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TEMPERATURE EFFECTS ON MICROALGAL PHOTOSYNTHESIS-LIGHT RESPONSES MEASURED BY O_2 PRODUCTION, PULSE-AMPLITUDE-MODULATED FLUORESCENCE, AND ^{14}C ASSIMILATION[1]

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Short-term temperature effects on photosynthesis were investigated by measuring O_2 production, PSII-fluorescence kinetics, and ^{14}C-incorporation rates in monocultures of the marine phytoplankton species *Prorocentrum minimum* (Pavill.) J. Schiller (Dinophyceae), *Prymnesium parvum* f. *patelliferum* (J. C. Green, D. J. Hibberd et Pienaar) A. Larsen (Coccolithophyceae), and *Phaeodactylum tricornutum* Bohlin (Bacillariophyceae), grown at 15°C and 80 μmol photons · m^{-2} · s^{-1}. Photosynthesis versus irradiance curves were measured at seven temperatures (0°C–30°C) by all three approaches. The maximum photosynthetic rate (P^C_{max}) was strongly stimulated by temperature, reached an optimum for *Pro. minimum* only (20°C–25°C), and showed a similar relative temperature response for the three applied methods, with Q_{10} ranging from 1.7 to 3.5. The maximum light utilization coefficient (α^C) was insensitive or decreased slightly with increasing temperature. Absolute rates of O_2 production were calculated from pulse-amplitude-modulated (PAM) fluorometry measurements in combination with bio-optical determination of absorbed quanta in PSII. The relationship between PAM-based O_2 production and measured O_2 production and ^{14}C assimilation showed a species-specific correlation, with 1.2–3.3 times higher absolute values of P^C_{max} and α^C when calculated from PAM data for *Pry. parvum* and *Ph. tricornutum* but equivalent for *Pro. minimum.* The offset seemed to be temperature insensitive and could be explained by a lower quantum yield for O_2 production than the theoretical maximum (due to Mehler-type reactions). Conclusively, the PAM technique can be used to study temperature responses of photosynthesis in microalgae when paying attention to the absorption properties in PSII.

Key index words: **^{14}C assimilation; microalgae; O_2 production; PAM fluorescence; phi-max; photosynthetic parameters; quantum yield; temperature**

Abbreviations: **ETR, electron transport rate; PAM, pulse amplitude modulated; *P-E,* photosynthesis-irradiance; POC, particular organic carbon; PQ, photosynthetic quotient; Q_{10}, temperature coefficient**

Pelagic photosynthesis can be estimated by measuring O_2 evolution, PSII-fluorescence kinetics, or ^{14}C assimilation. Each of the methods has its advantages and disadvantages, and all have been applied to assess the ecosystem primary production in various environments. The techniques, however, measure different products of the photosynthetic pathway and reflect different physiological processes with potentially different responses to environmental variables, such as temperature or salinity (Geider and Osborne 1992, Geel et al. 1997, Morris and Kromkamp 2003).

O_2-evolution measurements using O_2 electrodes allow for net O_2-production measurements in light and O_2-respiration measurements in the dark (Glud et al. 2000). Gross O_2 production can then be estimated as the net production added to the respiration (assuming constant respiration in light and dark). As such, the approach quantifies the O_2-production rate from the water-splitting complex in PSII. PSII fluorescence can be measured by PAM fluorometry and can be used to measure the operational quantum yield of PSII (Φ_{PSII}, Schreiber et al. 1986). From multiplying Φ_{PSII} with the quanta absorbed in PSII, the electron transfer rate in PSII can be calculated (Genty et al. 1989). The electron transfer rate (ETR) is a proxy for the gross photosynthetic rate (Kroon et al. 1993). The electrons generated in PSII are closely coupled to O_2 evolution but follow several pathways, among those, reduction of CO_2 via NAD(P)H production (Falkowski and Raven 1997). ^{14}C-assimilation rate measurements quantify the amount of dissolved inorganic carbon (DIC) converted into cell biomass and reflect an activity intermediate to net and gross photosynthesis,

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dependent on the incubation time (Falkowski and Raven 1997). For 1 h incubations, the technique is for convenience commonly assumed to indicate gross rates.

Photosynthetic O_2 production, Φ_{PSII}, and/or ^{14}C assimilation have been compared in a number of studies of vascular plants (Demmig and Bjorkman 1987, Seaton and Walker 1990), macroalgae (Hanelt and Nultsch 1995, Longstaff et al. 2002), microphytobenthos (Hartig et al. 1998, Barranguet and Kromkamp 2000, Glud et al. 2002a), and marine phytoplankton (Falkowski et al. 1986, Kroon et al. 1993, Geel et al. 1997, Flameling and Kromkamp 1998, Rysgaard et al. 2001, Morris and Kromkamp 2003). Although the investigations have been conducted under a variety of experimental conditions, a majority of studies on microalgae find a linear relationship between O_2 evolution and Φ_{PSII} under moderate irradiance (Falkowski et al. 1986, Genty et al. 1989, Geel et al. 1997), sometimes with deviation at very low (Schreiber et al. 1995, Flameling and Kromkamp 1998, Masojidek et al. 2001) or very high irradiance conditions (Falkowski et al. 1986, Flameling and Kromkamp 1998). Different explanations for the deviation have been proposed: spectral difference in PAR sources, changes in O_2 consumption in the light, cyclic electron transport around PSII, and Mehler-type reactions [see Flameling and Kromkamp (1998) for an overview]. The relationship between O_2 production and Φ_{PSII} is far from universal, and apparently there exists interspecies variance in the shape of the relationship and of the slope-coefficient (Barranguet and Kromkamp 2000, Masojidek et al. 2001). Additionally, it must be expected that environmental variables, such as temperature, can affect established relations for a given species. Even so, detailed comparison studies accounting for environmental variables, such as temperature, are still very limited (Barranguet and Kromkamp 2000, Morris and Kromkamp 2003). If fluorescence measurements are to be applied successfully for quantifying photosynthetic production, more careful and detailed studies of the temperature effect on the relationship between O_2 evolution, Φ_{PSII}, and ^{14}C assimilation are required (Schofield et al. 1998, Kuhl et al. 2001, Glud et al. 2002b, Morris and Kromkamp 2003).

The aim of this study was to investigate the relationship between temperature and photosynthetic parameters derived from measurements of O_2 production, Φ_{PSII}, and ^{14}C assimilation, using three culture-grown phytoplankton species—*Pro. minimum, Pry. parvum* f. *patelliferum*, and *Ph. tricornutum*—selected to represent typical species of Scandinavian waters. Photosynthetic activity was quantified from (i) measured rates of O_2 production by O_2 microsensors (P^C_{O2}, μmol $O_2 \cdot$ [mg POC]$^{-1} \cdot h^{-1}$, where POC stands for particulate organic carbon), (ii) calculated rates of O_2 production based on Φ_{PSII} in combination with biooptical determination of quanta absorbed in PSII (P^C_{PSII}, μmol $O_2 \cdot$ [mg POC]$^{-1} \cdot h^{-1}$), and (iii) measured rates of ^{14}C assimilation (P^C_{14C}, μmol $^{14}C \cdot$ [mg POC]$^{-1} \cdot h^{-1}$). The temperature influence on photosynthetic parameters is discussed in a physiological context.

MATERIALS AND METHODS

Algal cultures. Unialgal cultures of *Pro. minimum* (strain 79A, Oslofjord, isolated by K. Tangen, culture at Trondhjem Biological Station [TBS]), *Pry. parvum* f. *patelliferum* (isolated in Ryfylke, S-Norway, culture from University of Oslo), and *Ph. tricornutum* (unknown origin, TBS culture collection) were grown in semicontinuous cultures in f/2 medium (Guillard and Ryther 1962), prefiltered (0.2 μm sterile filters [Minisart, Santorius, Goettingen, Germany] pasteurized at 80°C in 3 h), and enriched with silicate (*Ph. tricornutum* only). All cultures were subsampled from the culture collection of TBS and grown at 15 ± 1°C, 33 ppt salinity seawater, and constantly bubbled with filtered air. The illumination was continuous white fluorescent light (Philips TL 40 W/55 tubes, Guilford, Surrey, UK), providing 80 μmol photons $\cdot m^{-2} \cdot s^{-1}$ as measured by means of a QSL-100 quantum sensor (Biospherical Instruments, San Diego, CA, USA) placed inside the culture flasks. The growth rate and the chl *a* concentration were maintained semiconstant by diluting the cultures once per day corresponding to a specific growth rate of 0.2 $\mu \cdot d^{-1}$ for *Pro. minimum* and *Pry. parvum*, and 0.7–0.8 $\mu \cdot d^{-1}$ for *Ph. tricornutum* both prior to and during the time of the experiments. The cultures were enriched with 1 g $NaHCO_3 \cdot L^{-1}$ to avoid a depletion of inorganic carbon and limiting pH conditions caused by high rates of photosynthesis (Olsen et al. 2006).

While growing, the physiological state of the cultures was monitored daily by measuring the ratio of in vivo chl *a* fluorescence before and after addition of DCMU (3[3,4 dichlorophenyl]-1, 1-dimethylurea, 50 μM final concentration) in a Turner Designs (Sunnyvale, CA, USA) fluorometer. DCMU blocks the electron transport in PSII and results in a maximal fluorescence. The ratio of fluorescence measured before and after the addition of DCMU >2.5 indicates a healthy state of the cell (Sakshaug and Holm-Hansen 1977). In our study, the ratio generally ranged from 2.7 to 3.5.

Experimental conditions. Cultures were subsampled every morning to perform parallel measurements of photosynthesis versus irradiance (*P-E* curves) from O_2-evolution, PAM, and ^{14}C-assimilation measurements. The subsamples were placed in a water bath set at one of the seven experimental temperatures (0, 5, 10, 15, 20, 25, and 30°C), and the experiment started after the respective temperatures had stabilized within the sample (<30 min). Incident irradiance was maintained. Subsequently, the sample was simultaneously introduced to each of the experimental setups.

O_2-evolution and ^{14}C-assimilation rates were measured in parallel after placing samples in a photosynthetron (Lewis and Smith 1983) in the dark and at 10 levels of irradiance from 3 to 570 μmol photons $\cdot m^{-2} \cdot s^{-1}$ (PAR), determined by the QSL-100 quantum sensor (Biospherical Instruments). The photosynthetron was placed in a temperature-controlled laboratory at the respective temperature. The samples were illuminated from below with an adjustable xenon light source (Osram 250 W, München, Germany), while a water-flow-through system prevented radiation heat. The correct temperature was ensured by continuous (1 s frequency) temperature measurements using small waterproof data loggers (TidbiT; Onset Computer Corp., Pocasset, MA, USA) installed in dummy samples.

Triplicate samples were incubated in 20 mL polyethylene scintillation vials for 1 h. Vials for O_2-evolution measurements were filled completely and closed with a lid mounted with a miniature pipe (internal diameter = 0.8 mm, length = 5 mm). The miniature pipe excluded headspace of air, avoided potential pressure accumulation from photosynthetic O_2 production, and allowed for insertion of an O_2 microsensor. Two milliliters of sample was incubated for carbon-assimilation measurements.

O_2-microsensor measurements. All oxygen measurements were carried out using Clark-type O_2 microelectrodes with a guard cathode (Revsbech 1989), having an external tip diameter of ~100 μm, stirring sensitivity of <1.5%, and a 90% response time of <4 s. The electrodes were calibrated using anoxic and air-saturated solutions at the specific temperature setting, as oxygen electrode signals are sensitive to temperature (Gundersen et al. 1998, Glud et al. 2000). The sensor current was measured using a picoammeter (Unisense, Aahus, Denmark) connected to a strip-chart recorder (Kipp & Zonen, Delft, the Netherlands) and a PC (Revsbech and Jørgensen 1986). The gross O_2-production rate (P^C_{O2}) was estimated by adding the dark respiration to the net O_2-evolution rate (both measured at each temperature), determined from the O_2-concentration change corrected for incubation time. All samples were mixed gently with a Pasteur pipette introduced through the miniature pipe prior to measuring, ensuring a homogeneous O_2 concentration within the vial. In several cases, the concentration of O_2 was monitored continuously during incubation by an electrode installed in a randomly selected sample, confirming linear O_2 evolution.

PAM measurements. Fluorescence was measured using a PAM-101 fluorometer with a 102 and 103 module (Walz, Effeltrich, Germany; Schreiber et al. 1986) equipped with a photomultiplier detector (PM-101/N, PMT, Walz, Germany). A red light-emitting diode (655 nm peak, <0.15 μmol photons $\cdot m^{-2} \cdot s^{-1}$, at 1.6 kHz) was used as probe light at an intensity too low to induce variable fluorescence. In the following, we used the nomenclature of van Kooten and Snel (1990). The minimum fluorescence (F_o) and the maximum fluorescence (F_m) was measured at the end of a dark-acclimation period (15 min), when approximately all reaction centers were closed. F_m was measured during a saturating light pulse from a halogen lamp (0.6 s, at >5,000 μmol photons $\cdot m^{-2} \cdot s^{-1}$; KL1500, Schott, Mainz, Germany) exposed to the sample via an optical fiber. The maximum quantum yield of PSII charge separation (Φ_{PSII_max}) in the dark-acclimated cells was calculated as follows:

$$\Phi_{PSII\_max} = F_v/F_m = \frac{F_m - F_0}{F_m} \quad (1)$$

Under actinic illumination, the operational quantum yield of PSII (Φ_{PSII}) was calculated from the steady-state fluorescence (F_s) and the maximum fluorescence after a saturation pulse ($F_m{}'$) at each incubation irradiance (Genty et al. 1989):

$$\Phi_{PSII} = \Delta F/F_m{}' = \frac{F_m{}' - F_s}{F_m{}'} \quad (2)$$

The incubation light was provided by a slide projector (Prodovit; Leica, Wetzlar, Germany) equipped with a halogen lamp and slide frames with different layers of neutral filters. After F_o and F_m were measured, the samples were exposed for 5 min at each of the irradiances (1–500 μmol photons $\cdot m^{-2} \cdot s^{-1}$), before measuring F_s and $F_m{}'$. The incubation irradiance (E, PAR) was measured inside the incubation chamber using a cosine-corrected (2π) light collector of the DIVING-PAM (Walz, Effeltrich, Germany). The spectral distribution of the incubation light was measured using a RAMSES spectroradiometer (TRIOS, Oldenburg, Germany) from 400 to 700 nm [$E(\lambda)$, 1 nm resolution]. The irradiance and the spectral distribution of the incubation light were used for further calculations of the amount of light absorbed by PSII. A Peltier cell (US-T/S Walz) kept the temperature constant (±0.2°C) during incubations.

Biooptics. To calculate O_2 evolution per biomass and time from Φ_{PSII}, the light absorbed by PSII was quantified in absolute units from the in vivo chl *a*–specific absorption coefficient, $a^*_\varphi(\lambda)$, ($m^2 \cdot [\text{chl } a]^{-1}$), and the PSII-scaled in vivo fluorescence excitation spectrum $F^*_{PSII}(\lambda)$ ($m^2 \cdot [\text{chl } a]^{-1}$). The optical density (OD) was measured on glass fiber filters (GF/F; Whatman Inc., Florham Park, NJ, USA), according to Yentsch (1962) and Mitchell and Kiefer (1988), and converted to OD in suspension (Mitchell 1990). Absorption was calculated according to Mitchell and Kiefer (1988) and normalized to chl *a* to give $a^*_\varphi(\lambda)$. In vivo fluorescence excitation spectra were measured according to Neori et al. (1988) and Johnsen and Sakshaug (1993), and quantum corrected using the dye Basic Blue 3 (Kopf and Heinze 1984). $F^*_{PSII}(\lambda)$ was obtained from scaling the fluorescence excitation spectrum to the corresponding $a^*_\varphi(\lambda)$ using the ''no overshoot'' procedure by matching the two spectra at wavelengths between 540 and 650 nm (Bidigare et al. 1989, Johnsen et al. 1997). The light absorption in PSII ($\bar{a}^*_{PSII}$, $m^2 \cdot [\text{chl } a]^{-1}$) was obtained by spectrally weighting $F^*_{PSII}(\lambda)$ against the incubator light source according to the following equation:

$$\bar{a}^*_{PSII} = \frac{\left[\sum_{400}^{700} F^*_{PSII}(\lambda) \cdot E(\lambda) d\lambda\right]}{E(PAR)} \quad (3)$$

where $E(\lambda)$ is the spectral irradiance of the incubator light source, and E(PAR) is the integrated irradiance from 400 to 700 nm. The applied biooptical procedure above is described in detail in Hancke et al. (in press). Definitions of biooptical parameters used are given in Table 1.

TABLE 1. Definitions of the productivity, photosynthetic, and biooptical parameters used in the text. Photosynthetic parameters according to Sakshaug et al. (1997).

P^C_{O2}	Carbon-specific measured O_2 production (net production + dark respiration; μmol $O_2 \cdot [\text{mg POC}]^{-1} \cdot h^{-1}$)
P^C_{PSII}	Carbon-specific O_2 production calculated from Φ_{PSII} and $\bar{a}^*_{PSII}$ in absolute units (μmol $O_2 \cdot [\text{mg POC}]^{-1} \cdot h^{-1}$)
P^C_{14C}	Carbon-specific ^{14}C assimilation (μmol $^{14}C \cdot [\text{mg POC}]^{-1} \cdot h^{-1}$)
α^C	Maximum light utilization coefficient normalized to carbon (μmol O_2 or $^{14}C \cdot [\text{mg POC}]^{-1} \cdot h^{-1} \cdot [\mu\text{mol} \cdot m^{-2} \cdot s^{-1}]^{-1}$)
P^C_{max}	Maximum photosynthetic rate normalized to carbon (μmol $O_2 \cdot [\text{mg POC}]^{-1} \cdot h^{-1}$)
E_k	Light-saturation index (μmol photons $\cdot m^{-2} \cdot s^{-1}$)
Φ_{PSII}	Operational quantum yield for PSII charge separation (eq. 2, mol electrons $\cdot$ [mol quanta]$^{-1}$)
$\Phi_{O2,\ PSII,\ 14C\_max}$	Maximum quantum yield for O_2, PSII, or ^{14}C, respectively (mol product $\cdot$ [mol quanta]$^{-1}$)
$^{PSII}\phi_{O2\_max}$	Maximum quantum yield for O_2 calculated from $\bar{a}^*_{PSII}$ (eq. 9, mol $O_2 \cdot$ [mol quanta]$^{-1}$)
$\bar{a}^*$	Spectrally weighted in vivo chl *a*–specific absorption ($m^2 \cdot [\text{mg chl } a]^{-1}$)
$\bar{a}^*_{PSII}$	Spectrally weighted in vivo PSII-specific absorption ($m^2 \cdot [\text{mg chl } a]^{-1}$)

POC, particulate organic carbon.

Calculation of O_2 evolution from PAM measurements in combination with biooptics. Electron transport rate is equal to the product of Φ_{PSII} and the amount of quanta absorbed by PSII ($\bar{a}^*_{PSII}$). By knowing the stoichiometric ratio of oxygen evolved per electron generated in PSII, the rate of O_2 evolution ($P^C{}_{PSII}$) can be quantified (Kroon et al. 1993). Instead of calculating ETR, we directly calculated the O_2-production rate in absolute units ($P^C{}_{PSII}$, μmol $O_2 \cdot$ [mg POC]$^{-1} \cdot h^{-1}$), from equation 4. (See Hancke et al. in press for a discussion on different approaches for quantifying the amount of quanta absorbed by PSII.)

$$P_{PSII} = \Phi_{PSII} \cdot E \cdot \Gamma \cdot \bar{a}^*_{PSII} \quad (4)$$

where Γ is the stoichiometric ratio of oxygen evolved per electron generated at PSII. According to the standard Z-scheme of photosynthesis, four stable charge separations take place in both PSI and PSII, to evolve one O_2 molecule (i.e., eight electrons to yield one molecule of oxygen). According to this assumption, Γ will be 0.25 O_2 electrons^{-1} (Kroon et al. 1993, Gilbert et al. 2000). Empirically, a higher number than eight electrons has been found, which may be due to alternative electron ''loss'' (e.g., Mehler-type reactions; Kromkamp et al. 2001, Longstaff et al. 2002, Hancke et al. in press). For simplicity, we assumed Γ to be 0.25 in this study.

Most papers that use PAM-estimated Φ_{PSII} to calculate O_2-evolution rates assume that absorbed irradiance is distributed between PSII and PSI, with a ratio of 0.5 (Gilbert et al. 2000). This is a rough estimate, and the ratio is higher for most phytoplankton classes, with the consequence of underestimating the O_2 evolution from PSII (Johnsen and Sakshaug 2007). In this study, we have applied a biooptical procedure to measure the PSII-specific absorption directly.

Photosynthetic O_2-production rates obtained in the photosynthetron and in the PAM cuvette were compared in a pilot study by measuring *P-E* curves of O_2 evolution in both experimental setups. An O_2 microsensor was inserted directly in the PAM cuvette (Hancke et al. in press), and measured rates were compared with the O_2-production rates measured in the photosynthetron. The *P-E* curves calculated from the two experimental setups showed equivalent shapes and similar rates and had an average difference and a standard deviation for $P^C{}_{max}$ and α^C of 2.2 ± 21.3% and 22.7 ± 23.8%, respectively. Simultaneous measurements of Φ_{PSII} verified reproducible photosynthetic responses between the pilot study and this study.

^{14}C assimilation. Carbon-assimilation rate ($P^C{}_{14C}$) was calculated from equation 5 (Geider and Osborne 1992):

$$P^C{}_{14C} = f\left(\frac{dpm_{org}}{dpm_{tot}}\right) \cdot [TCO_2]\left(\frac{1}{dt}\right) \quad (5)$$

where *f* is the isotope discrimination factor, assumed to be 1.06; dpm_{org} is the ^{14}C activity in organic matter (disintegrations per minute); dpm_{tot} is the total ^{14}C activity added to the sample; [TCO_2] is the total inorganic carbon concentration; and d*t* is the incubation time.

After incubation, the samples were acidified with HCl to pH between 1.5 and 2 and left overnight in a fume hood without caps to remove all inorganic C (Geider and Osborne 1992). Samples were back-titrated with NaOH to pH ~8 before scintillation cocktail (Ultima Gold; Perkin-Elmer, Waltham, MA, USA) was added, and the activity was measured in a scintillation counter (Packard Tri-Carb 1900; GMI, Ramsey, MN, USA). [TCO_2] was estimated from measured pH and total alkalinity (AT). AT was calculated after titration with HCl (Wedborg et al. 1999) and total inorganic carbon from Andersson et al. (1999). The dark-incubated uptake was generally <20% (<10% at temperature >15°C) of the light-incubated uptake and was subtracted in the rate calculations. We observed no temperature influence on the dark-incubated ^{14}C uptake.

Curve fit regression and calculations of Q_{10}. The *P-E* curves were fitted from equation 6 (Webb et al. 1974), as no tendency of reduction of *P* at irradiance >E_k (photoinhibition) was observed for the applied range of irradiance (0–566 μmol photons $\cdot m^{-2} \cdot s^{-1}$).

$$P^C = P^C{}_{max}\left(1 - \exp\left\{\frac{-\alpha^C \cdot E}{P^C{}_{max}}\right\}\right) \quad (6)$$

The maximum photosynthetic rate ($P^C{}_{max}$; μmol O_2 or $^{14}C \cdot$ [mg POC]$^{-1} \cdot h^{-1}$), the maximum light utilization coefficient (α^C; μmol O_2 or $^{14}C \cdot$ [mg POC]$^{-1} \cdot h^{-1} \cdot$ [μmol photons $\cdot m^{-2} \cdot s^{-1}$]$^{-1}$), and the light saturation index ($E_k = P^C{}_{max}/\alpha^C$; μmol photons $\cdot m^{-1} \cdot s^{-1}$) were calculated from fit of the *P-E* curves. All curve fitting was carried out using ordinary least-squares criterion in SigmaPlot 9.0 (SYSTAT Software Inc., San Jose, CA, USA).

For α^C or $P^C{}_{max}$ (response variables), the relationship with temperature and the covariance with method was analyzed using the statistical tool analysis of covariance (ANCOVA), with method as the test factor. Calculations were computed using S-Plus 6.2 (Insightful Corporation, Seattle, WA, USA).

The temperature response of $P^C{}_{max}$ was quantified by calculating the apparent activation energy (E_a, kJ $\cdot mol^{-1}$) and the corresponding Q_{10} from each method and species. E_a was calculated as the slope of the data between 5°C and 20°C in an Arrhenius plot (eq. 7), where ln(*k*) was plotted as a function of temperature $(R \cdot T)^{-1}$, according to Raven and Geider (1988) as follows:

$$\ln(k) = \ln(A) + \left(\frac{-E_a}{R \cdot T}\right) \quad (7)$$

where *k* is the rate of the reaction, *A* is the Arrhenius constant, *R* is the gas constant (8.3144 $J^{-1} \cdot mol^{-1}$), and *T* is the absolute temperature (K). Q_{10} was calculated from equation 8, for the temperature interval of 10°C to 20°C (Isaksen and Jørgensen 1996).

$$Q_{10} = \exp\left\{\frac{E_a \cdot 10}{R \cdot T(T+10)}\right\} \quad (8)$$

The maximum quantum yield for O_2 production ($^{PSII}\Phi_{O2\_max}$; mol $O_2 \cdot$[mol quanta]$^{-1}$) was calculated from the PSII-specific light absorption ($\bar{a}^*_{PSII}$) and was calculated for each temperature as follows:

$$^{PSII}\Phi_{O2\_max} = \frac{\alpha^*}{115 \cdot \bar{a}^*_{PSII}} \quad (9)$$

where 115 is a constant required to obtain consistent dimensions.

RESULTS

P-E *data.* *P-E* curves were fitted to POC normalized production rates derived from O_2-microsensor measurements ($P^C{}_{O2}$, μmol $O_2 \cdot$ [mg POC]$^{-1} \cdot h^{-1}$), quantum yield of charge separation in PSII (Φ_{PSII}) by PAM fluorescence ($P^C{}_{PSII}$, μmol $O_2 \cdot$ [mg POC]$^{-1} \cdot h^{-1}$), and ^{14}C assimilation ($P^C{}_{14C}$, μmol $^{14}C \cdot$ [mg POC]$^{-1} \cdot h^{-1}$) at temperatures from 0°C to 30°C, at 5°C intervals. *P-E* curves at 5°C and 20°C are shown for *Pro. minimum, Pry. parvum,* and *Ph. tricornutum* (Fig. 1). O_2-microsensor and ^{14}C-assimilation rates were measured in triplicate, and

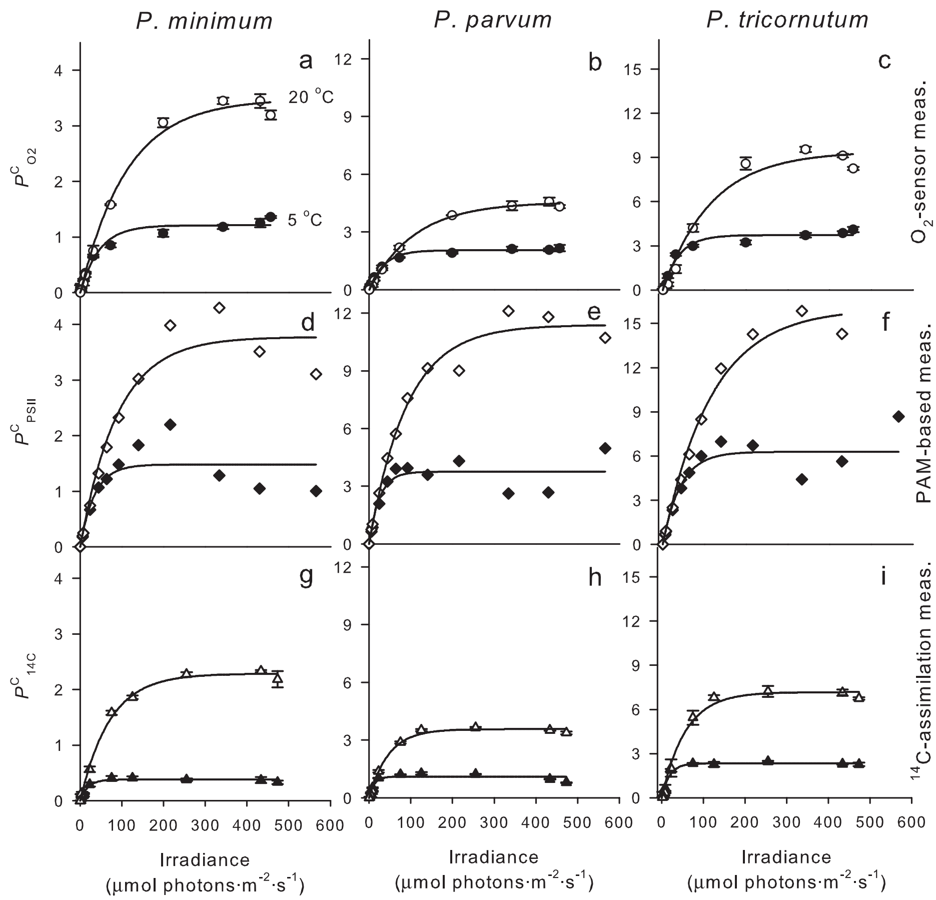

FIG. 1. Photosynthesis versus irradiance (*P-E*) curves measured by (a–c) O_2 microsensors (P^C_{O2}); (d–f) calculated from Φ_{PSII} (based on PAM measurements) in combination with biooptical measurements (P^C_{PSII}); and (g–i) measured ^{14}C assimilation (P^C_{14C}) at 5°C (filled symbols) and 20°C (open symbols), respectively. The study was conducted on three unialgal cultures of *Prorocentrum minimum* (left column), *Prymnesium parvum* (middle column), and *Phaeodactylum tricornutum* (right column). Units for P^C_{O2} and P^C_{PSII} are in µmol O_2 · (mg POC)$^{-1}$ · h^{-1}, and for P^C_{14C} in µmol ^{14}C · (mg POC)$^{-1}$ · h^{-1}. POC, particulate organic matter.

error bars are shown (Fig. 1, a–c, g–i). Evident for all three species and three methods, the maximum production rates were clearly higher (2.2–6.0 times) at 20°C than at 5°C. We observed no sign of photoinhibition for the applied irradiance range (0–566 µmol photons · m^{-2} · s^{-1}). The relationship between temperature and the photosynthetic parameters, calculated from O_2 evolution, Φ_{PSII}, and ^{14}C assimilation, was first investigated for relative values (excluding the significance of the light absorption) normalized at 5°C, being the lowest temperature with minimal scatter (Fig. 2), and then for absolute values (calculated by the use of $\bar{a}^*_{PSII}$, Fig. 3).

Temperature effects on relative P-E *parameters.* The relative response of the maximum photosynthetic rate (P^C_{max}) increased 2.5–6.0 times relative to the rate at 5°C, with increasing temperature, for all of the three investigated algal species and varied overall little between species and method (Fig. 2, a–c). P^C_{max} showed a temperature optimum at 20°C–25°C for *Pro. minimum*, followed by a decrease (Fig. 2a), whereas no clear sign of a temperature optimum was observed for *Pry. parvum* or *Ph. tricornutum* within the investigated temperature range (Fig. 2, b and c). The relative values for $P^C_{14C\_max}$ increased more with temperature than $P^C_{O2\_max}$, indicating a slightly stronger temperature response for ^{14}C assimilation than for O_2 production, most apparent for *Pro. minimum.* The relative response of $P^C_{PSII\_max}$ with increasing temperature fell in between $P^C_{14C\_max}$ and $P^C_{O2\_max}$ for *Pry. parvum* and showed slightly lower temperature responses for *Pro. minimum* and *Ph. tricornutum.*

The temperature response on P^C_{max} was quantified by the Q_{10} factor (Table 2) calculated from Arrhenius plots (not shown). The average Q_{10} was 2.1 ± 0.2 (mean ± SE), and Q_{10} showed only small variance between methods and species, with an exception of $P^C_{14C\_max}$ for *Pro. minimum.* Apparently, Q_{10} values for $P^C_{14C\_max}$ were higher than for $P^C_{O2\_max}$ and $P^C_{PSII\_max}$, supporting the observation of a stronger temperature response for C assimilation than for the two other methods.

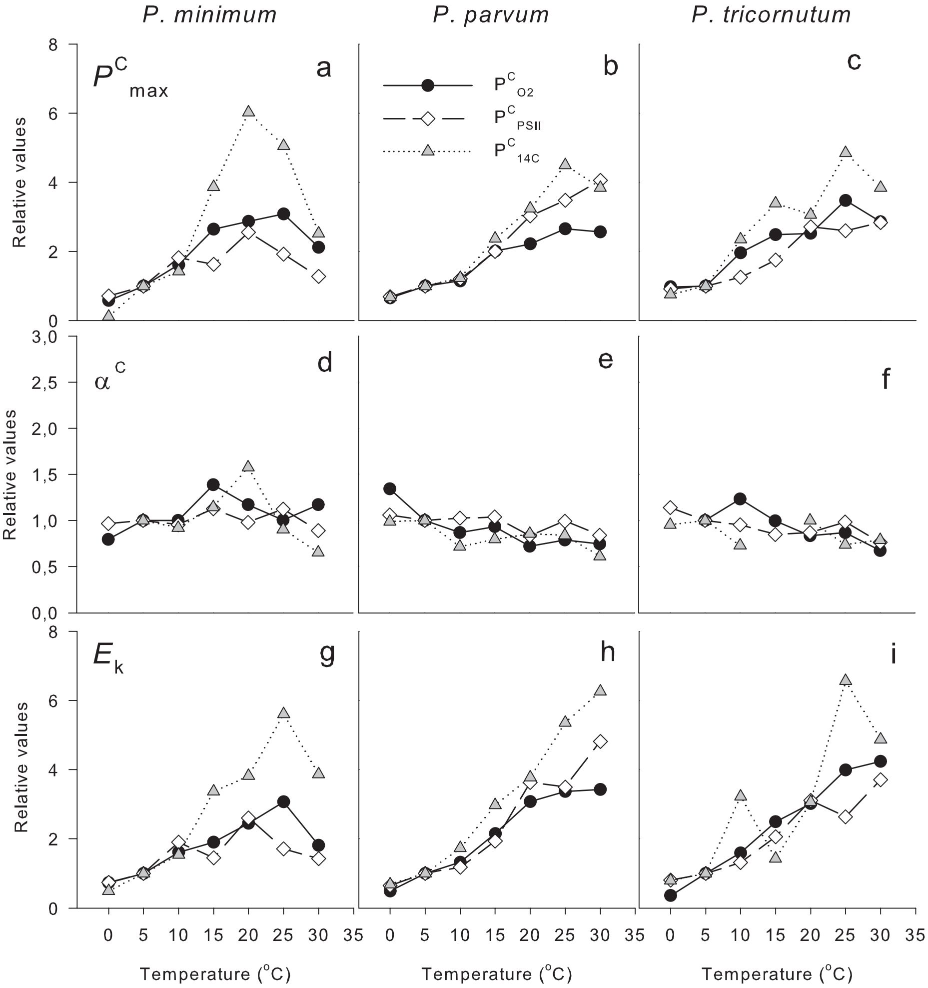

FIG. 2. Relative effect of temperature on the maximum photosynthetic rate ($P^C{}_{max}$, upper panel), the maximum light utilization coefficient (α^C, middle panel), and the light-saturation index (E_k, lower panel) for *Prorocentrum minimum* (left), *Prymnesium parvum* (middle), and *Phaeodactylum tricornutum* (right). The photosynthetic parameters were calculated from rates of measured O_2 production ($P^C{}_{O2}$, filled circles), Φ_{PSII} ($P^C{}_{PSII}$, open diamonds), and ^{14}C assimilation ($P^C{}_{14C}$, grey triangles). All parameters were normalized to 1.0 at 5°C. All cultures were grown at 15°C and 80 µmol photons · m^{-2} · s^{-1}.

Temperature had no or only little effect on relative values of α^C, showing similar temperature responses for each of the three species and an average Q_{10} of 1.0 ± 0.2 (mean ± SE). Q_{10} values of 0.9 for *Pry. parvum* and *Ph. tricornutum* indicated a slight decrease of α^C for this species. No difference was observed among the three methods as a function of temperature for any of the species, arguing for an equivalent temperature response on photosynthetic O_2 production, Φ_{PSII}, and ^{14}C assimilation in the light-limited part of the photosynthesis versus irradiance curve.

Relative values of E_k showed a strong temperature response (Fig. 2, g–i) and increased 2.6–6.5 times (relative to the rate at 5°C). As α^C generally was insensitive to temperature, the temperature response of E_k mirrored $P^C{}_{max}$. Similarly, as α^C did not differ between methods, the temperature response of E_k tended to be stronger for ^{14}C assimilation than for O_2- and Φ_{PSII}-based production rates.

Temperature effects on absolute values of P-E *parameters.* Increased temperature significantly increased the absolute values of $P^C{}_{max}$ for the three investigated species (Fig. 3, a–c), in accordance with the relative response, but varied more between species and in some cases between methods. The absolute values of $P^C{}_{max}$ supported the observation of a temperature optimum for *Pro. minimum* at 20°C–25°C and no temperature optimum for *Pry. parvum* and *Ph. tricornutum* within the investigated temperature range. The absolute values of $P^C{}_{max}$ were overall lowest for *Pro. minimum* (Fig. 3a) and highest for *Ph. tricornutum* (Fig. 3c). $P^C{}_{max}$ for the latter decreased slightly at 30°C, giving a weak indication of a temperature optimum at 25°C for $P^C{}_{O2\_max}$ and $P^C{}_{14C\_max}$. As $P^C{}_{max}$ values are carbon specific, the rates do correlate directly to maximum growth rates and reflect the

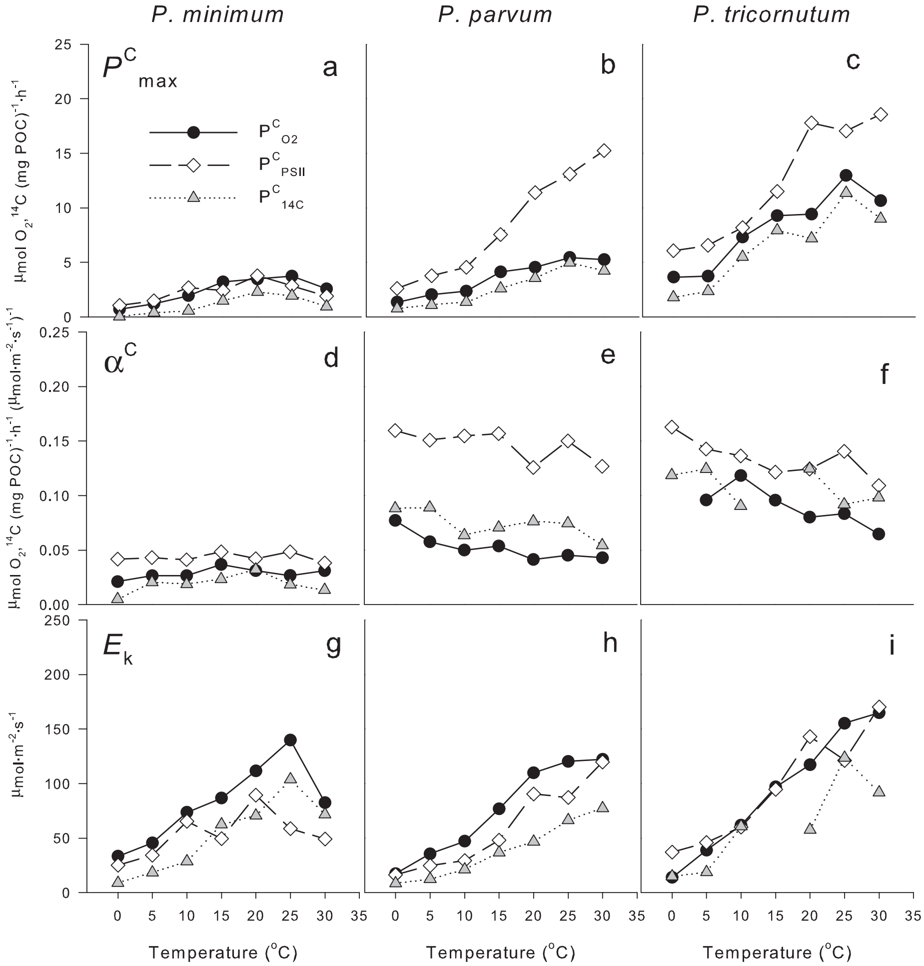

FIG. 3. Effect of temperature on the absolute values for the maximum photosynthetic rate (P^C_{max}, upper panel), the maximum light utilization coefficient (α^C, middle), and the light-saturation index (E_k, lower panel) for *Prorocentrum minimum* (left), *Prymnesium parvum* (middle), and *Phaeodactylum tricornutum* (right). Calculation of photosynthetic parameters and growth conditions as in Figure 2.

TABLE 2. The temperature effect expressed as Q_{10} for the maximum photosynthetic rate of P^C_{O2}, P^C_{PSII}, and P^C_{14C} for *Prorocentrum minimum*, *Prymnesium parvum*, and *Phaeodactylum tricornutum*, respectively.

	Pro. minimum	*Pry. parvum*	*Ph. tricornutum*
$P^C_{O2\_max}$	2.1	1.8	1.8
$P^C_{PSII\_max}$	1.7	2.1	1.9
$P^C_{14C\_max}$	3.5	2.3	2.1

Q_{10} was calculated from the slope of P^C_{max} as a function of temperature, from 5°C to 20°C, in an Arrhenius plot. The maximum photosynthetic rates of P^C_{O2}, P^C_{PSII}, and P^C_{14C} were calculated from measured rates of O_2 production, Φ_{PSII}, and ^{14}C assimilation, respectively.

productivity of the studied species (MacIntyre et al. 2002).

Between methods, the absolute values showed some interspecies variation of P^C_{max} as a function of temperature. The method used had a significant effect on P^C_{max} for all three species ($P < 0.05$); however, the interaction between temperature and method (temperature × method) was significant for *Pry. parvum* only, as $P^C_{PSII\_max}$ showed 1.8–2.9 times higher absolute values than for the two other methods as a function of temperature ($P << 0.05$, Fig. 3b). The response of $P^C_{O2\_max}$ and $P^C_{14C\_max}$ was not significantly different. The temperature × method interaction was nonsignificant for *Pro. minimum* ($P = 0.43$, Fig. 3a) and for *Ph. tricornutum* ($P = 0.07$, Fig. 3c), emphasizing that there was no difference of P^C_{max} among the three methodological approaches. Despite the statistical insignificance, $P^C_{PSII\_max}$ for *Ph. tricornutum* (seemed to) show slightly higher absolute values than $P^C_{O2\_max}$ and $P^C_{14C\_max}$ (*P*-values are shown in Table 3).

The temperature effect on absolute values of α^C was insignificant (*Pro. minimum*, Fig. 3d) or slightly decreasing with increasing temperature (*Pry. parvum* and *Ph. tricornutum*, Fig. 3, e–f). The slight decrease of α^C was observed as α^C_{O2} (*Pry. parvum*), and α^C_{O2} and α^C_{PSII} (*Ph. tricornutum*) decreased marginally. The additional values of α^C did not change with increasing temperature (*P*-values are shown in

TABLE 3. *P*-values of statistical tested variance and covariance (ANCOVA) for the significance of temperature, method, and the interaction between temperature and method (temperature × method).

	Prorocentrum minimum (0–20°C)	*Pro. minimum* (0–30°C)	*Prymnesium parvum* (0–30°C)	*Phaeodactylum tricornutum* (0–30°C)
P^C_{max}				
Temperature	*** ($P < 0.001$)	–	*** ($P < 0.001$)	*** ($P < 0.001$)
Method	*** ($P < 0.001$)	–	*** ($P < 0.001$)	*** ($P < 0.001$)
Temperature × method	N.S. ($P = 0.43$)	–	*** ($P < 0.001$)	NS ($P = 0.07$)
α^C				
Temperature	–	NS ($P = 0.23$)	*** ($P < 0.001$)	*** ($P = 0.001$)
Method	–	*** ($P < 0.001$)	*** ($P < 0.001$)	*** ($P < 0.001$)
Temperature × method	–	NS ($P = 0.71$)	NS ($P = 0.96$)	NS ($P = 0.50$)

A significance of temperature × method indicates that the relationship between the response variable (α^C or P^C_{max}) and temperature depended on the method used.
***A significant effect ($P < 0.05$).
NS, nonsignificant ($P > 0.05$).

Table 3). The temperature × method interaction was not significant for all of the species, demonstrating no difference between the slopes for the three methods applied. Consequently, the temperature response on the three methods was the same. The method, however, had a significant effect on α^C, resulting in significantly higher absolute values of α^C_{PSII} compared with α^C_{O2} and α^C_{14C} for all three species. This offset was especially clear for *Pry. parvum*, as α^C_{PSII} was 1.7–3.3 times higher than α^C_{O2} and α^C_{14C} (Fig. 3e). The two latter values were not significantly different. For *Ph. tricornutum*, α^C_{PSII} was 1.1–1.7 times higher than values for α^C_{O2} and α^C_{14C} (Fig. 3f). Two outliers of α for *Ph. tricornutum* (α^C_{O2} at 0°C, and α^C_{14C} at 15°C) have been eliminated from the data set due to unrealistic values caused by high scatter at low irradiances.

As α^C was constant or slightly decreasing with increasing temperature, the light saturation index (E_k) vaguely increased or mirrored the P^C_{max} temperature response (Fig. 3, g–i). E_k for *Pro. minimum* increased linearly to a temperature optimum at 20°C–25°C followed by a subsequent decrease. For *Pry. parvum* and *Ph. tricornutum*, E_k increased continuously with increasing temperature for all three methods. The relatively higher values of α^C_{PSII} and $P^C_{PSII\_max}$ compared with the two other methods, for *Pry. parvum* and *Ph. tricornutum*, counteracted each other, resulting in very similar values of E_k for the three methods as a function of temperature.

Temperature effects on the maximum quantum yield. The temperature effects on the maximum quantum yield (Φ_{max}) seemed to be negligible (*Pro. minimum*) or lead to a minor decrease with increasing temperature (*Pry. parvum* and *Ph. tricornutum*; Fig. 4). Φ_{PSII_max} values were in the range of 0.6–0.75 and were the lowest for *Pro. minimum.* $^{PSII}\Phi_{O2\_max}$ was the lowest for *Pry. parvum* (0.06–0.13), but within the same range for *Pro. minimum* and *Ph. tricornutum* (0.08–0.15), respectively. The lower $^{PSII}\Phi_{O2\_max}$ lead to a higher minimum quantum requirement (QR, the inverse of the maximum quantum yield; $1/\Phi_{max}$) for *Pry. parvum* than for the two other species: 0.8–2.7 times higher than for *Pro. minimum* (1.9 ± 0.7 times, mean ± SD) and 1.7–3.1 times higher than for *Ph. tricornutum* (2.2 ± 0.5

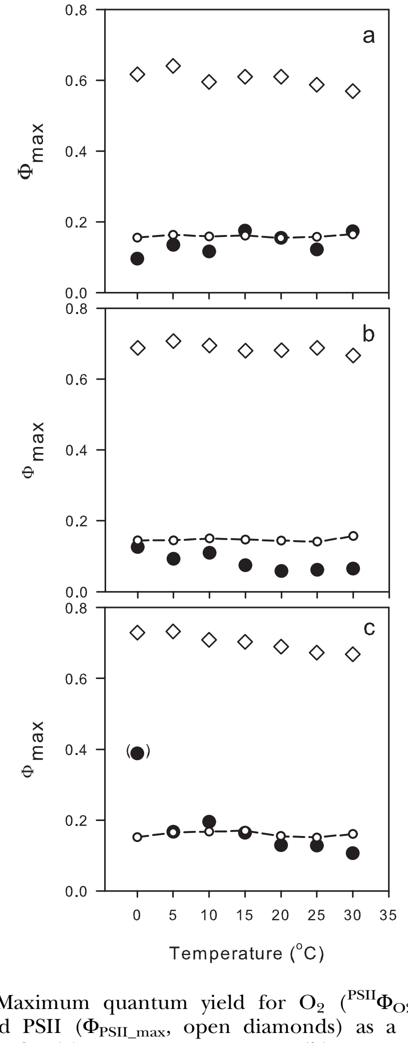

FIG. 4. Maximum quantum yield for O_2 ($^{PSII}\Phi_{O2\_max}$, filled circles) and PSII (Φ_{PSII_max}, open diamonds) as a function of temperature for (a) *Prorocentrum minimum*, (b) *Prymnesium parvum*, and (c) *Phaeodactylum tricornutum*. $^{PSII}\Phi_{O2\_max}$ was calculated based on the light absorption in PSII ($\bar{a}^*_{PSII}$) as was the theoretical maximum quantum yield for O_2 production (small open circles), which was calculated for each temperature (eq. 10, details in text).

times, mean ± SD). The QR for *Pro. minimum* and *Ph. tricornutum* was similar.

The calculated $^{PSII}\Phi_{14C\_max}$ was lower than $^{PSII}\Phi_{O2\_max}$ for *Pro. minimum* but slightly higher for the two other species, in contradiction to established theory. We have no obvious explanation for this finding other than it is likely that α^C_{14C} was overestimated because of few measuring points and high scatter within the light-limited part of the *P-E* curve, which would lead to an overestimation of Φ_{14C_max}. Data for Φ_{14C_max} are not shown.

DISCUSSION

The relationship between *P-E* parameters calculated from rates of O_2 production, Φ_{PSII}, and ^{14}C assimilation was investigated as a function of short-term changes in temperature. The results demonstrated that P^C_{max} increased and α^C was more or less insensitive to increasing temperature for all three species investigated, as is typical for most eukaryote algae (Davison 1991). Generally, this observation is not surprising as α^C represents light-limited photosynthesis and, as such, is primarily a function of photochemical light reactions (not enzyme dependent), and P^C_{max} describes the light-saturated processes of photosynthesis and appears to be limited by enzyme activity associated with the carbon metabolism of the dark reactions (assuming excess nutrients; Davison 1991, Sakshaug et al. 1997).

Temperature effects on P^C_{max}. The relative values for $P^C_{14C\_max}$ tended to increase more with temperature than $P^C_{O2\_max}$, indicating a slightly stronger temperature response for ^{14}C assimilation than for O_2 production, most apparent for *Pro. minimum* (Fig. 2). This observation was supported by the Q_{10} values (Table 2). Theoretically, this finding was expected since P^C_{14C} expresses gross carbon-uptake rates excluding respiratory activity (Sakshaug et al. 1997), whereas P^C_{O2} probably underestimated the gross O_2-production rate due to an enhanced O_2 consumption in the light compared with the dark, which P^C_{O2} did not account for. Enhanced O_2 consumption in the light is well documented for marine microalgae, as both intercellular (photorespiration and mitochondrial activity) and extracellular (e.g., bacterial metabolism) O_2 consumption is stimulated by photosynthesis (Weger et al. 1989, Beardall et al. 1994, Lewitus and Kana 1995, Xue et al. 1996). On average, for several algae classes, true gross O_2 production (i.e., measured by the dual isotope technique) has been observed to yield 20%–30% higher rates compared with rates obtained by adding the dark respiration to the net O_2-production rate (Weger et al. 1989, Lewitus and Kana 1995). All the above processes are stimulated by temperature, and, hence, the discrepancy between the dark and the light O_2-consumption rate will increase with increasing temperature (Davison 1991, Morris and Kromkamp 2003). This trend explains the relatively stronger temperature response for $P^C_{14C\_max}$ than for $P^C_{O2\_max}$, which will be further enhanced if the temperature response (Q_{10}) on the O_2-consumption processes exceeds the response of photosynthesis, as found for benthic microphytes (Hancke and Glud 2004).

The potential for photorespiration increases with increasing temperature, as the affinity of RUBISCO for O_2 is reduced relative to the affinity for CO_2 with increased temperature (Berry and Raison 1981). However, the importance of photorespiration in microalgae might be suppressed by the occurrence of a CO_2-concentrating mechanism (Lewitus and Kana 1995).

Although the maximum photosynthetic rate is related only to the number of photosynthetic units (n) and the minimum turnover time for electrons (τ), $P_{max} = n \cdot \tau^{-1}$ (Dubinsky et al. 1986), the rate-limiting step of the photosynthetic pathway has been widely debated (Sakshaug et al. 1997). The relative temperature response of $P^C_{PSII\_max}$ followed the temperature response of the two other techniques. This observation demonstrated that Φ_{PSII} from intact algae cells responded similarly to the rate of O_2 evolution and ^{14}C assimilation, to a short-term temperature change. This is consistent with the hypothesis that the overall rate-limiting reaction for light-saturated photosynthesis is carbon fixation rather than electron transport, as suggested by Sukenik et al. (1987). For our data, this finding implies that Φ_{PSII} as well as O_2 production must be limited by carbon-fixing enzymes (i.e., the RUBISCO complex), and stresses that Φ_{PSII} and O_2-production rates were not separated from the ^{14}C-fixation rate, as a function of short-term temperature changes. These data are consistent with the observation of a linear relationship between P^B (chl *a* normalized rates of P^C_{O2}) and ETR as function of temperature, for temperatures between 10°C and 30°C (Morris and Kromkamp 2003). However, their data deviated from linearity at the extremes of the investigated temperature range (5°C and 35°C).

For absolute values of the maximum photosynthetic rate, the relationship between rates of O_2 production and ^{14}C assimilation is known as the photosynthetic quotient, PQ (Laws 1991). Calculating PQ as the ratio between $P^C_{O2\_max}$ and $P^C_{14C\_max}$ resulted in values between 1.2 and 3.6 (average for all data = 1.8 ± 0.7), which is consistent with a general PQ of ~1.4 (Laws 1991, Sakshaug et al. 1997). As mentioned above, $P^C_{O2\_max}$ might be an underestimate of the gross O_2-production rate. However, $P^C_{14C\_max}$ may underestimate the gross carbon uptake, as 15 min incubations have been shown to result in higher carbon-uptake rates than 60 min incubations, which are used in this study (Lewis and Smith 1983, MacIntyre et al. 2002). PQ tended to decrease with increasing temperature for the three species investigated, with a slope coefficient of −0.03 to −0.05 (~ Q_{10} of 0.81–0.90), and was thus shown to be temperature sensitive. This finding could be explained by a more pronounced increase in $P^C_{14C\_max}$ compared with $P^C_{O2\_max}$ as seen from the Q_{10} (Table 2). An alternative explanation to a light-enhanced O_2 consumption decreasing PQ with increasing temperature is a potential increase in electron cost for nutrient uptake (Laws 1991).

In this study, we quantified the PSII electron flow and calculated the absolute rate of O_2 production in PSII (μmol $O_2 \cdot$ [mg POC]$^{-1} \cdot h^{-1}$) by combining Φ_{PSII} (from PAM measurements) with the biooptically determined quanta absorbed in PSII, $\bar{a}^*_{PSII}$ (eq. 4; Genty et al. 1989, Johnsen and Sakshaug 2007, Hancke et al. in press). The aim was to compare absolute rates of calculated O_2 production from PSII with measured rates of O_2 production and ^{14}C assimilation, where most studies relate only to relative rates of PSII efficiency (e.g., relative ETR) due to the challenge of measuring the light absorption in PSII. The results demonstrated a species-specific correlation among the three methods, with P^C_{PSII} showing higher absolute values of P^C_{max} and α^C than those determined from measured O_2 production (P^C_{O2}) and ^{14}C assimilation (P^C_{14C}) in most cases (Fig. 3).

The absolute values of P^C_{PSII} showing a species-specific offset compared with P^C_{O2} and P^C_{14C}, might originate in assuming that $\Gamma = 0.25$ (eq. 4). Assuming that Φ_{PSII} is accurately measured by the PAM technique, which is reasonable (Genty et al. 1989), the divergence between measured O_2 production and calculated O_2 production (from PSII fluorescence) can only be caused by two parameters: the absorption properties ($\bar{a}^*_{PSII}$) or the amount of O_2 evolved per electron generated in PSII (Γ). As we believe that $\bar{a}^*_{PSII}$ is a good measure of the PSII absorption (Johnsen and Sakshaug 2007, Hancke et al. in press), we suggest that the electrons needed per O_2 evolved are the major source for the difference between measured and calculated rates of O_2 production. [See Johnsen and Sakshaug (2007) for a discussion on the absorption by nonphotosynthetic versus photosynthetic efficient pigments and the relation to PSII and light-harvesting complexes.]

The calculated $^{PSII}\Phi_{O2\_max}$ for *Pry. parvum* was in the range of 0.06–0.13 (Fig. 4), corresponding to a QR of 8.0–17.3 mol photons $\cdot$ (mol O_2 produced)$^{-1}$. This rate is 1.1–2.5 times higher than the theoretical minimum (see below) and was on average 1.9 and 2.2 times higher than the QR for *Pro. minimum* and *Ph. tricornutum*, respectively. For the two latter species, the QR was in the range of 5.7–10.4 and 5.1–9.4, respectively. As Φ_{PSII_max} did not differ markedly between the three species, the higher $^{PSII}\Phi_{O2\_max}$ for *Pry. parvum* (of 1.1–2.5 times) is likely the explanation for the offset of P^C_{PSII} compared with P^C_{O2} and P^C_{14C} for this species. The offset was apparently temperature insensitive, which is consistent with the above explanation and is further supported by the equivalent Q_{10} values of the three methods.

The theoretical maximum quantum yield for O_2 when calculated from total absorption ($\bar{a}^*$, not the PSII-specific absorption) is 0.125 O_2 electron^{-1} (equivalent to a QR = 8 electrons O_2^{-1}). To correct

TABLE 4. Measured $\bar{a}^*$ and $\bar{a}^*_{PSII}$ for each subsample incubated in the pulse-amplitude-modulated (PAM) fluorometry setup (halogen light source) and O_2-production/^{14}C-assimilation setup (xenon light source) for each experimental temperature, for *Prorocentrum minimum, Prymnesium parvum,* and *Phaeodactylum tricornutum.*

	Temp (°C)	PAM incub. setup (halogen lamp)			O_2, ^{14}C incub. setup (xenon lamp)		
		$\bar{a}^*$	$\bar{a}^*_{PSII}$	$\bar{a}^*/\bar{a}^*_{PSII}$	$\bar{a}^*$	$\bar{a}^*_{PSII}$	$\bar{a}^*/\bar{a}^*_{PSII}$
Pro. minimum	0	0.0075	0.0058	1.29	0.0067	0.0054	1.24
	5	0.0065	0.0047	1.38	0.0058	0.0044	1.32
	10	0.0071	0.0053	1.34	0.0063	0.0050	1.26
	15	0.0073	0.0056	1.30	0.0065	0.0050	1.30
	20	0.0068	0.0055	1.24	0.0060	0.0049	1.22
	25	0.0074	0.0057	1.30	0.0066	0.0052	1.27
	30	0.0062	0.0049	1.27	0.0057	0.0043	1.33
Pry. parvum	0	0.0087	0.0074	1.18	0.0078	0.0068	1.15
	5	0.0085	0.0073	1.16	0.0077	0.0066	1.17
	10	0.0093	0.0076	1.22	0.0083	0.0070	1.19
	15	0.0092	0.0077	1.19	0.0083	0.0070	1.19
	20	0.0093	0.0080	1.16	0.0083	0.0072	1.15
	25	0.0098	0.0087	1.13	0.0088	0.0078	1.13
	30	0.0131	0.0109	1.20	0.0121	0.0096	1.26
Ph. tricornutum	0	0.0075	0.0062	1.21	0.0070	0.0057	1.23
	5	0.0075	0.0059	1.27	0.0072	0.0054	1.33
	10	0.0073	0.0057	1.28	0.0071	0.0053	1.34
	15	0.0074	0.0057	1.30	0.0072	0.0053	1.36
	20	0.0094	0.0076	1.24	0.0088	0.0071	1.24
	25	0.0099	0.0083	1.19	0.0092	0.0076	1.21
	30	0.0101	0.0078	1.29	0.0094	0.0073	1.29

All cultures were grown at 80 μmol photons $\cdot$ m$^{-2} \cdot$ s^{-1} at 15°C.

for light absorption by PSI and photoprotective pigments, we based the quantum yield calculation on the light absorption in PSII ($\bar{a}^*_{PSII}$) only. Consequently, the theoretical maximum quantum yield must be between 0.125 and 0.25, and we propose that it can be calculated from equation 10 as follows:

$$\text{theoretical}^{PSII}\Phi_{O2\_max} = 0.125 \cdot \left(\frac{\bar{a}^*}{\bar{a}^*_{PSII}}\right) \quad (10)$$

Applying this equation to our data gave theoretical maximum quantum yields for O_2 in the range of 0.155–0.165, 0.141–0.157, and 0.151–0.170 mol $O_2 \cdot$ (mol photons)$^{-1}$ for *Pro. minimum*, *Pry. parvum*, and *Ph. tricornutum*, respectively (Fig. 4, small open circles). The theoretical maximum quantum yield for O_2 was temperature insensitive, as $\bar{a}^*_{PSII}$ (Table 4). The average of the corresponding theoretical minimum QR was then 6.3 ± 0.2, 6.8 ± 0.2, and 6.3 ± 0.3 for the three species, respectively.

Values for the QR for O_2 production well higher than the theoretical minimum have commonly been published (Myers 1980, Gilbert et al. 2000). For freshwater phytoplankton, Gilbert et al. (2000) determined that absolute ETRs obtained from PSII fluorescence tend to overestimate primary production rates of ^{14}C fixation. They ascribe the discrepancy to the package effect of pigments in phytoplankton cells and to a noncarbon-related electron flow (e.g., nitrogen fixation), photorespiration, and the Mehler reaction. They assumed a PSII:PSI ratio of 0.5 but corrected the absorbance spectra for nonphotosynthetic pigments according to Schofield et al. (1996).

Dividing Φ_{PSII_max} by $^{PSII}\Phi_{O2\_max}$ yields the exact number of electrons generated in PSII needed to produce one O_2 molecule. However, since Φ_{PSII_max} and $^{PSII}\Phi_{O2\_max}$ were measured in two different experimental setups, our data do not support such a calculation. However, as Φ_{PSII_max} differed by only little, the result would follow the trend of $^{PSII}\Phi_{O2\_max}$. The higher QR for *Pry. parvum* than for *Pro. minimum* and *Ph. tricornutum* would influence both P^C_{max} and α^C. The temperature effect on Φ_{max} is discussed below.

The lower quantum yield for O_2 production than the theoretical maximum, leading to the offset between P^C_{PSII} and P^C_{O2}, can be caused by several electron-consuming or oxygen-consuming pathways (e.g., cyclic electron transport in PSII, pseudocyclic transport in the Mehler reaction, and light-dependent mitochondrial respiration; Flameling and Kromkamp 1998, Longstaff et al. 2002). Our data do not offer a separation between these processes, but it seems likely that cyclic electron transport around PSII or a Mehler-type of reaction (where the O_2 produced at PSII is reduced again at PSI) could contribute to the offset.

Nutrient-enriched treatments have been shown to lower the quantum requirement from ~8 to 5 (mol electrons absorbed per mol O_2) in experiments with the marine macroalga *Ulva lactuca* (Chlorophyta, Longstaff et al. 2002). In our experiments, all of the cultures were grown in f/2 medium, and, hence, we assumed that the nutrients were not limited and that no reduction of the quantum yield was caused by this reason.

Temperature acclimation of light-harvesting properties in the form of pigment complexes involves adjustment in both number and ratio of several photosynthetic pigments (Davison 1991). However, it is unlikely that the light-harvesting properties changed in our short-term temperature incubations, as all the cultures were grown at a constant temperature (15°C) and irradiance regime (80 μmol photons $\cdot m^{-2} \cdot s^{-1}$). Besides, neither $\bar{a}^*$ or $\bar{a}^*_{PSII}$ showed any correlation with temperature, nor did the relationship between them. Additionally, $\bar{a}^*_{PSII}$ excludes the absorption by PSI and any photoprotective carotenoids, including both diadinoxanthin and diatoxanthin (Johnsen et al. 1997, Johnsen and Sakshaug 2007). Hence, a potential change in the absorption properties caused by photoacclimation, during the incubations, would not influence $\bar{a}^*_{PSII}$ or the rate of P^C_{PSII}.

Temperature effects on α^C *and* E_k. The relative and absolute values of α^C showed an analogous response to a short-term temperature change and were demonstrated to be insensitive (*Pro. minimum*) or slightly decreasing (*Pry. parvum* and *Ph. tricornutum*) with increasing temperature. This trend was tested using a statistical test of covariance (Table 3). As the slope of α^C as a function of temperature was similar for the three methods and the interaction of temperature × method was insignificant ($P = 0.5$–0.96), we concluded that the temperature response for the three methods was the same for all three species. This is visually evident as seen from the plot of the relative values, as normalized at 5°C (Fig. 2, d–f). The absolute values of α^C demonstrated an offset of α^C_{PSII} compared with α^C_{O2} and α^C_{14C}, which was constant for the entire temperature range, arguing for a linear temperature-insensitive relationship between rates obtained from the three methods, in the light-limited part of the *P-E* curve. The offset of α^C_{PSII} was similar to the offset of $P^C_{PSII\_max}$, and we therefore conclude that the offset was general for the Φ_{PSII}-based O_2-production rates (P^C_{PSII}), for the entire irradiance range.

A linear offset of P^C_{PSII} compared with P^C_{O2} argues for a linear relation between the PSII electron transport and the measured O_2 production; however, our experimental setup did not support a direct comparison, as P^C_{PSII} and P^C_{O2} were measured at different irradiance levels (but within the same range). However, in a previous study, we observed a linear relationship between P^C_{PSII} and P^C_{O2} (as well as for Φ_{PSII} and $^{PSII}\Phi_{O2}$) for the same

TABLE 5. Chl *a* to C ratios (w/w) for *Prorocentrum minimum*, *Prymnesium parvum*, and *Phaeodactylum tricornutum* for each subsample incubated at one of the experimental temperatures (see Materials and Methods).

Temperature (°C)	*Pro. minimum*	*Pry. parvum*	*Ph. tricornutum*
0	0.0113	0.0252	0.0324
5	0.0124	0.0262	0.0294
10	0.0127	0.0258	0.0317
15	0.0117	0.0285	0.0304
20	0.0115	0.0275	0.0244
25	0.0117	0.0265	0.0238
30	0.0115	0.0192	0.0232
Mean	0.0118	0.0256	0.0279
SD (CV)	0.0005 (4.4%)	0.0030 (11%)	0.0040 (14%)

Growth conditions as in Table 4.

species when measured simultaneously in the same incubation chamber, under equivalent growth conditions (Hancke et al. in press).

A linear relation between P^C_{PSII} and P^C_{O2} aligns with Geel et al. (1997) who also found a linear relation between PSII ETRs and O_2-production rates at light-limited conditions in several marine phytoplankton species, including *Ph. tricornutum.* The relation between ETR and photosynthetic O_2 evolution has been investigated in a range of studies. Although the investigations were conducted under a variety of experimental conditions, a majority of these studies describe a linear relationship between O_2 production and Φ_{PSII} under moderate irradiances (Falkowski et al. 1986, Genty et al. 1989, Geel et al. 1997). Nonlinear or curvilinear correlations are described at high irradiance conditions (Falkowski et al. 1986, Schreiber et al. 1995, Flameling and Kromkamp 1998, Masojidek et al. 2001), with an excess of electron transport compared with O_2 production, or at very low irradiance presumably due to light-enhanced dark respiration (Flameling and Kromkamp 1998). A close coupling between the quantum yield for O_2 production and charge separation in PSII, but not between the quantum yield for O_2 production and ^{14}C fixation, has also been reported (Kroon et al. 1993). For the deviations, explanations such as spectral difference in PAR source, changes in O_2 consumption in the light, cyclic electron transport around PSII, and Mehler-type reactions have been proposed.

The slight decrease of α^C with temperature for *Ph. tricornutum* could be explained by an apparent decrease of the chl *a* to C ratio, as α^C (carbon-specific) often is correlated with the chl *a* to C ratio, since light absorption is correlated with chl *a* (MacIntyre et al. 2002). The chl *a* to C ratio for *Pro. minimum* and *Pry. parvum* was constant across the temperature range (except for a drop at 30°C for *Pry. parvum*, Table 5).

A mathematical consequence of the similar offset of P^C_{PSII} compared with P^C_{O2} and P^C_{14C}, for both P^C_{max} and α^C, resulted in similar values for E_k for the three methods. Hence, E_k for the three applied methods responded in parallel across the entire range of temperature, and we conclude that temperature responses on E_k can be studied quantitatively by the PAM technique, applying the present procedure to calculate O_2-production rates from Φ_{PSII}. Contradictory results have been published (Gilbert et al. 2000, MacIntyre et al. 2002). Gilbert et al. (2000) found that Φ_{PSII}-based O_2-production rates most often overestimated the measured O_2-production rates during light saturation, while the rates were similar during light-limited photosynthesis.

CONCLUSIONS

(i) Both calculated and measured O_2-production rates along with ^{14}C-assimilation rates showed the same relative response to a short-term temperature change, for the three studied microalgae species. This finding implies that the PAM technique analogous to O_2-production and ^{14}C-assimilation measurements can be applied to study relative temperature responses of photosynthesis versus irradiance relations. (ii) Absolute rates of calculated O_2 production based on Φ_{PSII} showed a species-specific correlation and overestimated the measured O_2-production rates of ~1–3 times during both light-limited (α^C) and light-saturated (P^C_{max}) photosynthesis. The offset of the Φ_{PSII}-based measurements was due to a lower quantum yield for O_2 production than the theoretical maximum and seemed to be insensitive to temperature. The lower quantum yield for O_2 production can possibly be ascribed to irradiance-induced Mehler-type reactions. (iii) The maximum quantum yield for both PSII and O_2 production decreased with increasing temperature, the latter considerably stronger than the first. (iv) Φ_{PSII} obtained with the PAM technique in combination with biooptically determined light absorption in PSII can be used as a valuable tool for studying temperature dependence of photophysiological processes in combination with O_2 and ^{14}C studies.

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