Comparison by PAM Fluorometry of Photosynthetic Activity of Nine Marine Phytoplankton Grown Under Identical Conditions

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Comparison by PAM Fluorometry of Photosynthetic Activity of Nine Marine Phytoplankton Grown Under Identical Conditions

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ABSTRACT

The photosynthetic activity of marine phytoplankton from five algal classes (Phaeodactylum tricornutum, Skeletonema costatum, Thalassiosira oceanica, Thalassiosira weissflogii, Dunaliella tertiolecta, Mantoniella squamata, Emiliania huxleyi, Pavlova lutheri and Heterosigma akashiwo) was investigated under identical growth conditions to determine interspecies differences. Primary photochemistry and electron transport capacity of individual species were examined by pulse amplitude–modulated (PAM) fluorescence. Although few differences were found in maximal photosystem II (PSII) photochemical efficiency between various species, large differences were noticed in their PSII–photosystem I (PSI) electron transport activity. We found that species such as T. oceanica and M. squamata have much lower photochemical activity than H. akashiwo. It appeared that processes involved in electron transport activity were more susceptible to change during algal evolution compared with the primary photochemical act close to PSII. Large variations in the nonphotochemical energy dissipation event among species were also observed. Light energy required to saturate photosynthesis was very different between species. We have shown that M. squamata and H. akashiwo required higher light energy (>1300 μmol m⁻² s⁻¹) to saturate photosynthesis compared with S. costatum and E. huxleyi (ca 280 μmol m⁻² s⁻¹). These differences were interpreted to be the result of variations in the size of light-harvesting complexes associated with PSI. These disparities in photosynthetic activity might modulate algal community structure in the natural environment where light energy is highly variable. Our results suggest that for an accurate evaluation of primary productivity from fluorescence measurements, it is essential to know the species composition of the algal community and the individual photosynthetic capacity related to the major phytoplankton species present in the natural phytoplankton assemblage.

INTRODUCTION

Marine phytoplankton biomass represents only 0.2% of the photosynthetically active carbon biomass on Earth but accounts for about 45–50% of the global net primary production (1). The large variability in the accessory light-harvesting pigments of phytoplankton participating in primary production may induce heterogeneity concerning the efficiency of light absorption and energy dissipation processes in photosynthesis (2). Furthermore, when phytoplankton are grown under different environmental conditions, such as light, temperature and nutritional status, photosynthetic activity can change through adaptation or acclimation processes (3). On the other hand, although phytoplankton species are phylogenetically diverse (see Fig. 1 for a diagram representing the evolution of photosynthetic eukaryote groups used in this study), the molecular structure of the photosystem II and I (PSII and PSI) core complexes responsible for primary photochemistry has been remarkably conserved (4,5). Such specificity in photosynthetic activity could lead to a problem if one compares energy storage and dissipation processes through photosynthesis between different algal species and attempts to evaluate global net primary productivity in aquatic ecosystems. Therefore, study of the differences in photosynthetic activity between algal species could facilitate the understanding of how different algal species can contribute to primary productivity in aquatic ecosystems.

Evaluation of primary productivity or photosynthetic capacity by either classical (¹⁴C-uptake or O₂ evolution) or recently developed methods on the basis of chlorophyll a fluorescence has shown wide variations among algal species. For example, the maximal PSII photochemical yield, , which is proportional to maximal photosynthetic efficiency, can change from 0.4 to 0.8 for different phytoplankton classes (6). Green algae such as Dunaliella tertiolecta and Dunaliella salina have values around 0.8, whereas the values for diatoms Phaeodactylum tricornutum and Thalassiosira weissflogii were shown to be between 0.6 and 0.7 (7–10). On the other hand, very low maximal PSII photochemical yield () was reported for the prasinophyte Mantoniella squamata (11). The operational PSII photochemical yield, ,
which characterizes steady state photosynthesis, appeared also to be widely variable among algal species, as seen for Emiliania huxleyi, Phaeocystis globosa and P. tricornutum ($\Phi_M$: 0.35, 0.5 and 0.6 respectively) (9,12). These variations in the maximal and operational PSII photochemical yields were explained to be the result of functional and structural properties specific for the photosynthetic apparatus of different species (2,6). One could consider also that these differences in photosynthetic activity might be influenced by various algal growth conditions (13), but so far little information is known on how the differences in the maximal and operational PSII photochemical yields are attributed to species properties when growth and measurement conditions are the same. Furthermore, besides the maximal and the operational PSII photochemical yields, it is also worthwhile to investigate how other photosynthetic fluorescence parameters reflecting energy storage and dissipation processes are variable among algal species.

In this study we investigated various aspects of photosynthetic activity and energy dissipation mechanisms of several ecologically important phytoplankton belonging to different taxonomic groups. Different photosynthetic parameters were examined by pulse amplitude–modulated (PAM) fluorometry for algal species grown under the same light, temperature and nutrient conditions. This investigation of the photosynthetic capacity of individual algal species will contribute to better estimates of primary productivity in aquatic ecosystems.

**MATERIALS AND METHODS**

The different marine phytoplankton strains used in this study are listed in Table 1. Cultures (except E. huxleyi) were grown in filter-sterilized modified EAW medium (14) in which the concentrations of nitrate, silicate and phosphate were 0.2, 0.5 and 0.1 mM respectively. For E. huxleyi the medium was modified to minimize coccolith detachment (15); the trace metal and selenium concentrations were respectively 1/50 and 1/100 of the original EAW concentrations, whereas nitrate and phosphate concentrations were reduced to 0.03 and 0.02 mM respectively, and silicate was omitted (16). Algae were grown in 40 mL culture tubes under continuous illumination provided by white fluorescent lamps (Vita-Lite 40 W, DURO-TEST, Philadelphia, PA). The light intensity was 130 µmol m$^{-2}$ s$^{-1}$, and the temperature was 17°C. Algae were grown at or near their maximum growth rates (Table 1) and were sampled in their midexponential growth phase. Similar results were obtained when the algae were grown in 1 L flasks (data not shown). Growth rates were calculated from plots of the log of fluorescence against incubation time with a Turner Designs fluorometer (Sunnyvale, CA). All measurements were carried out at the same time of day to minimize the error associated with any endogenous rhythms within the cultures.

Fluorescence induction measurements were made with a PAM fluorometer 101 (Heinz Walz GmbH, Effeltrich, Germany) after dark adaptation of the algae for 25 min, according to Schreiber et al. (17). Following dark adaptation, the dark-adapted state constant fluorescence yield, $F_0$, was recorded under modulated light (2 µmol m$^{-2}$ s$^{-1}$). The maximal fluorescence yield for a dark-adapted sample, $F_{M0}$, was obtained with a saturating flash (700 ms, 3000 µmol m$^{-2}$ s$^{-1}$) permitting all the plastoquinone (PQ) pool to be in maximum reduced state. The flash intensity was saturating because the addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (20 µM) did not permit further increases of the fluorescence level (data not shown). Fluorescence induction dependent on PSII–PSI electron transport was measured under continuous actinic light (130 µmol m$^{-2}$ s$^{-1}$). Simultaneously, saturating flashes (700 ms, 3000 µmol m$^{-2}$ s$^{-1}$) given periodically (every 30 s) provided the maximal fluorescence yield for a light-adapted sample, $F_{M0}$. At steady state fluorescence yield ($F_3$), actinic light was turned off and, following a short dark period, the light-adapted state constant fluorescence yield, $F_0$, was evaluated. Fluorescence parameters were calculated according to the following equations after subtraction of the blank fluorescence value obtained by measuring the fluorescence of a 0.22 µm filtered sample.

$$
\Phi_M = (F_{M0} - F_0)/F_{M0} = F_M/F_{M0} (18)
$$

$$
\Phi_M = (F_{M0} - F_0)/F_{M0} (19)
$$

$$
\phi_{PSII} = (F_{M0} - F_3)/(F_{M0} - F_0) (20)
$$

$$
\phi_{PSII} = (F_{M0} - F_3)/(F_{M0} - F_0) (21)
$$

$F_M$ is variable fluorescence, $\phi_{PSII}$ and $\phi_{PSII}$ are the relative photochemical and nonphotochemical quenching values and $UQF_{FACII}$ is the relative unquenched fluorescence. Triplicate cultures were grown and data were analyzed by one-way analysis of variance.

The relative photosynthetic electron transport rate (ETR) was estimated from the operational PSII photochemical yield measured at different photosynthetic photon flux densities (PPFD),

$$
ETR = \Phi_M \times PPFD
$$

The photosynthesis/irradiance (P/E) curve measurements were taken on the same algal sample by increasing the actinic light intensity stepwise between 0 and 1600 µmol m$^{-2}$ s$^{-1}$ with saturating flashes (700 ms) every 30 s according to White and Critchley (22). After each increase in actinic

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**Table 1.** Strains and growth rates of the different species used in this study. The strain numbers are from the Northeast Pacific Culture Collection (NEPCC) and Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP). Values are means ± 1 SD ($n = 3$)

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain number</th>
<th>Growth rate (day$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillariophyceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phaeodactylum tricornutum</td>
<td>NEPCC 640</td>
<td>1.11 ± 0.03</td>
</tr>
<tr>
<td>Skeletonema costatum</td>
<td>NEPCC 676</td>
<td>1.18 ± 0.04</td>
</tr>
<tr>
<td>Thalassiosira oceanica</td>
<td>CCMP 1003</td>
<td>0.93 ± 0.05</td>
</tr>
<tr>
<td>Thalassiosira weissflogii</td>
<td>NEPCC 636</td>
<td>0.90 ± 0.01</td>
</tr>
<tr>
<td>Chlorophyceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dunaliella tertiolecta</td>
<td>NEPCC 001</td>
<td>1.11 ± 0.03</td>
</tr>
<tr>
<td>Prasinophyceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mantoniella squamata</td>
<td>NEPCC 524</td>
<td>0.76 ± 0.03</td>
</tr>
<tr>
<td>Prymnesiophyceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emiliania huxleyi</td>
<td>NEPCC 732*</td>
<td>0.52 ± 0.01</td>
</tr>
<tr>
<td>Pavlova lutheri</td>
<td>NEPCC 634</td>
<td>0.72 ± 0.01</td>
</tr>
<tr>
<td>Raphidophyceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterosigma akashiwo</td>
<td>NEPCC 522</td>
<td>0.59 ± 0.05</td>
</tr>
</tbody>
</table>

*This isolate was kept in low macro- and micronutrient North Pacific (Station Papa; 50°N, 145°W) water to retain the organism’s ability to form coccoliths.*
RESULTS AND DISCUSSION

It has been reported for different algal species that $\Phi_M$ can vary by up to 100% depending on the taxonomic group (6). This wide variation was proposed to be due to different organization and functional properties of the photosynthetic apparatus (6,24). As seen from our study, when algae were grown under the same conditions, much smaller variations in $\Phi_M$ values were noticed among different algal species (~15%) compared with earlier studies (Table 2). Therefore, we can assume that the previously reported large variations were probably caused not only by specificity of different photosynthetic apparatus but by various culture conditions.

However, fluorescence induction of *E. huxleyi*, *Thalassiosira weissflogii* and *Pavlova lutheri* revealed important variations in the maximal fluorescence yields measured after dark and light adaptation ($F_M$ and $F_S$ respectively; Fig. 2). Important variations were also noticed in the fluorescence yield at steady state electron transport ($F_S$). Smaller differences in the fluorescence kinetics were also observed for the same species (data not shown). These variations might indicate real differences among species concerning the balance of the energy dissipation associated with PSII–PSI electron transport. The high value of $F_S$ seen for *E. huxleyi* (Fig. 2A) could reflect that an important fraction of PSII reaction centers was closed, indicating that few PSII participate in electron transport toward PSI. On the other hand, the decrease in $F_S$ close to $F_0$ seen in *P. lutheri* (Fig. 2C) could indicate high PQ pool oxidation state and an active electron transport activity toward PSI.

One might expect that fluorescence indicators obtained at steady state electron transport vary importantly between algal species because the variations in the fluorescence kinetics were evident. Indeed, variations were noticed for $\Phi_M$, $\Phi_P$ and $\Phi_Q$ (17). For example, unlike $\Phi_M$, which tends to be similar between species (Table 2), $\Phi_P$ and $\Phi_Q$ showed variation of 55, 68, 75 and 100% respectively. Therefore, low variation of $\Phi_M$ values reflect functional properties of the conservative part of PSII electron transport (PSII core complex), whereas other parameters are influenced by various factors related to organization and function of photosynthetic electron transport and related processes. The quenching components evaluated by $q_P$ and $q_N$ showed larger variations compared with the relative quenching coefficients ($q_{\text{rel}}$ data not shown). This might be explained by the fact that $q_P$ and $q_N$ are not normalized to the same reference signal and because the calculation of $q_P$ is based on a photosynthetic unit model (puddle model) known not to be representative of the real situation (20,21,25).

We found that all algal species except *T. oceanica* and *M. squamata* have similar $\Phi_M$ and photochemical quenching ($q_{\text{rel}}$). This might be explained by PSII and PSI core complexes that are highly conserved among all classes of eukaryotic algae (5,26). However, reasons for lower photochemical energy dissipation in *T. oceanica* and *M. squamata* compared with the other species are not clear yet. It has been found that *M. squamata* has high chlororespiratory activity (27), which might explain the high nonphotochemical energy dissipation that was noticed in our study. Furthermore, *M. squamata* appears to lack some of the light-harvesting complexes associated with PSI (28), which might reduce the light energy funnelled to the PSI reaction centers and consequently diminish PSII–PSI electron transport. On the other hand, the low PSII electron transport for *T. oceanica* (see $\Phi_M$ and $q_{\text{rel}}$ in Table 2) could be interpreted by the higher PSII:PSI ratio characteristic for this species compared with the other algal species (29–31). Decreased PSII–PSI electron transport will induce a high reduced state of PSII and therefore the high $F_S$ yield and high UQF value seen for *T. oceanica* (Fig. 2A, Table 2).

Although the photochemical energy dissipation ($q_{\text{rel}}$) of *P. tricornutum*, *S. costatum*, *T. weissflogii*, *D. tertiolecta*, *E. huxleyi*, *P. lutheri* and *Heterosigma akashiwo* was similar, significant differences were found in nonphotochemical energy dissipation ($q_{\text{rel}}$), which varied from 0.222 to 0.512. It is known that nonphotochemical energy dissipation processes are affected by mechanisms and structures that are not linked only to the PSII and PSI core complexes (32,33). Therefore we may suppose that these processes have passed through important modifications during the evolution of algal species. The relative unquenched fluorescence parameter also showed wide variation among species, suggesting that the processes involved in the control of the proportion of closed PSII reaction centers at steady state electron transport were not conserved during the evolution of phytoplankton.

Significant differences in the photosynthetic capacity and structural properties among the examined species are also shown by the change in the P/E response (Fig. 3). The initial slope of the P/E curve was reported to be proportional to the PSII antenna size (34). Therefore, the smaller initial slopes of the P/E curves for *T. oceanica*, *H. akashiwo* and *M. squamata* compared with *P. tricornutum*, *S. costatum* and *E. huxleyi* can be interpreted as smaller PSII antenna sizes. This might explain the high energy...
radiation required to reach photosynthetic saturation for these species (Fig. 4). Indeed, a good correlation between the energy to saturate photosynthesis (ESAT) and the initial slope of the P/E curve (a) was obtained for the investigated species. However, the fact that *T. oceanica*, which has a small antenna size, shows saturation of photosynthesis at relatively low energy (280 $\mu$mol m$^{-2}$ s$^{-1}$/C0$_2$; see in Figure 4), might be explained by its high PSII:PSI ratio noted earlier (30). It appeared that photosynthesis of the majority of investigated algae was saturated at intensities >1300 $\mu$mol m$^{-2}$ s$^{-1}$. However for some species such as *H. akashiwo* and *M. squamata*, the electron transport rate was saturated above 1300 $\mu$mol m$^{-2}$ s$^{-1}$. The saturation at relatively low light intensity for *E. huxleyi* and *S. costatum* might suggest that modest requirements in ATP and NADPH are necessary for CO$_2$ fixation. Variations in light intensity in the natural habitat could influence the contribution of individual algae to global photosynthesis in aquatic ecosystems. Indeed, as indicated by the decline in photosynthesis (see Fig. 3), some species could be photoinhibited at relatively low light intensity, making them less likely to be productive near the surface of the ocean during sunny days. However, light intensity is only one of the possible ecological factors that can influence the contribution of individual algae to the entire algal community. Therefore additional effects on the photosynthetic capacity of other environmental factors, such as temperature and nutrient status, have also to be considered.

We have shown that the photosynthetic activity of different algal species can differ widely even if they were grown under the same conditions. Consequently, for an accurate evaluation of primary productivity on the basis of fluorescence measurements, it is useful
to know the structure of the algal community and individual photosynthetic capacity related to the major phytoplankton species. Indeed, the knowledge of algal species composition is essential if one wants to obtain an accurate evaluation of primary productivity because, as we have shown here, an estimate of the photosynthetic capacity of an algal assemblage is closely linked to specific properties of each algal species.

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REFERENCES


