:00), respectively. Squares for $1/\tau_{Q_a}$ represent the mean value for all casts at 1 m.
would be acclimated to the average light level within the mixed layer, thereby exhibiting a medium value of $1/\tau_{Q_a}$. Such distinct profiles of $1/\tau_{Q_a}$ in stratified vs. mixed water columns have been found in temperate shelf regions, from FRRF measurements of discrete samples, in which the protocol could detect Chl $a$ fluorescence decay for the same cells (Moore et al., 2006). It is important to note that an imbalance between $1/\tau_{Q_a}$ and $1/\tau_{PSII}$ is expected when a significant cyclic electron flow occurs.

The onset of cyclic electron flow and its contribution to the imbalance of these two rates would depend on in situ irradiance, but profiles of $1/\tau_{Q_a}$ showed no diurnal variation (Fig. 2). Therefore, given a degree of spatially homogeneous balance between $1/\tau_{Q_a}$ and $1/\tau_{PSII}$, thus the observed variations in $1/\tau_{Q_a}$, we assumed that using the in situ FRRF protocol would reflect the real (i.e. natural) variations in the maximum electron transport of cells; however, the measurements per se yielded overestimation.

Here, we used the “$1/\tau_{Q_a}$-like” parameter from the FRRF as an index of $1/\tau_{PSII}$ for cells, by assuming a simple relationship:

$$
1/\tau_{PSII} = A \times 1/\tau_{Q_a, FRRF} \quad (0 < A < 1).
$$

Fig. 3. Comparisons of chlorophyll-normalized, daily oxygen production rate ($P^B$) from bottle experiments (DO method; $n = 3$), with FRRF-based estimates from daytime observations. Two types of FRRF-$P^B$ estimates were plotted: one was calculated using the $\varphi_e$ variable model, composed of equations (1)–(3) (open symbols), the other by using only equation (1), with the constant $\varphi_e = 0.25$ (grey symbols). The dashed line represents a 1:1 relationship.

$1/\tau_{Q_a, FRRF}$ is the mean vertical profile data (binned over 1 m depth intervals) at each station, assuming that the alteration of $1/\tau_{PSII}$, associated with photoacclimation, might occur on the time scale of a day $A$ is a correction factor for the calculation of $1/\tau_{PSII}$ and may be instrument- and/or protocol-specific for the FRRF instrument in question (therein denoted as $A_{Diving \ Flash}$). In order to determine the optimum $A_{Diving \ Flash}$, we calculated $P^B$ from the photosynthetic model [equations (1)–(3)] when $A$ changed from 0 to 1, and compared the results with the measured $P^B$ from incubations. The estimated hourly $P^B$ data around the 0, 5 and 10 m depths were taken from all casts and integrated for the time interval 06:00–18:00 h to calculate gross daily (i.e. daytime) productivity. By comparison with FRRF- and bottle incubation-derived daily $P^B$, we determined $A_{Diving \ Flash}$ in equation (3), so that the difference between both estimates was minimized. This resulted in an $A_{Diving \ Flash}$ of 0.25 with RMSE of 1.8 mmol O$_2$ mg Chl $a$ $^{-1}$ day $^{-1}$ ($n = 5$; Fig. 3). When this factor is <0.2 or >0.25, FRRF $P^B$ deviates more from incubation results, both for Stns 1 and 2 (not shown). Setting $A_{Diving \ Flash}$ to 1 would result in $\varphi_e = 0.25$ for almost all data, in which the charge separation does not exceed $1/\tau_{PSII}$, even under high irradiance, and would lead to large overestimations of FRRF $P^B$ (RMSE $>7.4$ mmol O$_2$ mg Chl $a$ $^{-1}$ day $^{-1}$; $n = 5$), especially for data sets with relatively high values of $0.2–1.2$ ms $^{-1}$, which is expected when a significant cyclic electron flow occurs. Additionally, the spectral correction for $\sigma_{PSII}$ was not conducted in this study due to the lack of data for in situ irradiance spectra and the light absorption coefficient for phytoplankton, which could lead to a $\sim$1.5-fold overestimation of modelled productivity in the upper layer (Suggett et al., 2006). Meanwhile, as described above, the value for modelled FRRF-$P^B$, with the constant $\varphi_e$ ($=0.25$), was circa 3-fold higher than the measured value.
(Fig. 3), highlighting the importance of considering variations in \( \varphi \), when estimating productivity, even with the corrected \( \sigma_{\text{PSII}} \). In the following analyses, we calculated \( P^B \) using \( A_{\text{Diving Flash}} = 0.25 \).

**FRRF-derived \( P^B \)–irradiance curves**

The relationship between \( P^B \) and PAR (\( P \cdot E \)) was derived from daytime FRRF casts, to discuss the photoacclimation strategy at the observation sites. Hereafter, the unit of \( P^B \) was converted to a carbon base (mg C mg Chl\(^{-1}\) h\(^{-1}\)), assuming a photosynthetic quotient (PQ) of unity (Table I). The data used in this analysis were from above the optical depth (OD) of 2 (<1.8 and <4.8 m, for Stns 1 and 2, respectively) and binned over log PAR intervals of 0.1. To derive the parameters of the \( P \cdot E \) curves, the binned data were fitted to the following exponential model of Platt et al. (Platt et al., 1980):

\[
P^B = P^B_s \times \left( 1 - \exp \left( -\frac{\alpha \times E}{P^B_s} \right) \right) \times \exp \left( -\frac{\beta \times E}{P^B_s} \right)
\]

(4)

\( P^B \) is the light-saturated rate of photosynthesis without photoinhibition (mg C mg Chl\(^{-1}\) h\(^{-1}\)), \( \alpha \) the initial slope of the \( P \cdot E \) curve (mg C mg Chl\(^{-1}\) h\(^{-1}\), \( \mu \)mol quanta m\(^{-2}\) s\(^{-1}\) h\(^{-1}\)) and \( \beta \) a photoinhibition parameter (mg C mg Chl\(^{-1}\) h\(^{-1}\), \( \mu \)mol quanta m\(^{-2}\) s\(^{-1}\) h\(^{-1}\)). From these parameters, the maximum photosynthetic rate, \( P^B_{\text{max}} \) (mg C mg Chl\(^{-1}\) h\(^{-1}\)), and the light saturation parameter, \( E_k \) (\( \mu \)mol quanta m\(^{-2}\) s\(^{-1}\)), were calculated:

\[
P^B_{\text{max}} = P^B_s \times \left( \frac{\alpha}{\alpha + \beta} \right) \times \left( \frac{\beta}{\alpha + \beta} \right)^{(\beta/\alpha)}
\]

\[
E_k = \frac{P^B_{\text{max}}}{\alpha}.
\]

Fitting the model to the binned data for the stratified Stn. 1 and mixed Stn. 2 accounted for a high proportion of the variance, with standard residuals of 0.51 and 0.58 mg C mg Chl\(^{-1}\) h\(^{-1}\), respectively (Fig. 4a and b). Both the binned data and modelled curves displayed a clear site-dependent difference, in terms of the light dependence of \( P^B \).

The estimates of the \( P \cdot E \) parameters in the UGOT are listed in Table III, all of which are comparable to values previously reported for natural phytoplankton assemblages (Platt et al., 1980; Sakshaug and Holm-Hansen, 1986; Marañón and Holligan, 1999; Moran and Estrada, 2001; Shaw and Purdie, 2001; van Håsten and Smith, 2002). The value of \( \alpha \) for Stn. 2 was higher than that for Stn. 1 by a factor of 1.7 [0.063 vs. 0.104 mg C mg Chl\(^{-1}\) h\(^{-1}\) (\( \mu \)mol quanta m\(^{-2}\) s\(^{-1}\) h\(^{-1}\)]. A comparable magnitude of difference (1.8-fold) was found for \( P^B_{\text{max}} \), but was higher at Stn. 1 than at Stn. 2, where \( P^B_{\text{max}} \) was 1.4-fold higher (10.6 vs. 7.8 mg C mg Chl\(^{-1}\) h\(^{-1}\)). These differences were in accordance with the higher \( E_k \) for Stn. 1, more than twice that for Stn. 2 (169 vs. 75 \( \mu \)mol quanta m\(^{-2}\) s\(^{-1}\)). This indicates that cells were acclimated to relatively bright light in the stratified upper waters of Stn. 1, and to low light in the mixed column of Stn. 2, even for layers at an identical OD (OD <2). This may relate to the fact that the average irradiance for cells at Stn. 2 was lower than at Stn. 1, due to deep mixing.

The difference in \( \alpha \) between Stns 1 and 2 corresponds to a lower \( \sigma_{\text{PSII}}' \) for the upper waters of Stn. 1, compared with mixed Stn. 2 (Fig. 2c and h). As already noted, \( \sigma_{\text{PSII}}' \) is principally subject to the properties of antenna pigments in cells, which are closely associated with photoacclimation/adaptation responses (Moore et al., 2006). Thus, the light-harvesting capacity of cells that are acclimated or species that are adapted to the bright conditions of Stn. 1 might be suppressed, as a result of higher amounts of photoprotective carotenoids, resulting in lower \( \alpha \).

Estimates of \( P^B_s \) and \( P^B_{\text{max}} \) largely depend on the quantum yield of \( O_2 \) evolution, \( \varphi \), in equation (3), since the decrease in \( \varphi \) accounts for most of the depression of \( P^B \) under high irradiance (i.e. photoinhibition). For cells in the stratified Stn. 1, we assumed a maximum \( \varphi = 0.25 \) for PAR <300 \( \mu \)mol quanta m\(^{-2}\) s\(^{-1}\), while \( \varphi = 0.25 \) for Stn. 2 dropped to <0.25 for PAR <130 \( \mu \)mol quanta m\(^{-2}\) s\(^{-1}\) (Fig. 4d and e); that is, a higher light threshold for a decrease \( \varphi \) at Stn. 1 would enable a higher \( P^B \) and \( P^B_{\text{max}} \), relative to those in Stn. 2. This change in the threshold itself could be attributed to the fact that the average 1/\( \tau_{\text{PSII}} \) for the upper layer of Stn. 1 (~0.68 m s\(^{-1}\)) was faster than for Stn. 2 (0.52 m s\(^{-1}\)). Actually, incorporating 0.68 m s\(^{-1}\) into 1/\( \tau_{\text{PSII}} \) for Stn. 2 would elevate the PAR threshold for a decrease \( \varphi \) from ~130 to 200 \( \mu \)mol quanta m\(^{-2}\) s\(^{-1}\) (Fig. 4e), and thus, \( P^B_{\text{max}} \) to 10.0 mg C mg Chl\(^{-1}\) h\(^{-1}\), comparable to estimates for Stn. 1 (Fig. 4c). Such changes in 1/\( \tau_{\text{PSII}} \), \( E_k \) and \( P^B_{\text{max}} \) mainly reflected the theoretical relationship \( P^B_{\text{max}} = E_k / \alpha = \eta_{\text{PSII}}' \times (1/\tau_{\text{PSII}}) \), in which \( \eta_{\text{PSII}}' \) is the ratio of “functional” RCII to Chl \( a \) (Falkowski and Raven, 1997). This means that the lower \( P^B_{\text{max}} \) for Stn. 2 resulted from the relatively slow 1/\( \tau_{\text{PSII}} \), associated with acclimation of cells to weak light by the increase in RCII relative to carbon-fixation capacity (Sukenik et al., 1987; Falkowski and Raven, 1997). Conversely, the faster 1/\( \tau_{\text{PSII}} \) of cells in Stn. 1 resulted in a higher \( P^B_{\text{max}} \). However, “downstream” processes of PSII would ultimately constrain the carbon-fixation capacity, \( P^B_{\text{max}} \) such as the concentration and/or activity of RUBISCO (Rivkin, 1990; Orellana and Perry, 1992; Geider and MacIntyre, 2002). We have no information on the biochemical characteristics of...
RUBISCO for the predominant phytoplankton species, but the impact of N- and P-limitation (Falkowski et al., 1989; Beardall et al., 1991; Geider et al., 1998) would not likely cause a difference in the cellular RUBISCO contents among our sampling sites. Thus, we attribute the significant site-differences in P–E parameters (\(a\), \(P_{B \text{max}}\) and \(E_k\)) from our FRRF measurements, primarily to a different photoacclimation response, with both an alteration of antenna pigment structures and the number of RCII.

Fig. 4. Relationships between underwater PAR and instantaneous FRRF-based \(P_B\) data (diamonds) (a) for Stn. 1, (b) for Stn. 2. Circles in (a and b) represent the binned \(P_B\) data from an OD <2. The dashed line represents the \(P-E\) curve estimated by fitting the binned data in Stn.1, and the solid line represents that for Stn. 2. (c) Comparison of \(P-E\) curves between Stn. 1 (dashed line) and Stn. 2 (solid line), and Stn. 2 with 1/\(\tau_{PSII}\) set at 0.68 m s\(^{-1}\) (grey line). (d) Relationship between \(\varphi_0\) and underwater PAR at Stn. 1 (open circles), (e) at Stn. 2 (closed circles) and at Stn. 2 with 1/\(\tau_{PSII}\) = 0.68 m s\(^{-1}\) (grey squares). Note that the x-axis is logarithmic in c–e.

Table III. The estimated photosynthesis vs. irradiance (\(P-E\)) parameters for Stns 1 and 2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Stn. 1</th>
<th>Stn. 2</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td>0.063 ± 0.003</td>
<td>0.104 ± 0.009</td>
<td>mg C mg Chl(^{-1}) h(^{-1})</td>
</tr>
<tr>
<td>(\beta)</td>
<td>0.008 ± 0.002</td>
<td>0.002 ± 0.001</td>
<td>mg C mg Chl(^{-1}) h(^{-1})</td>
</tr>
<tr>
<td>(P_{B \text{max}})</td>
<td>10.6 ± 1.4</td>
<td>7.8 ± 7.2</td>
<td>mg C mg Chl(^{-1}) h(^{-1})</td>
</tr>
<tr>
<td>(E_k)</td>
<td>169 ± 23</td>
<td>75 ± 12</td>
<td>(\mu\text{mol}) photon m(^{-2}) s(^{-1})</td>
</tr>
</tbody>
</table>

Initial slope of the \(P-E\) curve (\(a\)), the photoinhibition parameter (\(\beta\)), maximum photosynthetic rate (\(P_{B \text{max}}\)) and the light saturation parameter (\(E_k\)).

On the whole, variations in \(P_B\) in the layer of OD >2 also followed the \(P-E\) curve we modelled for the layer of OD <2 (Fig. 4a and b), implying that the vertical and diurnal changes in \(P_B\) in the UGOT can be described by the single light-dependent equation of \(P_B\) for each site. Given that a diurnal change in the light field within the water column was modelled, a \(P_B\) profile for each time point could be reproduced using a single \(P-E\) curve, derived from only one noon cast of FRRF when incident light is highest. By integrating the data over both depth and time, it is possible to estimate the daily water column productivity (Tripathy et al., 2010). In the field, however, more frequent casts would be required to identify the \(P_B\) profile with the highest surface PAR, especially for days characterized by highly variable cloud cover.
Average EZ photosynthetic rate and light utilization efficiency

The instantaneous average, Chl α-normalized, photosynthetic rate for the EZ \( P_{\text{EZ}}^B \) (mg C mg Chl \(^{-1}\) h \(^{-1}\)) was calculated by dividing the depth-integrated rate by the total amount of Chl \( a \) in the EZ (Fig. 5a). The depth of the EZ was 7 and 12 m, for Stns 1 and 2, respectively (Table II). Between \( P_{\text{EZ}}^B \) and surface PAR, there was a clear logarithmic relationship \( (\rho^2 = 0.80) \), which suggests that photosynthesis in the UGOT is controlled principally by irradiance (Fig. 5a). Consequently, the index of hourly light utilization efficiency of EZ \( \Psi_{c,\text{EZ}} \) (mg C mg Chl \(^{-1}\) h \(^{-1}\) (\( \mu \)mol quanta m \(^{-2}\) s \(^{-1}\)) \(^{-1}\)) is also related to PAR \( (\rho^2 = 0.89) \), and decreased as PAR increased (Fig. 5b). This also emphasizes the significance of irradiance for light utilization by cells, but one question remains: Why do the average EZ parameters show the same behaviour at both sites, despite the distinct site differences in the photoacclimation state of cells, as indicated by the \( P - E \) curves?

To confirm this consistency between observation sites, both the diurnal changes in \( P_{\text{EZ}}^B \) and \( \Psi_{c,\text{EZ}} \) of Stns 1 and 2 were modelled by using the site-specific \( P - E \) parameters listed in Table III and the average Chl \( a \) profiles (Fig. 6c), given ideal changes (sinusoidal variation) in insolation, but with different mean light dissipation between sites (Figs 2a and f, and 6a). The modelled \( P_{\text{EZ}}^B \) and \( \Psi_{c,\text{EZ}} \) of both stations were described by a similar relationship with surface PAR (Fig. 6e and g), as found in observed values (Fig. 5). However, such consistencies disappeared in the model (Fig. 6f and h), which introduced a vertically uniform Chl \( a \) distribution at both stations (average EZ concentrations of 5.1 and 3.4 mg Chl m \(^{-3}\), for Stns 1 and 2, respectively; Fig. 6d), but retained the site-specific \( P - E \) parameters. This stresses that a reciprocal relationship between profiles of \( P_{\text{EZ}}^B \) and Chl \( a \) are needed for the same behaviour of \( P_{\text{EZ}}^B \) (and \( \Psi_{c,\text{EZ}} \)) against PAR, as we observed between stations.

On the supposition that phytoplankton biomass depends mainly on the photosynthetic activity of cells, the following relationships could be proposed: In the stratified upper water of Stn. 1, the cells confined to bright light would obtain a higher \( P_{\text{EZ}}^B \) at depths \(<\) 2 m, relative to the cells in the shaded, deeper layer. This would result in a \( P_{\text{opt}}^B \) of 111 mg C mg Chl \(^{-1}\) day \(^{-1}\) at a depth of 1 m (Fig 6b). The cells with high photosynthetic activity would accumulate in the upper waters, contributing to the shallow peak of Chl \( a \) (6.2 mg Chl m \(^{-3}\); Fig. 6c), which in turn would cause shading in the deeper layers of the EZ, and thereby affect the \( P_{\text{opt}}^B \). On the other hand, in the mixed water at Stn. 5, a relatively low \( P_{\text{opt}}^B \) of 85 mg C mg Chl \(^{-1}\) day \(^{-1}\) was situated at a depth of \( \approx \) 4 m (Fig 6b) because cells at that depth had acclimated to lower light, while the upper layer suffered photoinhibition. Mixing across the EZ would limit the accumulation of cells in such a narrow layer, and keep the profile of Chl \( a \) relatively uniform at an average concentration of 3.4 mg Chl m \(^{-3}\) (Fig 6c).

Such a scenario can partly explain the general patterns between the \( P_{\text{EZ}}^B \) and Chl \( a \) profiles in coastal environments, both of which are highly dependent on water column stability and distribution of irradiance. Cells will acclimate, within limits, to any irradiance to maximize their photosynthetic rate at the time scale of the growth rate (Sakshaug and Holm-Hansen, 1986). Such adequate acclimation to light would potentially lead to the optimum EZ-average photosynthetic rate and light utilization efficiency. Both potentials can be limited by photosynthetic resources other than light, such as macro- and micro-nutrients (Hucky and Kilham, 1988; Sunda and Huntsman, 1995), but these effects would be negligible in a eutrophic region such as the UGOT. It is therefore possible to say that, due to little stress from nutrient limitation, comparable potentials for \( P_{\text{EZ}}^B \) and \( \Psi_{c,\text{EZ}} \) were found for the different light regimes at the two sites (Fig 5). This hypothesis should be examined, in view of both energetic costs for acclimation and repair of photo-damaged parts (i.e. RCII; Raven, 2011), and differences among species (Six et al., 2008; Dimier et al., 2009).
Additionally, control of the grazing pressure on phytoplankton abundance, and thereby Chl \( a \) profile, should be considered. However, if such a relationship between \( P_{B}^{E} \) and surface PAR, as observed and modelled here, was robust even for a limited area, such as in this study, which is coastal and highly eutrophic, it would make a significant contribution to estimation of daily local productivity and material cycling in the coastal environment.

**SUPPLEMENTARY DATA**

Supplementary data can be found online at [http://plankt.oxfordjournals.org](http://plankt.oxfordjournals.org).

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