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FEATURE ARTICLE

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Effects of temperature on photosynthetic parameters and TEP production in eight species of marine microalgae

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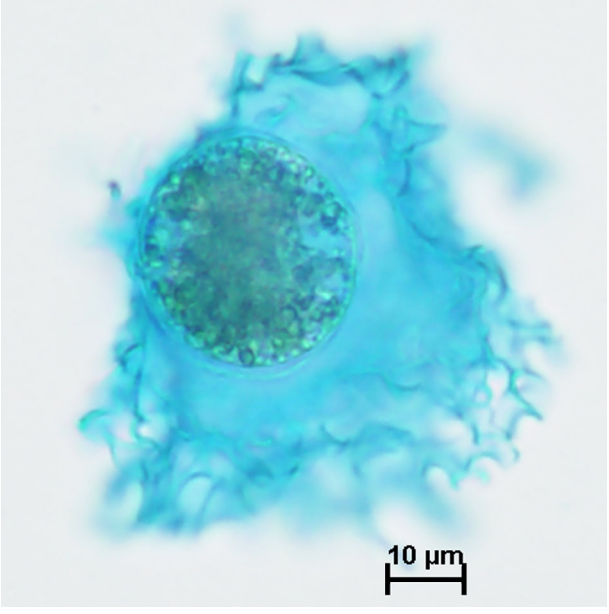
ABSTRACT: The effects of temperature on photosynthesis and transparent exopolymeric particle (TEP) production for 8 planktonic species belonging to 3 microalgal phyla (*Heteronkontophyta*, *Dinophyta* and *Haptophyta*) were investigated. Nutrient-replete semi-continuous cultures were grown at 13 temperatures between 5 and 25°C or 35°C (depending on the lethal temperature). A non-linear parametric model was applied to data on growth rate, photosynthetic parameters (electron transport rate, ETR), light utilization efficiency, α) and TEP production. The maximal photosynthetic activity at optimal temperature of production varied from 2.70 (*Pavlova lutheri*) to 4.64 (*Thalassiosira pseudonana*) mmol e^- (mg chl *a*)$^{-1}$ h^{-1}. The variation in the photoacclimation state confirmed the similarity of acclimation trends at low temperature to those at high irradiance. However, different responses were observed between species, highlighting the fact that photoacclimation mechanisms vary interspecifically for both light harvesting and downstream photosynthetic metabolism. TEP production was lowest in *Isochrysis galbana* and greatest in *Lepidodinium chlorophorum* (6 vs. 380 mg xanthan equiv [mg chl *a*]$^{-1}$ d^{-1}). The proportion of carbon fixed by photosynthesis and excreted as TEP was 70.8% for *L. chlorophorum*, while other species excreted 6.7 to 30%. A linear relationship was found between the ETR(T) and TEP(T) models for the 3 diatoms, indicating a coupling between photosynthetic activity and TEP production. This provides a new outlook on carbon excretion, which has classically been described as a consequence of nutrient stress.

KEY WORDS: Transparent exopolymeric particle · Excretion · Electron transport rate · ETR · Diatom · *Dinophyta* · *Haptophyta*



TEP excretions by *Lepidodinium chlorophorum* stained with alcian blue.
Photo: P. Claquin

INTRODUCTION

Microalgae and bacteria may excrete large quantities of polysaccharides, which represent a considerable amount of organic carbon (Passow 2002a). The colloidal fraction of these microbially derived dissolved polysaccharides is the main source for the abiotic formation (by coagulation) of transparent exopolymeric particles (TEPs), a type of exopolymeric substance (EPS). TEPs are heavily implicated in biogeochemical

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cycling of carbon and other elements in the marine environment, notably through involvement in aggregation mechanisms which can influence sedimentation of phytoplankton blooms (Thornton 2002). They are also important in ecological contexts since they may affect grazing (Prieto et al. 2001) and may inhibit viral infection (Brussaard et al. 2005).

The products of photosynthesis can be excreted within a few hours of formation (Underwood et al. 2004). Excretion of TEP precursors by microalgae is known to be enhanced under nutrient stress (Staats et al. 2000, Passow 2002b, Underwood et al. 2004), which is often considered to be the consequence of an overflow of photosynthate produced in excess of cellular requirements (Staats et al. 2000). Underwood et al. (2004) described the formation of 2 types of EPS depending on nutrient status; the first type was produced under non-limiting conditions and the second under limitation, revealing different mechanisms implicated in carbon excretion, some of which are not necessarily linked to metabolic overflow processes. Photosynthesis, like all metabolic processes, is affected by temperature (Davison 1991). Microalgae manifest a range of physiological responses to temperature changes (Thompson 2006), but the effect of temperature on carbon excretion has rarely been studied. In non-thermal acclimated cultures of benthic diatoms, Wolfstein & Stal (2002) observed that carbon excretion relative to biomass was higher at low temperature. Parallel temperature-dependent changes in photosynthesis and dissolved organic carbon (DOC) excretion were observed in batch cultures of the chlorophyte *Chlorella vulgaris* and the cyanobacterium *Synechococcus* sp., while DOC excretion was temperature independent in the haptophyte *Isochrysis galbana* (Zlotnik & Dubinsky 1989).

In the present study, we evaluated the effects of temperature in nutrient-replete conditions on photosynthetic parameters and on TEP production in 8 species belonging to the dominant marine microalgal groups: diatoms (*Bacillariophyceae*, *Heterokontophyta*), dinoflagellates (*Dinophyceae*, *Dinophyta*) and haptophytes (*Pavlovophyceae* and *Prymnesiophyceae*, *Haptophyta*). These planktonic species were selected on the basis of their relevance in ecological and biogeochemical contexts and/or for their use as live feed in shellfish aquaculture.

MATERIALS AND METHODS

Culture conditions. Eight species of microalgae — *Thalassiosira pseudonana* Hasle et Heimdal (*Bacillariophyceae*, AC589), *Skeletonema marinoi* Sarno et Zingone (*Bacillariophyceae*, AC174), *Pseudo-nitzschia fraudulenta* (Cleve) Hasle (*Bacillariophyceae*), *Emiliania huxleyi* (Lommann) Hay et Mohler morphotype A (*Prymnesiophyceae*, AC474), *Isochrysis galbana* Green (*Prymnesiophyceae*, AC34), *Isochrysis* aff. *galbana* (termed T-Iso. Tahitian isolate) (*Prymnesiophyceae*, AC102), *Pavlova lutheri* (Droop) Green (*Pavlovophyceae*, AC44) and *Lepidodinium chlorophorum* (Elbrächter et Schnepf) Hansen, Botes et de Salas (*Dinophyceae*, AC195) — obtained from the Algobank–Caen culture collection (University of Caen Basse-Normandie, France) were grown in semi-continuous culture at 13 different temperatures between 5 and 25°C or 35°C (depending on the lethal temperature). The cultures of *T. pseudonana*, *S. marinoi* and *P. lutheri* were axenic. The other cultures were not completely axenic, but the level of bacterial contamination was controlled and was extremely low. Cultures (50 ml) were grown in 150 ml borosilicate Erlenmeyer flasks in sterile natural seawater (salinity 35) enriched with f/2-medium supplements. The temperature gradient was obtained using a 2 cm thick, 1.5 × 0.6 m aluminium plate with a 1 cm diameter (0.6 m long) hole drilled 2 cm from each end, through which distilled water was pumped. Water passing through the hole at either side of the plate was circulated through a separate closed system water bath and the temperature gradient across the plate was regulated by controlling the temperature in each water bath. Cultures were acclimated for at least 1 wk at each temperature. The cultures were illuminated continuously at an intensity of 130 µmol photons m^{-2} s^{-1} provided by daylight fluorescent lamps. Light intensity was measured in the culture using a micro-spherical quantum sensor (US-SQS/L Walz). Cultures were manually mixed by gentle swirling 3 times per day. In order to maintain the cultures in exponential phase at a constant growth rate without nutrient limitation, they were diluted daily with f/2-medium as described in MacIntyre & Cullen (2005). After daily dilution, *in vivo* chlorophyll *a* (chl *a*) concentrations were equivalent in all cultures for all temperature conditions, thus minimising light variation between cultures. Biomass was estimated daily before and after dilution by fluorimetric measurement (Turner Designs) of *in vivo* chl *a*. Specific growth rates (µ, d^{-1}) were calculated using:

$$\mu = \ln(\text{chl } a_t/\text{chl } a_{t_0})/(t - t_0) \quad (1)$$

where t is time in days, chl a_{t_0} is initial chl *a* after dilution (i.e. at the initial time t_0), and chl a_t is chl *a* at time t before the dilution.

The cultures were assumed to be in steady state when daily growth rate and photosynthetic capacity (ETR_{max}) had been stable for at least 5 d. Triplicate samples were taken on 3 consecutive days once steady state had been attained in each semi-continuous culture.

Photosynthetic parameters. Chl *a* was measured spectrophotometrically after extraction in 90% acetone, and *in vivo* absorption was measured spectrophotometrically according to Shibata et al. (1954). Chlorophyll-specific absorption cross sections (a^*; m^2 [mg chl]$^{-1}$) were calculated from the chlorophyll concentration and *in vivo* absorption (Dubinsky et al. 1986).

ETR_{max} was quantified by measuring variable fluorescence. The maximum energy conversion efficiency, or quantum efficiency of PSII charge separation (F_v/F_m), was measured using a WATER/B PAM (Walz) (Schreiber et al. 1986). After a dark adaptation period of 15 min at growth temperature, a 2 ml sub-sample was placed in a darkened measuring chamber. The sample was excited by a weak blue light (1 µmol m^{-2} s^{-1}, 470 nm, frequency 0.6 kHz) and fluorescence was detected at wavelengths above 695 nm. F_v/F_m was calculated by (Genty et al. 1989):

$$F_v/F_m = (F_m - F_0)/F_m \quad (2)$$

where F_0 and F_m are the minimum and maximum fluorescence of a dark-adapted sample during a saturating light pulse (0.6 s, 470 nm, 1700 µmol m^{-2} s^{-1}), respectively.

A succession of rapid light curves relating the ETR to the irradiance (E) was performed. The samples were exposed to 9 different irradiances from 0 to 1000 µmol photon m^{-2} s^{-1} for 40 s each. The steady-state fluorescence (F_s) and the maximal fluorescence (F_m') were measured. According to Genty et al. (1989), the effective quantum efficiency of PSII in actinic irradiance was calculated as:

$$\Delta F/F_m' = (F_m' - F_s)/F_m' \quad (3)$$

$\Delta F/F_m'$ can be used to calculate the linear rate of photosynthetic electron transport (ETR) of a single active PSII unit (Genty et al. 1989):

$$ETR = \Delta F/F_m' \times E \times a^*_{PSII} \quad (4)$$

where a^*_{PSII} is the optical cross section of PSII. As we could not measure a^*_{PSII}, we calculated ETR per unit chlorophyll assuming that 50% of the absorbed photons are allocated to photoreactions in the PSII (Gilbert et al. 2000). ETR (mmol e^- [mg chl *a*]$^{-1}$ h^{-1}) was calculated as:

$$ETR = \Delta F/F_m' \times E \times a^* \times 0.5 \quad (5)$$

where a^* is the chlorophyll-specific absorption cross section (m^2 [mg chl *a*]$^{-1}$). As no significant photo-inhibition was observed, the Webb et al. (1974) model was applied to the data, ETR_{max} (maximum electron transport rate expressed in mmol e^- [mg chl *a*]$^{-1}$ h^{-1}) and the initial slope of the ETR(E) curve, or maximal light utilization efficiency (α) in mmol e^- (mg chl *a*)$^{-1}$ h^{-1} (µmol photons m^{-2} s^{-1})$^{-1}$, was then calculated as:

$$ETR = ETR_{max}\,(1 - e^{(-\alpha E/ETR_{max})}) \quad (6)$$

The light saturation parameter E_k was calculated using:

$$E_k = ETR_{max}/\alpha \quad (7)$$

Colorimetric determination of TEP. The method of Passow & Alldredge (1995) for determination of TEP concentration and its expression in xanthan equivalents per litre (Xeq l^{-1}) was adapted to incorporate the centrifugation protocol (instead of filtration) of Arruda Fatibello et al. (2004). Five ml of culture were centrifuged at 4000 rpm (3200 × *g*) for 20 min. Two ml of 0.02% Alcian blue (Sigma) in 0.06% acetic acid prepared as described in Passow & Alldredge (1995) was added to the pellet. The sample was centrifuged (3200 × *g*, 20 min) immediately in order to remove the excess dye. The pellet was rinsed with 1 ml of distilled water and centrifuged several times until excess dye was totally removed. Four ml of 80% H_2SO_4 were then added to the pellet. After 2 h, the absorption of the supernatant was measured at 787 nm. No precipitation of Alcian blue due to salt residue was observed in blanks. The calibration standard preparation described in Passow & Alldredge (1995) is applicable only for very low concentrations of TEP, i.e. calibration standard weight of xanthan gum ranging between 0 and 40 µg, which was not suitable for our samples. Moreover, these authors reported that only 16% of aqueous xanthan gum standard solution was retained on filters. In addition, the absorption of the blank obtained by Passow & Alldredge (1995) was always quite high. A protocol based on that of Passow & Alldredge (1995) but adapted to our experimental needs was consequently developed. A standard suspension of 1.0 g l^{-1} of xanthan gum in absolute ethanol was prepared. This standard suspension was mixed for 20 min and then sonicated in order to obtain small particles. Between 10 µl and 0.8 ml of this suspension was mixed with 2 ml of the solution of Alcian blue and then centrifuged at 3200 × *g* for 30 min. The pellet obtained was carefully rinsed with ethanol until the supernatant was clear (at least 3 times). The ethanol was then evaporated at 30°C overnight. Since xanthan gum does not dissolve in ethanol, the amount of xanthan gum in the dry residue was known. By weighing dried xanthan gum before and after treatment procedures, overall loss of xanthan during the treatment were estimated to be lower than 5%. Six ml of 80% H_2SO_4 were then added to the pellet and absorption was measured as described above. The standard curve was highly reproducible over a large range of concentrations (Fig. 1). The protocol was validated on several microalgal strains and each time a linear relationship

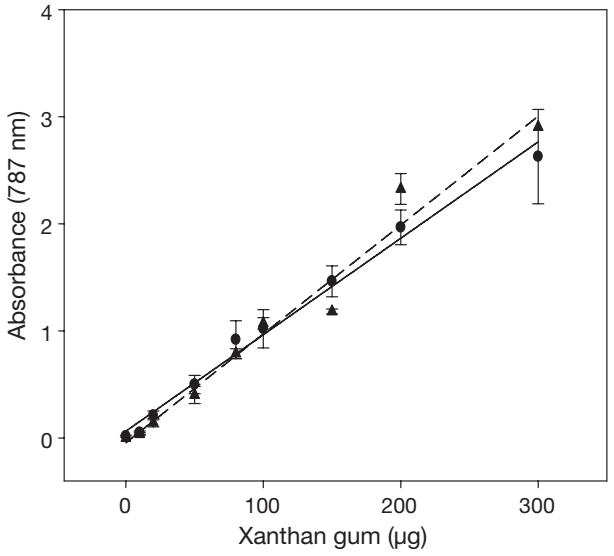

Fig. 1. Two standard curves made with xanthan gum coloured with Alcian blue after sulphuric acid treatment. Values of absorbance were corrected for blanks (the absorbance of blanks was <0.02). Linear regressions were fitted to experimental data: dashed line, $y = 0.010x - 0.043$ ($r^2 = 0.97$); solid line, $y = 0.009x - 0.065$ ($r^2 = 0.99$); Means ± SD (n = 3) are shown

was found between microalgal biomass (chlorophyll or cell number) and TEP concentration. Some examples are shown in Fig. 2.

TEP production (mg Xeq [mg chl *a*]$^{-1}$ d^{-1}) was estimated in steady state by measuring the TEP concentration per chl *a* unit (mg Xeq [mg chl *a*]$^{-1}$) before the daily dilution. Knowing the daily dilution rate (D) expressed in d^{-1}, it was possible to calculate the daily TEP production.

Temperature model. The non-linear parametric model of Blanchard et al. (1996) inspired from O'Neill (Straskraba & Gnauck 1985) was fitted on growth rates (µ, d^{-1}), ETR_{max} (mmol e$^-$ [mg chl *a*]$^{-1}$ h^{-1}) and TEP production (mg Xeq [mg chl *a*]$^{-1}$ h^{-1}) as a function of temperature (T, °C):

$$X(T) = X_{MAX}\left[\frac{(T_{let}-T)}{(T_{let}-T_{opt})}\right]^{\beta} \times \exp\left(-\beta\left\{\left[\frac{(T_{let}-T)}{(T_{let}-T_{opt})}\right]-1\right\}\right) \quad (8)$$

where $X(T)$ corresponds to $\mu(T)$, $ETR_{max}(T)$, $\alpha(T)$ or TEP production(T). X_{MAX} represents μ_{MAX}, ETR_{MAX}, α_{MAX} or the maximal TEP production at the optimal temperature (T_{opt}). T_{let} is the lethal temperature and the shape parameter β is a dimensionless parameter related to the Q_{10}. To simplify curve fitting, Morris & Kromkamp (2003) fixed β at 1.3. We did not fix β, but we fixed T_{let} as a function of experimental data.

E_k being calculated as the ratio of ETR_{max} and α, the E/E_k model was estimated using the $ETR_{max}(T)$ and $\alpha(T)$ models.

All curve fitting was carried out using the least squares criterion of SigmaPlot 10 (Systat Software). All fittings were tested using analyses of variance ($p < 0.001$), residuals being tested for normality and homogeneity of variance, and parameter significance by the Student's t-test ($p < 0.05$). In order to evaluate the relationship between $ETR_{max}(T)$ and $TEP(T)$, the shapes of the models were compared using the method of Ratkowski (1983) for non-linear models ($p < 0.05$), after normalization of the observations by their respective maximum values.

RESULTS

Growth and photosynthesis as a function of temperature

The growth rate of the 8 species varied as a function of temperature following the Blanchard et al. (1996) model, which allowed us to determine growth parameters of all of the species (Table 1). The fits of the model were always significant, indicating that growth of the cultures was controlled, as expected, by temperature. *Isochrysis galbana* presented the minimal value of μ_{max} (0.60 d^{-1}) and *Thalassiosira pseudonana* the maximal value (1.36 d^{-1}). The lowest optimal temperature for growth ($T_{opt(\mu)}$) was recorded for *Pseudo-nitzschia*

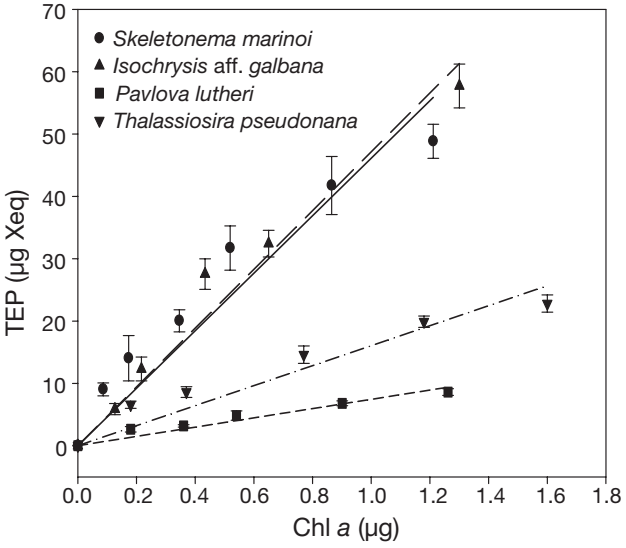

Fig. 2. Example of linear relationships found between microalgal biomass (µg chl *a*) of *Skeletonema marinoi* ($y = 46.13x$, $r^2 = 0.90$), *Isochrysis* aff. *galbana* ($y = 47.13x$, $r^2 = 0.97$), *Pavlova lutheri* ($y = 7.43x$, $r^2 = 0.93$), *Thalassiosira pseudonana* ($y = 16.03x$, $r^2 = 0.90$) and transparent exopolymeric particles (TEP; µg Xeq: xanthan equivalents) for validation of our TEP labelling protocol. Means ± SD (n = 3) are shown

fraudulenta, 20.8°C, and the highest, 30.7°C, for *I.* aff. *galbana.* The $T_{opt(\mu)}$ of the other tested species were within the range 22.0 to 24.7°C. *P. fraudulenta* also presented the lowest lethal temperature (24.1°C), and *I.* aff. *galbana* the highest (35.5°C). The ETR_{max} was determined at each temperature (Fig. 3). For the 8 species, the data obtained fitted significantly with the temperature model (Fig. 3, Table 1). ETR_{MAX} was measured at the optimal temperature of production ($T_{opt(ETR)}$) and varied from 2.70 mmol e^- (mg chl *a*)$^{-1}$ h^{-1} for *Pavlova lutheri* to 4.64 mmol e^- (mg chl *a*)$^{-1}$ h^{-1} for *T. pseudonana*. $T_{opt(\mu)}$ and $T_{opt(ETR)}$ were not significantly different for *T. pseudonana*, *Skeletonema marinoi*, *P. fraudulenta* and *P. lutheri* ($p > 0.05$), while they were significantly different for the other 4 species ($p < 0.05$). No significant variations of a^* were found as a function of tem-

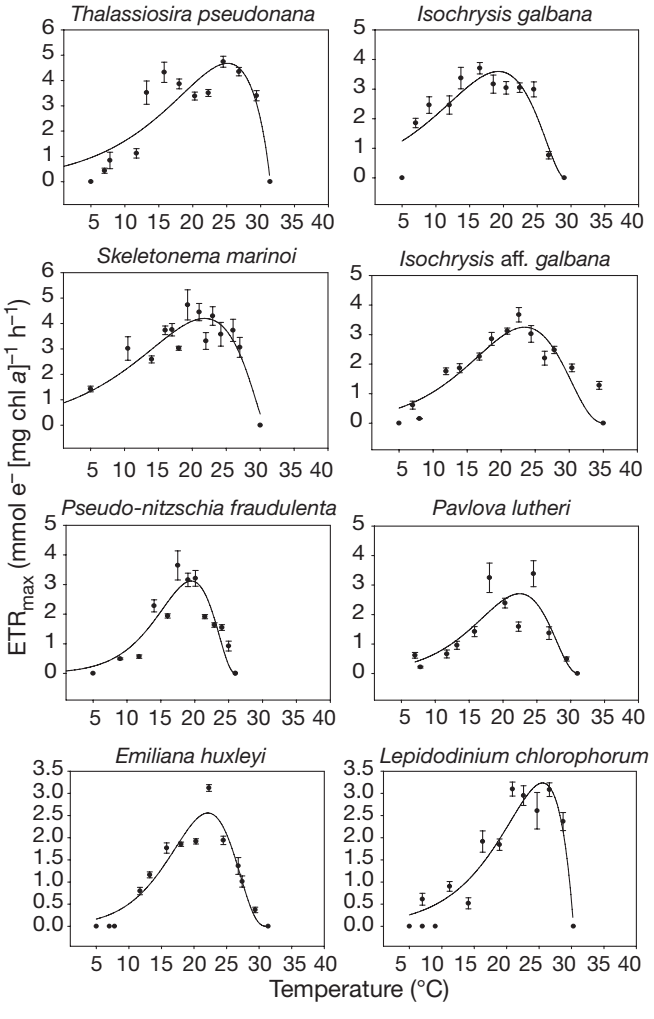

Fig. 3. Photosynthetic capacities (ETR_{max}) of 8 phytoplankton species grown in semi-continuous culture as a function of temperature. Means ± SE of triplicate cultures are shown; fitted lines represent the Blanchard et al. (1996) model $ETR_{max}(T)$

Table 1. Growth and photosynthetic parameters (mean ± SE). μ_{MAX} is the maximal growth rate (d^{-1}) at the optimal temperature ($T_{opt(\mu)}$; °C); $\beta_{(\mu)}$, $\beta_{(ETR)}$ and $\beta_{(\alpha)}$ are dimensionless parameters related to the Q_{10}; $T_{let(\mu)}$ (°C) and $T_{let(ETR)}$ (°C) are, respectively, the lethal temperature of growth and of photosynthetic activity; ETR_{MAX} (mmol e^- [mg chl *a*]$^{-1}$ h^{-1}) is the maximal photosynthetic capacity at optimal temperature of electron transport rate $T_{opt(ETR)}$; α_{MAX} (mmol e^- [mg chl *a*]$^{-1}$ h^{-1} [µmol photons m^{-2} s^{-1}]$^{-1}$) is the maximal light utilization efficiency at $T_{opt(\alpha)}$. All fittings were tested by analyses of variance ($p < 0.001$), residuals being tested for normality and homogeneity of variance, and parameter significance by the Student's *t*-test ($p < 0.05$)

Species	μ_{MAX}	$T_{opt(\mu)}$	$\beta_{(\mu)}$	$T_{let(\mu)}$	ETR_{MAX}	$T_{opt(ETR)}$	$\beta_{(ETR)}$	$T_{let(ETR)}$	α_{MAX}	$T_{opt(\alpha)}$	$\beta_{(\alpha)}$
Thalassiosira pseudonana	1.36±0.06	24.7±0.3	1.83±0.24	31.4	4.64±0.48	25.1±1.0	0.90±0.36	31.4	0.046±0.003	25.16±0.78	0.79±0.23
Skeletonema marinoi	1.24±0.06	22.5±0.3	1.88±0.27	30.7	4.19±0.23	21.7±0.7	1.38±0.33	30.7	0.023±0.001	14.83±3.14	0.65±0.20
Pseudo-nitzschia fraudulenta	0.79±0.07	20.8±0.3	3.70±0.82	24.1	3.11±0.26	19.4±0.4	2.50±0.59	26.1	0.028±0.002	22.46±0.40	0.74±0.17
Emiliania huxleyi	0.94±0.09	24.4±0.5	1.46±0.42	29.4	2.56±0.22	22.1±0.4	3.41±0.57	29.4	0.024±0.002	24.33±0.82	1.18±0.40
Isochrysis galbana	0.60±0.06	21.9±0.9	0.96±0.48	26.7	3.59±0.31	19.25±0.8	1.92±0.54	29.1	0.026±0.002	19.22±0.85	1.68±0.50
Isochrysis aff. *galbana*	1.17±0.06	30.7±0.3	0.68±0.10	35.5	3.25±0.26	23.4±0.7	3.06±0.79	35.5	0.029±0.002	25.84±0.98	1.36±0.37
Pavlova lutheri	1.30±0.1	22.8±0.4	3.41±0.70	31.3	2.70±0.37	22.4±1.0	2.62±1.05	31.3	0.027±0.001	22.87±0.68	1.19±0.27
Lepidodinium chlorophorum	1.16±0.09	22.0±0.5	2.67±0.60	30.3	3.23±0.24	25.6±0.5	0.95±0.26	30.3	0.031±0.001	24.90±0.70	0.86±0.25

perature for all species. The average values of a^* (expressed in m^2 chl a^{-1}) were 2.69×10^{-2} for *T. pseudonana*, 2.62×10^{-2} for *S. marinoi*, 2.79×10^{-2} for *P. fraudulenta*, 2.80×10^{-2} for *Emiliania huxleyi*, 2.62×10^{-2} for *I. galbana*, 2.26×10^{-2} for *I.* aff. *galbana*, 2.25×10^{-2} for *P. lutheri* and 3.32×10^{-2} for *Lepidodinium chlorophorum*. The chlorophyll content per cell tended to increase with rising temperature (data not shown) as classically described in the literature (Berges et al. 2002).

In contrast to μ and ETR_{max}, which followed the same trend for all species (i.e. O'Neill bell shape model), α did not present the same trend as a function of temperature for the 8 species (Fig. 4). The classic bell shape of the model fitted significantly with the observations for *Thalassiosira pseudonana*, *Emiliania huxleyi*, *Pavlova lutheri*, *Isochrysis* aff. *galbana*, *I. galbana* and *Lepidodinium chlorophorum*, α increasing with temperature, reaching a steady state before $T_{opt(ETR)}$ and starting to decrease at high temperature up to the lethal temperature. For the 2 other species, the model also fitted significantly; however, for *Pseudo-nitzschia fraudulenta*, α increased continuously with temperature up to T_{let} and stayed relatively constant around 3.2×10^{-3} mmol e^- (mg chl *a*)$^{-1}$ h^{-1} (μmol photons m^{-2} s^{-1})$^{-1}$ over the temperature range for *Skeletonema marinoi*.

The parameter E/E_k (corresponding to the ratio between the experimental growth irradiance and the light-saturation parameter, E_k) showed various patterns as a function of species, as shown by the E/E_k model (Fig. 5). For *Thalassiosira pseudonana* and *Isochrysis galbana* the E/E_k ratio was quite stable. For *Skeletonema marinoi* and *Lepidodinium chloropho-*

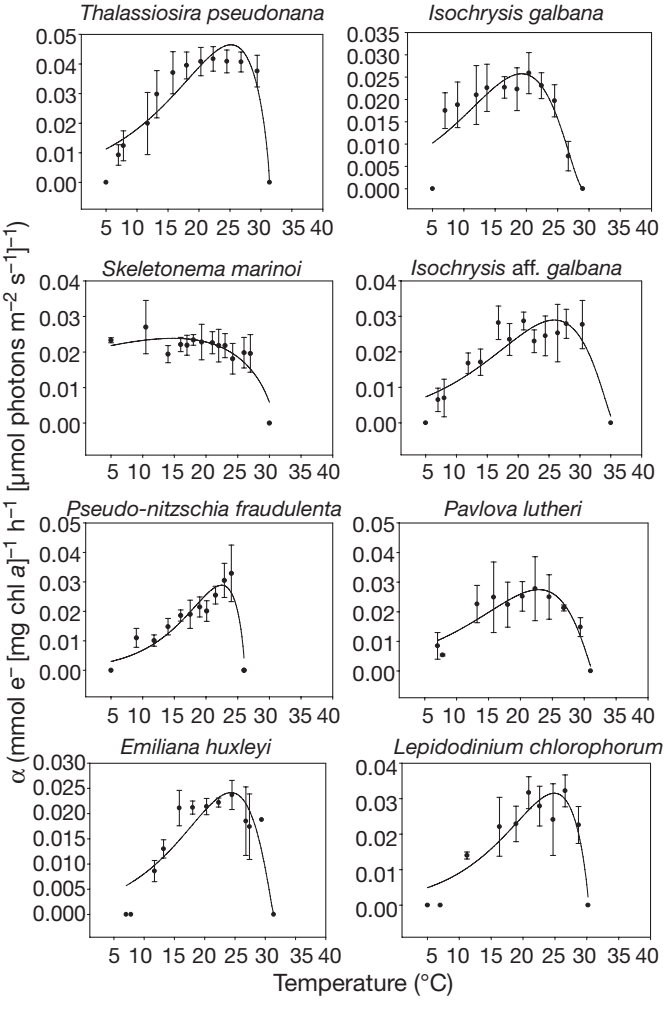

Fig. 4. Photosynthetic efficiency (α) of 8 phytoplankton species grown in semi-continuous culture as a function of temperature. Means ± SE of triplicate cultures are shown; fitted lines represent the Blanchard et al. (1996) model $\alpha(T)$

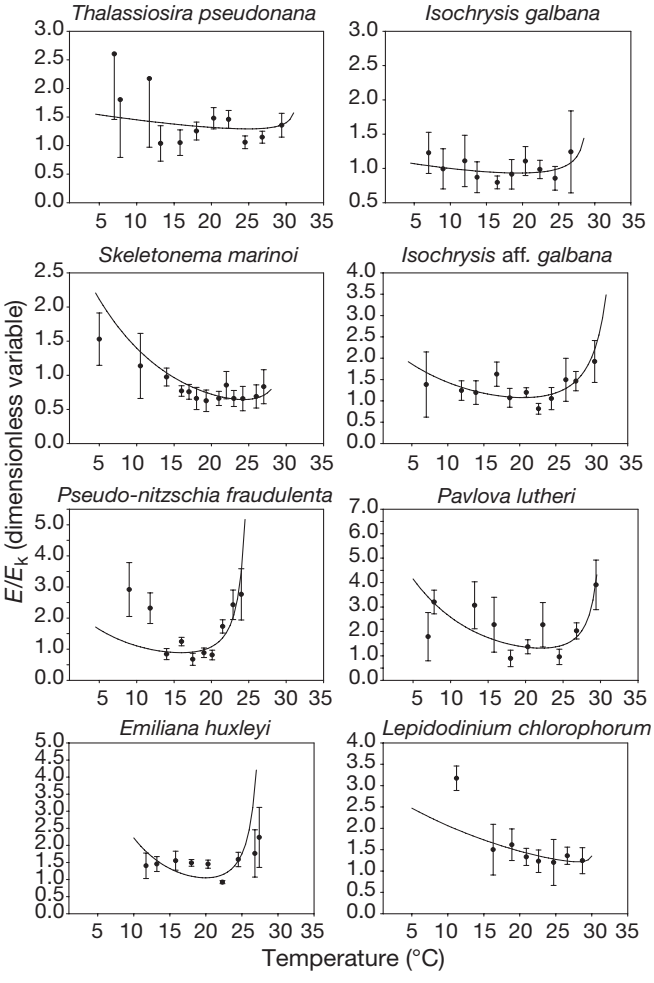

Fig. 5. Ratio of irradiance of growth (E) to the light saturation parameter (E_k) for 8 phytoplankton species grown in semi-continuous culture as a function of temperature. Means ± SE of triplicate cultures are shown; fitted lines represent the evolution of the E/E_k model estimated using the $ETR_{max}(T)$ and $\alpha(T)$ models

rum, the ratio was relatively high at low temperature. For the 4 other species, the E/E_k ratio increased at both low and high temperatures, e.g. the E/E_k ratio of *Pseudo-nitzschia fraudulenta* rose above 20°C and reached 2.7 at 24°C.

TEP production as a function of temperature

TEP production per chl *a* unit varied as a function of temperature and the model fitted significantly with the observations for *Thalassiosira pseudonana*, *Pseudo-nitzschia fraudulenta*, *Skeletonema marinoi*, *Isochrysis galbana and I.* aff. *galbana* (Fig. 6). For these species, the production of TEP increased with temperature until a maximum and decreased at high temperature.

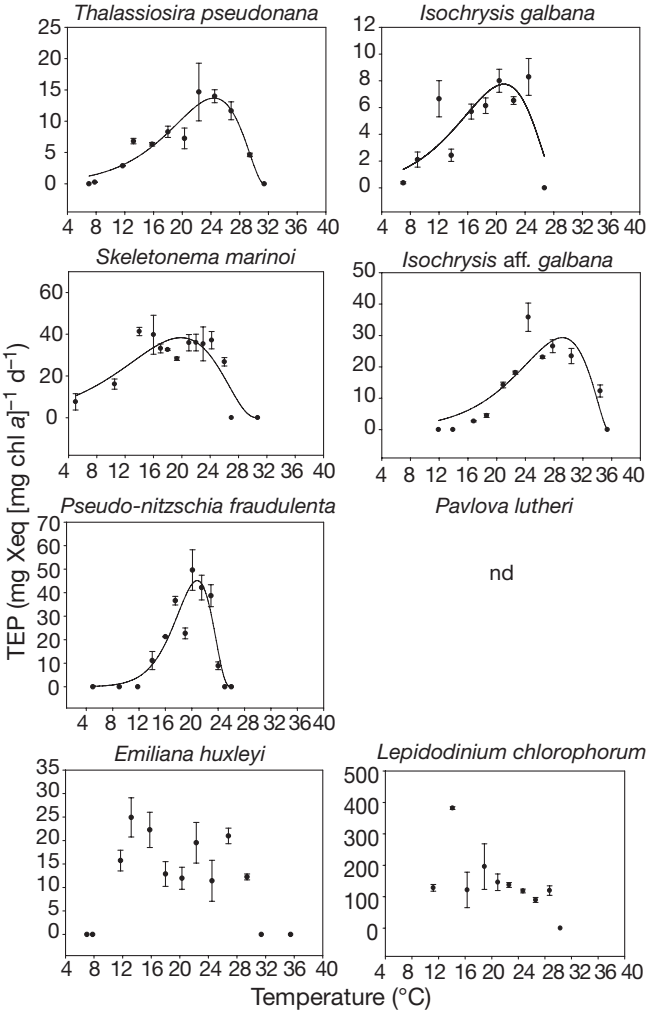

Fig. 6. Transparent exopolymeric particle (TEP) production of 8 phytoplankton species grown in semi-continuous culture as a function of temperature. Means ± SE of triplicate cultures are shown; fitted lines represent the Blanchard et al. (1996) model TEP(T). nd: not determined

The same trend was observed for *Lepidodinium chlorophorum*, but the model did not fit significantly with the data. For *Emiliania huxleyi* no relationship appeared between temperature and TEP production. The dinoflagellate *L. chlorophorum* produced a large amount (on average 10 times more) of TEP in comparison with the other tested species. The maximum production measured for this species was 380 mg Xeq (mg chl *a*)$^{-1}$ d^{-1}. The diatoms *P. fraudulenta* and *S. marinoi* produced up to ca. 40 mg Xeq (mg chl *a*)$^{-1}$ d^{-1}. *I.* aff. *galbana* produced 4 times more TEP than *I. galbana*, which produced only 6 mg Xeq (mg chl *a*)$^{-1}$ d^{-1}. *E. huxleyi* produced up to 25 mg Xeq (mg chl *a*)$^{-1}$ d^{-1} and *T. pseudonana* 15 mg Xeq (mg chl *a*)$^{-1}$ d^{-1}.

Relationship between photosynthesis and TEP production

Using the method of Ratkowski (1983), no differences were found in the shape of the normalized models between $ETR_{max}(T)$ and TEP(T) for *Skeletonema marinoi* and *Pseudo-nitzschia fraudulenta* (comparison for non-linear models, $p > 0.05$). This means that T_{opt} and β (dimensionless parameter related to Q_{10}) were similar for the 2 models. A difference was found for *Thalassiosira pseudonana* between the 2 models due to β ($p < 0.05$), while T_{opt} was not different. For all other species the comparison between the 2 normalized models was not significant. For the 3 diatoms, linear regressions were found between the 2 models ($p < 0.01$), ETR(T) at growth irradiance and TEP(T), the coefficients of determination, r^2, being respectively 0.94, 0.91 and 0.84 for *T. pseudonana*, *S. marinoi* and *P. fraudulenta* (Fig. 7).

DISCUSSION

In culture, the 8 tested microalgal strains were able to survive over a large temperature range, all growing at least between 7 and 24°C (Fig. 3). Consequently, they can be characterized as temperate eurythermal organisms, in contrast to stenothermal microalgae like, for example, Antarctic or Arctic diatoms (Suzuki & Takahashi 1995), some of which showed maximum growth at 0°C and full inhibition of cell division above 7°C (Longhi et al. 2003). The haptophyte *Isochrysis* aff. *galbana* exhibited the largest range of thermal tolerance, between 7 and 35.5°C, while the diatom *Pseudo-nitzschia fraudulenta* had the lowest range (5 to 24.1°C).

Relative ETR is frequently used to characterize ETR_{max} in algae under various growth conditions (Ralph & Gademann 2005). Knowing the a^*, the ETR

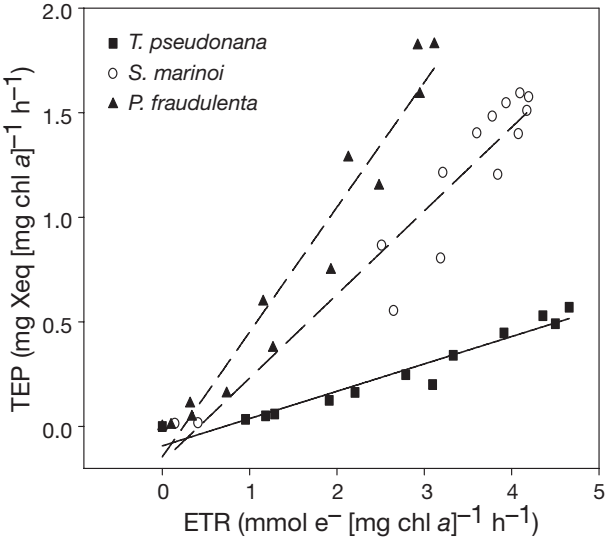

Fig. 7. Linear regression between both models, ETR(T) at growth irradiance (i.e. photosynthetic activity) and TEP(T) for the diatoms *Thalassiosira pseudonana* ($y = 0.13x - 0.09$, $r^2 = 0.94$, $p < 0.01$), *Skeletonema marinoi* ($y = 0.40x - 0.16$, $r^2 = 0.91$, $p < 0.01$) and *Pseudo-nitzschia fraudulenta* ($y = 0.59x - 0.14$, $r^2 = 0.95$, $p < 0.01$); ETR(T): model of photosynthetic activity as a function of temperature; TEP(T): model of TEP production as a function of temperature, expressed in mg Xeq (mg chl *a*)$^{-1}$ h^{-1} for reconciling the time unit; Symbols: estimated values of TEP production as a function of ETR at experimental growth temperatures

was expressed in the present study as a function of chl *a* concentration, which allows comparison of the photosynthetic parameters of tested species (Fig. 3). The values of ETR_{max} and α were within the range of values reported in the literature (Morris & Kromkamp 2003, Lefebvre et al. 2007). For example, ETR_{max} varied between 2.3 and 7.1 mmol e$^-$ (mg chl *a*)$^{-1}$ h^{-1} in the diatom *Skeletonema marinoi* in this study and between 1.3 and 7.2 mmol e$^-$ (mg chl *a*)$^{-1}$ h^{-1} in another diatom, *Cylindrotheca closterium* (Morris & Kromkamp 2003). The 2 centric diatoms, *Thalassiosira pseudonana* and *S. marinoi*, presented the highest μ_{MAX} and ETR_{MAX} (Table 1). The relative efficiency with which diatoms are able to transform photosynthetic energy to growth, due in part to the low loss of photosynthetic electrons to alternative pathways like photorespiration and the Mehler-reaction (Wilhelm et al. 2006), can probably partly explain the ecological success of this microalgal group in modern oceans.

Contrary to ETR_{max} (Fig. 3), which showed a common pattern of variation as a function of temperature, thermal acclimation of α appeared to be species dependent (Fig. 4). In particular, unlike for other species, α was quite stable with temperature for *S. marinoi*. Stability of $\alpha_{(ETR)}$ was also observed by Lefebvre et al. (2007) *in S. costatum* (= *S. marinoi* according to Sarno et al. 2005) and by Morris & Kromkamp (2003) in *C. closterium* at high growth rate. Contrary to the other species, *S. marinoi* was able to photosynthesise and grow under 5°C, indicating that it did not reach its minimal threshold temperature, which probably explains the high value of α at 5°C. This capacity may account for the wide geographic distribution of *Skeletonema* spp. (Suzuki & Takahashi 1995). α is known to be modulated as a function of irradiance and light spectrum (Sakshaug et al. 1997). Our experiments were performed at constant light (in quantity and in spectrum) in semi-continuous culture, which allowed maintenance of a stable level of biomass. Therefore, α variation cannot be explained by an auto-shading effect. The reduction of α observed in many species at low temperature (Fig. 4) was probably partly due to the decreased chlorophyll content per cell recorded with decreasing temperature (data not shown). A decrease in chlorophyll content is a typical algal response to high irradiance, along with increased content of photoprotective carotenoids whose function is to dissipate excess energy. Several previous studies have shown that acclimation to low temperature mimics adaptation to high irradiance (Anning et al. 2001, El-Sabaawi & Harrison 2006).

The E/E_k models applied on data allowed definition of statistically significant trends upon which the following interpretation is based. The E/E_k ratio was close to 1 around the optimal temperature for all species and the value was higher than 1 both below and above those optimal temperatures notably for the haptophytes *Emiliania huxleyi*, *Isochrysis* aff. *galbana*, *Pavlova lutheri* and the diatom *Pseudo-nitzschia fraudulenta* (Fig. 5). For *Lepidodinium chlorophorum and Skeletonema marinoi*, the E/E_k ratio increased with a decrease in temperature, while the ratio was quite stable for *I. galbana* and *Thalassiosira pseudonana*. A high E/E_k ratio indicates light saturation and an imbalance between light-harvesting and downstream photosynthetic reactions (Anning et al. 2001), while a ratio around 1 indicates optimization of light harvesting with photosynthetic metabolism as a function of the incident light. In the present study, it appears that, except for *I. galbana* and *T. pseudonana*, which exhibited a relatively stable value of E/E_k (Fig. 5), the other species were not able to acclimate their light-harvesting capacity at extreme temperatures after at least 1 wk of acclimation. Anning et al. (2001) described an increase of the ratio E/E_k in the marine diatom *Chaetoceros calcitrans* at low temperature, as we observed particularly for *S. marinoi* and *L. chlorophorum* and more generally for all tested species. This confirms the apparent similarity of acclimation at low temperature and high irradiance. The broad range of E/E_k responses confirms that temperature acclimation is

species dependent, i.e. various mechanisms and strategies implicating light harvesting and the whole downstream photosynthetic metabolism are responsible for this heterogenic response (Davison 1991, Thompson 2006). For example, RUBISCO activity, particularly at high temperature, depends on chaperon proteins which maintain RUBISCO's function, and these accompanying proteins are interspecifically variable (Thompson 2006). The use of rapid light curves defined as very short light steps of different irradiances may lead to a wrong estimation of ETR_{max} and consequently of E_k (Serôdio et al. 2005). However Perkins et al. (2006) showed that for diatoms light steps with durations longer than 30 s were suitable. In the present study, light steps of 40 s were applied, and we observed that the fluorescence steady state was reached at all irradiances for all species and for all temperature treatments.

The concentrations of TEP measured in the present study (1 to 382 mg Xeq (mg chl *a*)$^{-1}$) are within the range (1 to 3700 mg Xeq (mg chl *a*)$^{-1}$) presented by Passow (2002a) in a review synthesizing data on 22 microalgae belonging to various phyla. In our study, the dinoflagellate *Lepidodinium chlorophorum* produced the most TEP (159 mg Xeq [mg chl *a*]$^{-1}$ d^{-1}); this echoes the results of Passow (2002b), who measured high production of TEP per cell (1309 pg Xeq cell^{-1}, corresponding to 70 mg Xeq [mg chl *a*]$^{-1}$; Passow 2002a) in the dinoflagellate *Gonyaulax polyedra*. Passow (2002a) reported low TEP production (1 to 7 Xeq [mg chl *a*]$^{-1}$) in a non calcifying strain of *Emiliania huxleyi*, whereas we measured higher production (between 12 and 25 mg Xeq [mg chl *a*] $^{-1}$ d^{-1}) in a calcified strain of the same species.

In the present study, temperature influenced TEP production in the 3 diatoms and both *Isochrysis* strains, but did not affect TEP production in *Emiliania huxleyi* and *Lepidodinium chlorophorum* (Fig. 6). In the case of *I. galbana*, this appears to contradict the result of Zlotnik & Dubinsky (1989), who found that temperature did not affect DOC excretion. TEP formation, and carbon excretion in general, is known to be strongly influenced by nutrient status; however, in the present study, growth was not nutrient limited and was controlled only by temperature. Contrary to Wolfstein & Stal (2002), who observed higher EPS production per chl *a* unit at low temperature in batch culture for the diatom *Cylindrotheca closterium*, we observed for the tested diatoms an increase of EPS production up to an optimal temperature and then a decrease at high temperature. For the 3 diatoms, and in contrast to the other species, TEP production was significantly linearly related to photosynthetic activity (Fig. 7); this indicated that carbon excretion was not simply due to an overflow of carbon resulting from unbalanced growth, but on the contrary to a balance between production and excretion of carbon. This balance may be due to the semi-continuous culture conditions, which allow a better equilibrium between metabolic pathways than under batch culture (MacIntyre & Cullen 2005). Underwood et al. (2004) found that 2 distinct types of EPS were produced by *C. closterium* depending on nutrient status: one type being produced during nutrient-replete culture (EPS_{type1}) and the other type being produced in addition during nutrient stress (EPS_{type2}). Applying this model to our data for pelagic diatoms, it can be argued that under thermal acclimation the EPS_{type2} would not be formed, while EPS_{type1} production would be coupled with carbon production. In the study of Wolfstein & Stal (2002), EPS production of *C. closterium* may have decreased at low temperature with increasing age of the culture as a result of thermal acclimation and decreasing thermal stress. Analyses of TEP and EPS composition as a function of thermal acclimation would be an interesting next step for further investigation. The cultures performed in this study were conducted under continuous illumination, which could have amplified carbohydrate metabolism and thus carbon excretion. The use of a light/dark cycle in future studies would allow quantification of this potential effect.

The rate of photosynthesis estimated by PAM fluorometry and oxygen evolution or carbon fixation have been compared in several phytoplankton species (Flameling & Kromkamp 1998, Morris & Kromkamp 2003, Lefebvre et al. 2007), which allows estimation of the number of mol of C fixed per mol of electrons. Morris & Kromkamp (2003) found a value of 0.114 mol C (mol electron)$^{-1}$. In order to estimate carbon fixation in our study, we used this factor to convert the ETR (mmol e$^-$ [mg chl *a*]$^{-1}$ h^{-1}) at growth irradiance (i.e. photosynthetic activity) into carbon expressed in mg C (mg chl *a*)$^{-1}$ h^{-1}. TEP concentrations (Xeq [mg chl *a*]$^{-1}$) were also converted to carbon, in light of the work of Engel & Passow (2001), who determined ratios between TEP carbon (µg C) and TEP (µg Xeq) in several species. A ratio between TEP carbon (mg C chl *a*$^{-1}$) and TEP (Xeq [mg chl *a*]$^{-1}$) of 0.70 was applied. These conversions allow estimation of the percentage of photosynthetic carbon which was excreted in the form of TEPs. For the 3 diatoms, the linear relationship between calculated carbon production and TEP production was obviously similar to the one between ETR at growth irradiance and TEP (Fig. 7), but the absolute values of slopes changed. The slope values were, respectively, 0.067, 0.20 and 0.30 for *Thalassiosira pseudonana*, *Skeletonema marinoi* and *Pseudo-nitzschia fraudulenta*, which signifies that, respectively, 6.7, 20 and 30% of the photosynthetic carbon production was excreted as TEP. Estimations reported

in the literature for benthic diatoms, which are known to produce large amounts of EPS, range from 30 to 73% of photosynthate being excreted, whereas for pelagic phytoplankton estimations range from 1.5 to 22% (Goto et al. 1999, Smith & Underwood 2000). Because no relationships were found between photosynthetic production and TEP production for the other species, this percentage was estimated at $T_{opt(ETR)}$ at growth irradiance: 17.3% was calculated for *Emiliania huxleyi*, 6.8% for *Isochrysis galbana*, 15.9% for *I.* aff. *galbana* and 70.8% for *Lepidodinium chlorophorum*.

Even though dinoflagellates are known to excrete large amounts of carbon (Passow 2002b) and the presence of large amounts of TEPs in the *Lepidodinium chlorophorum* cultures was confirmed by light microscopic observations, the percentage of carbon production estimated to be excreted in *L. chlorophorum* seems abnormally high. There are a number of potential explanations for this: (1) heterotrophy, frequently described in dinoflagellates, would affect this estimation, but no organic material except that excreted by the microalgae was present in the medium; (2) the presence of bacteria, which are known to produce EPS and TEPs, would be another potential source of bias, but high levels of contamination would have been detected by microscopic observations; (3) dinoflagellates tend to be relatively fragile and a stress reaction during sampling may have influenced measurements. TEP concentration is often high in blooms dominated by dinoflagellates in the natural environment (Passow 2002a). Irrespective of the accuracy of this estimation, *L. chlorophorum* is known to produce large slimy blooms which can create anoxic conditions (Sournia et al. 1992), and ecdysis and the formation of mucocysts have been reported for this species (Elbrächter & Schnepf 1996). The high level of carbon excretion would facilitate aggregation and bloom sedimentation and hence contribute to the dramatic consequences of such blooms. According to our data, an increase in temperature would not amplify this potential phenomenon of aggregation (and other effects of TEPs) for *L. chlorophorum* and *Emiliania huxleyi*, but would do so for the 2 *Isochrysis* strains and diatoms.

CONCLUSIONS

In the present study, the growth and ETR_{max} of 8 common microalgal species of interest in ecological and/or aquacultural terms were characterised. The photosynthetic acclimation that we observed was roughly in accordance with literature reports, but the species-specific trends of E/E_k merit further study. The linear relationship observed in diatoms between TEP production and photosynthesis during balanced nutrient-replete growth provides a new way to consider carbon excretion, which has most frequently been described to be a consequence of stress. Underwood et al. (2004), focusing on EPS composition and metabolic pathways of EPS production as a function of environmental conditions, provide a basis for future work aimed at acquiring a better knowledge of the dynamics of excretion of organic compounds which play an important role in global carbon fluxes. In this context, our study demonstrates the differential interactions between photosynthesis and TEP production between species, highlighting the potential of inter-specific comparisons for developing our understanding of the metabolic mechanisms involved in TEP production. Because TEP formation influences aggregation mechanisms, grazing and virus attack, processes which are involved in the fate of phytoplankton blooms, accurate prediction of carbon excretion by phytoplankton is important for improving the simulation of bloom dynamics.

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