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PRIMARY RESEARCH PAPER

# Light control on phytoplankton production in a shallow and turbid estuarine system

**Carla Gameiro · Juan Zwolinski · Vanda Brotas**

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**Abstract** Tagus estuary is one of the largest estuaries of Western Europe. With the aim of unravelling the drivers of primary production in this shallow and turbid nutrient replete estuary, we tested the hypothesis that light availability is a major factor controlling phytoplankton production. Environmental parameters, phytoplankton biomass, community composition, and photosynthetic parameters were monitored at two sites in the estuary during a complete annual cycle. Despite the fact that nutrient concentrations were always above growth-limiting values, Chl *a* concentrations were relatively low throughout the study period. High water column turbidity, due to riverine inputs, promoted a rapid attenuation of light and created a compressed profile with optimal photosynthetic conditions. Therefore, the phytoplankton community, dominated by small cells, such as diatoms and cryptophycean flagellates, displayed highly photosynthetic efficiency and low light-saturated photosynthetic rates as a photo-acclimation response to low light conditions year-round. Primary production rate was unimodal, peaking in the summer months. In such estuarine system, gross primary production could thus be predicted by an existing robust empirical model based on pigment standing crop (Chl *a*), surface irradiance ($E_0$) and optical depth ($Z_{eup}$). Compared to other shallow estuaries, the Tagus can be classified as a low- to moderately productive estuary, being the turbidity-induced low light conditions the principal factor limiting phytoplankton growth.

**Keywords** P–E curves · Light limitation · Photo-Acclimation · Primary production · Tagus estuary

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C. Gameiro (✉) · V. Brotas
Centro de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisbon, Portugal
e-mail: clgameiro@fc.ul.pt

J. Zwolinski
Instituto de Investigação das Pescas e do Mar (IPIMAR), Av. Brasília, 1449-006 Lisbon, Portugal

## Introduction

One of the principal goals of phytoplankton ecology has been to understand the factors that regulate phytoplankton primary production in aquatic ecosystems. Rivers influence primary production in estuaries by providing a significant amount of nutrients, which together with in situ benthic remineralization makes them areas of high primary production (Nixon, 1995). On the other hand, tide turbulence and river-induced turbidity (suspended inorganic particles and coloured substances) together with high water inflows and rapid flushing times may limit phytoplankton growth. Other environmental factors such as temperature (Eppley, 1972), phytoplankton community

structure (species and cell size) (Côté & Platt, 1983) and zooplankton grazing (Verity, 1986) may also play an important role in controlling primary production in estuaries. Therefore, the determining factors controlling phytoplankton primary production in estuaries are more complex than those affecting production in the open ocean.

In turbid and nutrient-rich estuarine systems, light availability may be the most important factor controlling biomass-specific productivity (Wofsy, 1983). The high seston concentrations that typify these systems leads to a rapid attenuation of light with depth, which results in a compressed photosynthetic profile and a shallow euphotic zone in relation to the water column depth. In such systems, the restricted light availability may alter phytoplankton production in two ways: by regulating the maximum attainable biomass in the system (Wofsy, 1983) and by stimulating physiological acclimation to low light conditions (Falkowski, 1981). Furthermore, turbid waters have an impact in the way that phytoplankton use light, resulting in high photosynthetic efficiency under low light conditions (Grobbelaar, 1990).

Physiological acclimation to environmental conditions such as light (intensity and spectral quality) is an important factor determining variations in photosynthetic responses and growth of algae in nature (Harrison & Platt, 1986). The functional relationship between photosynthesis and light (P–E curves) is a useful tool to assess primary production at a given place and time. The shape and magnitude of the P–E curve reflects the underlying biophysical, biochemical and metabolic processes that regulate photosynthesis (Falkowski & Raven, 1997; Sakshaug et al., 1997). Two key photosynthetic parameters that describe the P–E relationship are: the light-saturated production rate plateau ($P^{B}_{max}$) which is a function of the enzymatic processes in photosynthesis, and the initial slope of the P–E relationship ($\alpha^{B}$) which is a function of the photochemical processes in photosynthesis and depends on the quantum yield and on the ability of the cells to trap incident light. The derived parameter, $P^{B}_{max}/\alpha^{B}$, provides the co-called photo-adaptation parameter ($E_k$) (Talling, 1957), which in variable light environments, phytoplankton have the capacity to adjust in order to maintain an optimum balance between light and dark reactions of photosynthesis (Sakshaug et al., 1997). As a result, phytoplankton assemblages are able to optimize production rate under suboptimal conditions through a physiological response/adjustment. This cellular physiologic adjustment, termed acclimation, may also serve to limit the damage that may occur as a consequence of exposure to adverse environmental conditions (Geider et al., 1998).

The Tagus estuary is one of the largest estuaries on the west coast of Europe and it plays an important role in the migration route of birds as well as in the life ecology of many coastal fish species. Despite the high nutrients concentration, the phytoplankton biomass in the estuarine waters is usually low (annual median $< 3.5$ μg $l^{-1}$) (Gameiro & Brotas, 2010). For that reason, the Tagus estuary has been characterized as oligo- (Nixon, 1995) and meso-trophic (Vollenweider et al., 1998). One of the reasons for the relatively low productivity of this nutrient-rich estuary could be related to the low light availability in the water column because of the elevated concentration of suspended particulate matter in the water column (mean 30 mg $l^{-1}$) resulting from the high freshwater input and strong tidal currents (Gameiro et al., 2007).

In this study, we hypothesized that light availability is the main environmental driver on the dynamics of phytoplankton biomass and primary production in the Tagus estuary. To test this hypothesis, P–E curves were derived from monthly experiments at two sites, and primary production rates were estimated on an hourly basis throughout 1 year. Photosynthetic parameters and phytoplankton community characteristics were used to determine its physiological response mechanisms of the phytoplankton community living in this low light environment. In addition, we took a robust, empirical model of phytoplankton production, the $BZ_{eup}E_0$ model (Cole & Cloern, 1984; Cloern, 1987), and adapted it to use with our data.

## Materials and methods

### Study area

The Tagus estuary is located on the west coast of Portugal (38°44′N, 9°08′W), with a broad shallow bay (10 m mean depth) covering an area of about 320 $km^2$ (Fig. 1). It is located in the most populated area of Portugal. The Tagus River is the main source of freshwater into the estuary and it drains a total area of 86,629 $km^2$, representing the second most important hydrological basin in the Iberian Peninsula.

Inflow to the estuary fluctuates seasonally with an average monthly flow varying from 120 $m^3$ $s^{-1}$ in summer to 653 $m^3$ $s^{-1}$ in winter (last 30 years of Water National Institute public database, INAG; www.inag.pt). Estimated water residence time ranges between 6 and 65 days for winter and summer, respectively (Martins et al., 1984). The Tagus estuary is mesotidal with semidiurnal tides ranging from 0.4 m at neap tide to 4.1 m at spring tide. Seawater enters to the estuary through a deep narrow inlet channel and the tidal influence can be measured up to 80 km inland from Lisbon. Shallowness and strong tidal currents contribute to a well-mixed water column, even during neap tides (Gameiro et al., 2004). The suspended sediment load for an average year has been estimated as $4 \times 10^5$ tons (Vale & Sündby, 1987).

## Sampling

Sampling was carried out at two sites (S1 and S4) located in the upper and mid part of the Tagus estuary, respectively (Fig. 1). Of the data collected in the Tagus estuary between 1999 and 2007, these two sites were the ones that diverged more in terms of environmental conditions (Gameiro et al., 2007), and thus are the most representative of the estuary heterogeneity. Surface water samples were collected monthly at high tides during neap tides, from July 2006 to June 2007. The sampling sites differed in relation to their average water depth during high tide: 3.5 and 5.5 m for S1 and S4, respectively. Sampling at S4 started only in August and was interrupted in November and December due to adverse meteorological conditions. Water was collected with a bucket and samples were immediately prefiltered on a 200 μm meshsize net to remove larger zooplankton and neuston.

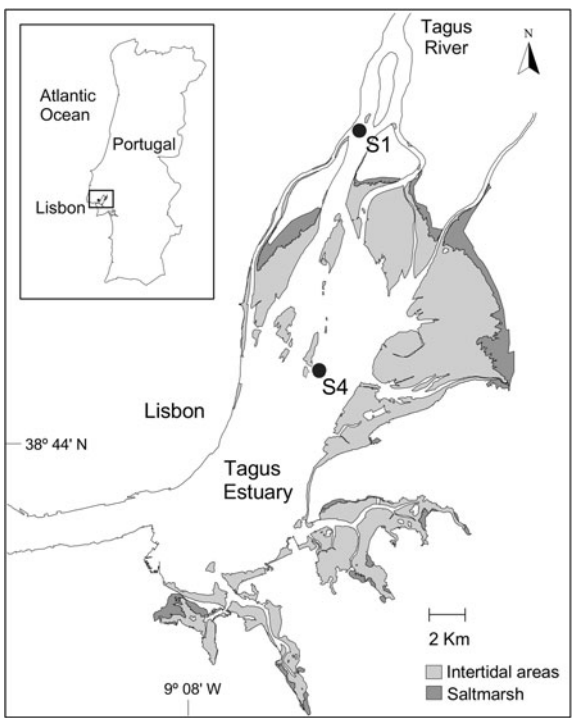


**Fig. 1** Map of Portugal with enlarged area of the Tagus estuary. *Shaded areas* represent intertidal zones and *circles* indicate the location of the sampling sites

## Environmental parameters

Water temperature ($T$) and salinity (Sal) were measured in situ with a thermometer and an ATAGO S/MillE refractometer, respectively. Surface incident PAR ($E_0$) and light intensity at depth $Z(E_z)$ were measured using a LiCor (LI192SA) underwater quantum sensor, from surface to the bottom, at 0.5 m depth intervals. We assumed an exponential decay of irradiance versus depth: $E_{(z)} = E_0 \exp(K_{par} Z)$, where $E_{(z)}$ is irradiance at depth $Z$ in μmol photons $m^{-2}$ $s^{-1}$, $E_0$ is the irradiance at the surface (μmol photons $m^{-2}$ $s^{-1}$) and $K_{par}$ is the light attenuation coefficient (Kirk, 1994). $K_{par}$ ($m^{-1}$) was estimated by solving the equation $K_{par} = (\ln E_0 - \ln E_{zt})/Z_t$, where $Z_t$ is the maximum depth in m. Mean water column irradiance ($E_m$) was determined as $E_m = E_0 [1 - \exp(K_{par} Z_t)]/K_{par} Z_t$ (μmol photons $m^{-2}$ $s^{-1}$) (Jumars, 1993). The euphotic depth ($Z_{eup}$), also known as optic depth, was taken as the depth where there is 1% of the surface irradiance and it was approximated as $4.6/K_{par}$ (Kirk, 1994). The depth of the mixed layer was considered the same as total depth at each sampling site, as no stratification in water column was previously observed at the same sites during neap tides (Gameiro et al., 2004). Freshwater inflow data and total daily rainfall were obtained from INAG public database.

Dissolved nutrient concentrations, nitrate ($NO_3$), nitrite ($NO_2$), phosphate ($PO_4$) and silicate ($SiO_2$) were determined in triplicate water samples prefiltered through Whatman GF/C filters, with a FossTecator FIAstar™ 5000 Analyser. Nitrate was

determined according to Grasshoff (1976), nitrite according to Bendschneider & Robison (1952), phosphates and silicates according to Murphy & Riley (1962) and Fanning & Pilson (1973), respectively. Dissolved ammonium ($NH_4^+$) concentration was determined using manual colorimetric method in filtered samples according to Koroleff (1969/1970). The definitions of nutrient limitation by Dortch & Whitledge (1992) were applied (N-limitation: N:P $\leq$ 10 and Si:N $>$ 1; P-limitation: N:P $>$ 30, Si:P $>$ 3; Si-limitation: Si:N $<$ 1, Si:P $<$ 3) together with the threshold defined by Fisher et al. (1988) for the uptake of phytoplankton in turbid estuarine zone (N-limitation: DIN $\leq$ 1; P-limitation: $PO_4 \leq 0.5$; Si-limitation: Si $<$ 5).

### Phytoplankton biomass and species composition

Triplicate surface water samples were filtered for phytoplankton biomass analyses onto Whatman GF/F glass fibre filters and Chl *a* was extracted in 90% acetone at 4°C in darkness for 24 h (Lorenzen, 1967). Chl *a* was quantified with a Shimadzu UV-1603 spectrophotometer before and after acidification for phaeopigments correction at 664 and 750 nm.

Water samples (100 ml) for phytoplankton identification and cell counts were collected and immediately fixed with Lugol's solution. Counts in sedimentation chambers were performed using an inverted light microscope Olympus I-70 at 400× magnification, according to the method of Utermöhl (Hasle, 1978). At least 250 cells were counted in each sample.

### Photosynthesis-light curves

Less than 1 h after collection, water samples were put in 50 ml glass flasks, which were spiked with 100 µl of $NaH^{14}CO_3$ with 20 µCi ml$^{-1}$ (764 kBq ml$^{-1}$) ($^{14}$C Centralen, DHI) and incubated at in situ temperature. Samples were homogenized by rotation (30 rpm), and incubation irradiance was provided by two 400 W quartz metal halide lamps with clear outer bulb (Philips HPI-T Pro). Eight different light intensities (*E*) (0, 20, 160, 225, 340, 445, 520 and 720 µmol photons m$^{-2}$ s$^{-1}$) were setup using white and black linen nets. Three replicates were used at each light intensity. Incubation was finished after 2 h by filtration through gravity on GF/F glass fibre filters (25 mm Ø). Filters were washed with 5 ml of filtered seawater, placed in scintillation vials and fumed in a HCl atmosphere for 20 min to remove precipitated inorganic $^{14}$C. Analysis for radioactivity was carried out in a Beckman scintillation counter (Model LS 7800) using RadySafe® as scintillation cocktail. Activity of $^{14}$C in dark bottle filters was subtracted from light bottles to correct for non-photosynthetic uptake. Dissolved inorganic carbon content of the sample water was calculated following Parsons et al. (1984). Carbon photosynthetic uptake (*P*) was calculated using an isotope discrimination factor of 1.05 and normalized to Chl *a* [$P^B$, mg C (mg Chl *a*)$^{-1}$ h$^{-1}$] (superscript B indicates photosynthetic rates normalized to Chl *a*). It was assumed that the measured rates of photosynthesis equalled gross rates because the incubation time of 2 h was relatively short (Williams, 1993).

Primary production data, obtained monthly, were plotted to construct light saturation curves. Each P–E curve was fitted according to Platt et al. (1980):

$$P^B = P_S^B \left[1 - e^{\frac{-\alpha \times E}{P_s^B}}\right] \times e^{\frac{-\beta \times E}{P_s^B}}$$

where $P^B$ [mg C (mg Chl *a*)$^{-1}$ h$^{-1}$] is the primary production rate normalized to biomass concentration (B, mg Chl *a* m$^{-3}$), *E* is the incubation irradiance (µmol photons m$^{-2}$ s$^{-1}$), $P_s^B$ [mg C (mg Chl *a*)$^{-1}$ h$^{-1}$] is the saturated rate of Chl *a* normalized photosynthesis in the absence of photoinhibition, $\alpha^B$ [mg C (mg Chl *a*)$^{-1}$ h$^{-1}$ (µmol photons m$^{-2}$ s$^{-1}$)$^{-1}$], representing photosynthetic efficiency, is the initial slope as *E* approaches zero, and $\beta^B$ [mg C (mg Chl *a*)$^{-1}$ h$^{-1}$ (µmol photons m$^{-2}$ s$^{-1}$)$^{-1}$] is the photoinhibition parameter. The attained maximum photosynthetic rate ($P_{max}^B$) was calculated using the formula: $P_s^B\,(\alpha/(\alpha+\beta))(\beta/(\alpha+\beta))^{\beta/\alpha}$ and the photo-adaptation parameter ($E_k$, µmol photons m$^{-2}$ s$^{-1}$) was estimated as the ratio $P_{max}^B/\alpha^B$. The light intensity which photosynthesis is maximum is regarded as optimum irradiance and obtained from the P–E curves ($E_{op} = (P_s^B/\alpha^B)$ $(\alpha/(\alpha+\beta))(\beta/(\alpha+\beta))^{\beta/\alpha}$). Fitting the P–E equation to the data was done by least-squares optimization using the "optim" routine available in R (R Development Core Team, 2005). All the parameters function fit the experimental data extremely well, with 90% of the models having $r^2$ values above 0.98.

Daily total photosynthetically active radiation at surface ($E$) for the study period was taken from global hourly radiation measurements at S. Julião do Tojal (ca. 10 km from the sampling sites) meteorological station (obtained from INAG). Maximum daily surface irradiances ($E_{max}$) were obtained from INAG, and changes in irradiance over the day were described according to Parsons et al. (1984). Assuming that light attenuation coefficient remained constant over the day, the irradiance at time $t$ and depth $z$ [$E_{(t,z)}$] was determined according to: $E_{(z,t)} = E_{max} \sin^3(\pi t/D) e^{-K_{par} z}$, where $E_{(t)}$ is the irradiance at time $t$ (μmol photos $m^{-2}$ $s^{-1}$), $E_{max}$ the maximum daily irradiance at the surface (μmol photos $m^{-2}$ $s^{-1}$), $t$ is the time past dawn (h) and $D$ is the daylength (h) (Parsons et al., 1984). Each daylength was divided in 50 time intervals. For integrating total daily water column productivity at depth $z$ ($P_{(t)}$; mg C $m^{-3}$ $h^{-1}$): $P_{(t)} = \sum_{z=0}^{z=z_{max}} P_{(z,t)} \Delta z$ with an integration step of $\Delta z = 0.1$ m. Then, $P_{(z,t)} = \int_0^{\infty} \int_0^{D} P_{(z,t)} dt dz$. The estimative of daily integral photosynthesis was computed as described in Platt et al. (1990).

The depth at which light is saturating, when irradiance was $> E_{op}$ was considered as the bottom of the light-saturated zone. Production rates estimative for dates other than the sampling days were calculated using daily irradiance data, and assuming that $K_{par}$ and Chl $a$ concentration measured on the sampling dates were constant for that month. Annual areal primary production was estimated by summing the values of daily rates obtained for each site. Tagus estuary annual areal primary production rates were calculated from the average of both sites.

### Statistical analyses

Basic statistics and linear regression analysis were calculated using the Statsoft Statistica 6.0. Spearman's rank correlation coefficients were used to investigate statistical correlations between the various parameters. Multiple comparisons among pairs of means were performed using the $t$-test. Multivariate phytoplankton community analyses were carried out by using PRIMER 6.1.9 (PRIMER-E Ltd., Plymouth). The data were fourth-root transformed to down-weight the importance of the very abundant species. Similarities were calculated between every pair of samples, using the Bray–Curtis coefficient of similarity (Bray & Curtis, 1957). Analysis of similarities (ANOSIM) was used to test the significance of the difference between the different *a priori* groups of samples (sites and seasons). The similarity percentages routine (SIMPER) was used to identify the level of within-group sample similarity and to identify the species that contributed most to this average similarity.

## Results

### Environmental parameters

Mean monthly surface incident irradiance calculated from daily measurements throughout the study period ranged from 135 (December and January) to 470 μmol photons $m^{-2}$ $s^{-1}$ (July) (Fig. 2a). The highest monthly average wind speed (1.6 m $s^{-1}$) was recorded during August and the lowest (0.6 m $s^{-1}$) at November. River discharge from Tagus river varied from an exceptionally high discharge in early winter (1,450 $m^3$ $s^{-1}$, November) to a very low flow during spring (80 $m^3$ $s^{-1}$, April), which according to the Martins et al. (1984) relationship, corresponds to residence times of 8 and 70 days, respectively. Total monthly rainfall displayed a similar seasonal pattern (Fig. 2b).

There was no difference in water temperature between sites ($P = 0.396$). Summer water temperatures ranged from 21°C up to 26°C, and 12°C was the mean temperature during winter (Table 1). Marked salinity differences were observed between the sites throughout the study period, more evident during summer ($P < 0.001$). A significant negative correlation between salinity and river discharge was found at both sites ($r = -0.79$; $P < 0.05$; $n = 10$ for S1 and $r = -0.86$; $P < 0.05$; $n = 7$ for S4). Vertical light attenuation coefficients and euphotic zone depth varied without any identifiable seasonal pattern. Light extinction coefficients were always higher at S1 than at S4 (Table 1). The depth of the euphotic zone increased from an annual mean of 2.6 m at S1 to 5.0 m at S4. The ratio euphotic depth:total depth ($Z_{eup}/Z_t$) at both sites ranged between 0.5 and 1.7; The average $Z_{eup}/Z_t$ ratio was 0.8 and 1.0 at S1 and S4, respectively (Table 1). The euphotic depth exceeded the actual water column depth ($Z_t < Z_{eup}$)

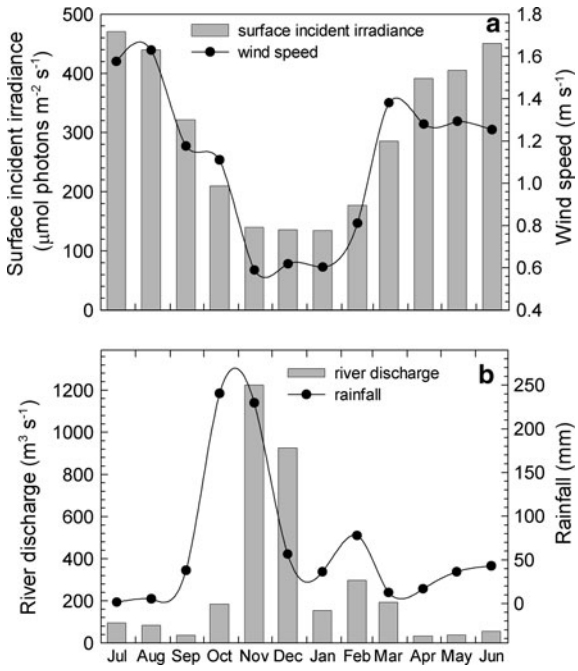


**Fig. 2** Environmental parameters for the Tagus estuary, from July 2006 to June 2007. **a** Mean monthly surface incident irradiance (µmol photons $m^{-2}$ $s^{-1}$) and monthly average wind speed (m $s^{-1}$) (data from National Meteorological Institute, IM); **b** mean monthly river discharge ($m^3$ $s^{-1}$) and total monthly rainfall (mm) (data from INAG)

in 17 and 44% of the sampling dates at S1 and S4, respectively. A significant negative correlation between light attenuation coefficient and salinity ($r = -0.76$; $P < 0.001$; $n = 21$) was found. $E_m$ ranged from 64 to 252 µmol photons $m^{-2}$ $s^{-1}$. Except for April, S1 always had the lowest mean water column irradiance (Table 1).

Higher concentrations of dissolved nutrients were generally found at S1 than at S4 ($P < 0.01$) (Fig. 3). Changes in DIN ($NO_3^- + NO_2^- + NH_4^+$) concentrations showed a clear seasonal variation, ranging from 36 to 158 µM at S1 and from 14 to 98 µM at S4 during summer and winter, respectively (Fig. 3a), suggesting that nitrogen is not a limiting nutrient. $NO_3$ was the major DIN component throughout the year (> 65%). Dissolved silicate concentration also showed a seasonal pattern reaching maximum values during winter (Fig. 3b). A significant negative correlation between salinity and DIN was found ($r = -0.77$; $P < 0.0001$; $n = 21$). Silicates concentrations displayed a seasonal evolution similar to that of DIN.

**Table 1** Water temperature (°C), salinity, light attenuation coefficient ($K_{par}$, $m^{-1}$), euphotic depth ($z_{eup}$, m), total depth:euphotic depth ratio ($z_{eup}/z_t$) and mean euphotic zone irradiance ($E_m$, µmol photons $m^{-2}$ $s^{-1}$) obtained at S1 and S4 from July 2006 to June 2007

| Date | Water temp. | Salinity | $K_{par}$ | $Z_{eup}$ | $Z_{eup}/Z_t$ | $E_m$ |
|---|---|---|---|---|---|---|
| S1 | | | | | | |
| Jul | 26.3 | 10 | 2.5 | 1.9 | 0.5 | 252 |
| Aug[a] | 24.0 | 11 | 1.4 | 3.4 | 1.1 | 150 |
| Sep | 21.5 | 15 | 1.9 | 2.5 | 0.6 | 131 |
| Oct[a] | 19.1 | 17 | 1.3 | 3.6 | 1.2 | 93 |
| Nov | 18.0 | 5 | 2.3 | 2.0 | 0.6 | 119 |
| Dec | 12.2 | 4 | 2.0 | 2.2 | 0.6 | 64 |
| Jan | 11.0 | 11 | 1.5 | 3.0 | 1.0 | 65 |
| Feb | 14.0 | 4 | 1.7 | 2.7 | 0.8 | 195 |
| Mar | 13.7 | 10 | 2.0 | 2.3 | 0.7 | 200 |
| Apr | 20.5 | 10 | 1.8 | 2.6 | 0.7 | 97 |
| May | 19.0 | 15 | 1.7 | 2.7 | 0.8 | 165 |
| Jun | 21.5 | 15 | 1.9 | 2.4 | 0.7 | 121 |
| S4 | | | | | | |
| Jul | – | – | – | – | – | – |
| Aug | 21.8 | 31 | 1.2 | 3.9 | 0.7 | 197 |
| Sep | 21.1 | 30 | 1.0 | 4.7 | 0.9 | 175 |
| Oct[a] | 18.9 | 34 | 0.8 | 5.7 | 1.4 | 98 |
| Nov | – | – | – | – | – | – |
| Dec | – | – | – | – | – | – |
| Jan[a] | 12.0 | 20 | 1.2 | 3.8 | 1.1 | 90 |
| Feb | 14.0 | 14 | 1.1 | 4.3 | 0.7 | 248 |
| Mar[a] | 14.5 | 23 | 0.7 | 6.4 | 1.1 | 102 |
| Apr[a] | 20.0 | 27 | 0.6 | 8.4 | 1.7 | 71 |
| May | 18.8 | 32 | 0.9 | 5.1 | 1.0 | 181 |
| Jun | 20.7 | 26 | 1.7 | 2.8 | 0.5 | 225 |

[a] Euphotic depth exceeds the total depth ($Z_{eup} > Z_t$)

Significant positive correlation was obtained between river discharge and silicates ($r = 0.72$; $P < 0.0001$; $n = 21$). An opposite seasonal pattern to that of DIN and silicate was observed for dissolved phosphate, with the highest values occurring during summer (Fig. 3c). At both sites, Si:P > 3 and silicate concentration below 5 was never found, indicating that silicate was not a limiting nutrient. Although Si:P > 3 was always found, the lowest $PO_4$ concentration was 2.8 µM, suggesting that phosphate was not a limiting nutrient.

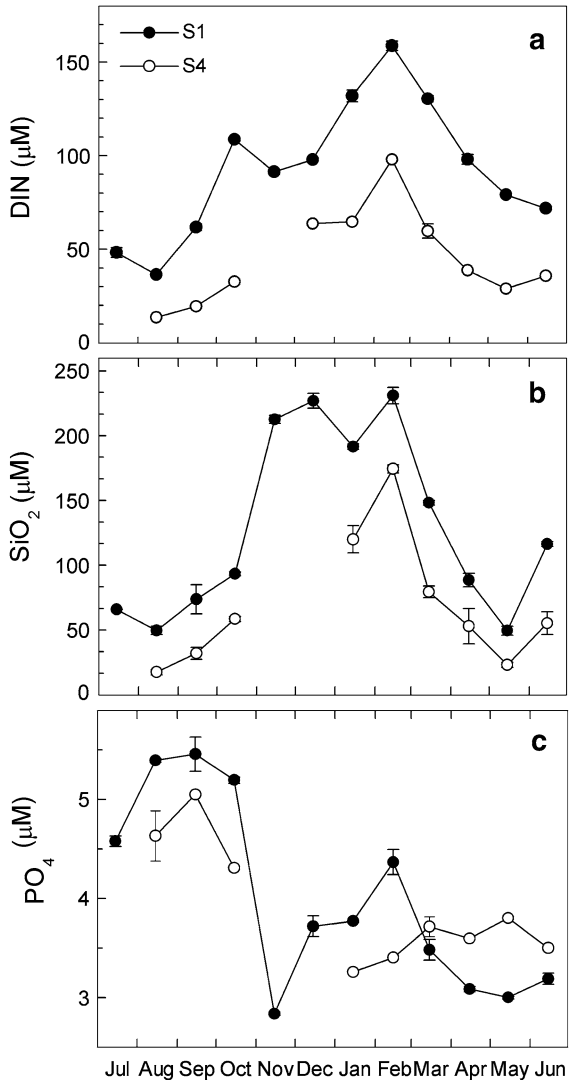


**Fig. 3** Seasonal variation of dissolved nutrients concentration (average ± standard deviation) during the sampling period. **a** DIN ($NO_3^- + NO_2^- + NH_4^+$, µM), **b** silicate ($SiO_2$, µM) and **c** phosphate ($PO_4$, µM) at sampling sites: S1 (*filled circle*) and S4 (*open circle*)

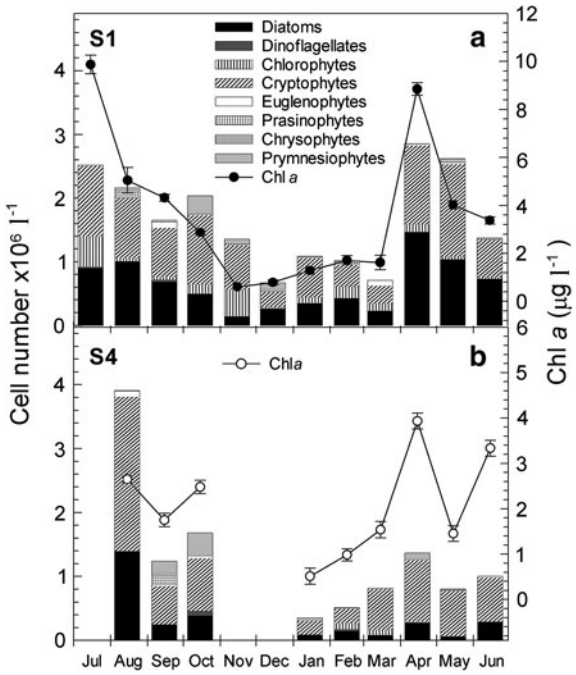


**Fig. 4** Seasonal variation of phytoplankton biomass in terms of Chl *a* (µg $l^{-1}$) (*line plot*) and in terms of cell number per litre of different groups estimated by microscopy (*bars plot*) at the two sampling sites of the Tagus estuary: **a** S1 (*filled circle*) and **b** S4 (*open circle*)

## Phytoplankton species composition

Phytoplankton cells were mostly diatoms and cryptophytes, both corresponding to an average of 84% of total cells at both sites (Fig. 4). At the riverine site (S1), diatoms were dominated by small species of the genera *Chaetoceros* (*Chaetoceros subtilis* var. *abnormis* f. *simplex*, C. *throndsenii* var. *throndsenia*), *Cylindrotheca closterium*, *Leptocylindrus minimus*, *Navicula* sp. and *Skeletonema costatum*. This assemblage comprised the major fraction of the community during August, December, February, April and June. During the remaining months, the phytoplankton composition was dominated by cryptophytes (Fig. 4a), mainly *Plagioselmis* sp., *Rhodomonas salina* and *Teleaulax acuta*. Chlorophytes, mainly *Ankistrodesmus falcatus*, *Monoraphidium contortum* and *Scenedesmus quadricauda*, were most common in November, reaching 0.42 × $10^6$ cells $l^{-1}$. Taxonomic composition at S4 was similar to S1 with the exception of spring months when *Ditylum brightwelii* contributed considerably to the phytoplankton community of site S4. Throughout the study period, chlorophytes represented an average of 4% of the total phytoplankton cells at S4, reaching the highest abundance (19%) during February (Fig. 4b). Prasinophytes and euglenophytes had highest cell abundances during the late summer–early autumn period (Fig. 4b).

Analysis of similarities (ANOSIM) showed no spatial differences in species composition between the sites. In contrast, significant dissimilarities were

found between seasons ($R = 0.419$; $P = 0.001$), revealing seasonality on species composition with summer and winter having the highest pairwise test $R$ value ($R = 0.669$; $P = 0.002$). According to the SIMPER analysis, *Plagioselmis* sp. and *Skeletonema costatum* were the most representative species in summer, whereas *Ankistrodesmus* sp. and small flagellates dominated the winter assemblages.

## Phytoplankton biomass and primary production rates

Phytoplankton biomass (Chl *a*) showed strong seasonal variation (0.6–9.9 μg $l^{-1}$) at both sites (Fig. 4), with a maximum in summer (July) and spring (April) at S1 (Fig. 4a). During the rest of the year, phytoplankton biomass was persistently low (< 4 μg $l^{-1}$). Chl *a* concentrations were generally higher at S1 than at S4 but not significantly so. Temperature and light were the only environmental parameters that were significantly correlated with biomass (Chl *a*) (Table 2). Accordingly, higher values of phytoplankton abundance were found at S1 (0.71–2.85 × $10^6$ cells $l^{-1}$) than at S4 (0.35–3.91 × $10^6$ cells $l^{-1}$), except during August (Fig. 4).

The highest primary production rates ($P^B$) were observed in summer and photoinhibition was present in all P–E curves (Fig. 5). The estimated mean daily primary production rate varied during the study between 778 mg C $m^{-2}$ $day^{-1}$ (in July) and 2 mg C $m^{-2}$ $day^{-1}$ (in November), both at S1, showing a marked seasonal pattern (Fig. 6). Primary production rates were extremely significant correlated with Chl *a* ($r = 0.89$, $P < 0.001$) and with mean daily irradiance ($E_0$) ($r = 0.75$, $P < 0.001$). Annual areal primary production was estimated as 92 g C $m^{-2}$ $year^{-1}$ for S1 and 77 g C $m^{-2}$ $year^{-1}$ for S4. Thus, a mean annual areal primary production for Tagus estuary was estimated as 85 g C $m^{-2}$ $year^{-1}$.

## Photosynthetic parameters

Chl *a* normalized P–E parameters values ($P^B_{max}$ and $\alpha^B$) showed a clear seasonality (Fig. 7). Highest $P^B_{max}$ and $\alpha^B$ values were observed during spring and summer and both sites exhibited similar photosynthetic parameters, except for September and April (Fig. 7a, b). $P^B_{max}$ average was 4.3 mg C (mg Chl *a*)$^{-1}$ $h^{-1}$ and ranged between 1.0 and 8.4 mg C (mg Chl *a*)$^{-1}$ $h^{-1}$ (December and July, respectively) (Fig. 7a; Table 3). Maximum $\alpha^B$ was observed in May (0.088 mg C (mg Chl *a*)$^{-1}$ $h^{-1}$ μmol photons $m^{-2}$ $s^{-1}$ as both sites mean) (Fig. 7b). The $E_k$ values ranged between 50 and 140 μmol photons $m^{-2}$ $s^{-1}$, in December and July, respectively (Fig. 7c).

The photosynthetic parameters $P^B_{max}$ and $\alpha^B$ were strongly correlated (Table 2). A strong positive linear correlation was found between water temperature and $E_k$ ($r = 0.92$; $P < 0.001$; $n = 21$) (Fig. 8a; Table 2), and the best fit for $P^B_{max}$ and temperature was an exponential model ($R^2 = 0.83$; $P < 0.001$; $n = 21$) (Fig. 8b). Most of the variability regarding $P_{max}$ and $\alpha$ parameters was a direct result of fluctuations in phytoplankton biomass, as shown by the high

**Table 2** Correlation coefficients (*r*) between photosynthetic parameters, biological and environmental factors

| | $\alpha^B$ | $P^B_{max}$ | $E_k$ | $T$ | $E_0$ | Sal | DIN | $PO_4$ | $SiO_2$ | Chl *a* |
|---|---|---|---|---|---|---|---|---|---|---|
| $\alpha^B$ | 1.00 | | | | | | | | | |
| $P^B_{max}$ | **0.86*** | 1.00 | | | | | | | | |
| $E_k$ | – | 0.73 | 1.00 | | | | | | | |
| $T$ | **0.92*** | **0.83*** | 0.92 | 1.00 | | | | | | |
| $E_0$ | 0.56 | 0.58 | 0.59 | 0.61 | 1.00 | | | | | |
| Sal | – | – | – | – | – | 1.00 | | | | |
| DIN | – | −0.58 | −0.63 | −0.63 | – | **−0.77*** | 1.00 | | | |
| $PO_4$ | – | – | 0.47 | – | – | – | – | 1.00 | | |
| $SiO_2$ | −0.64 | −0.74 | −0.64 | −0.70 | −0.46 | **−0.76*** | **0.83*** | – | 1.00 | |
| Chl *a* | – | 0.64 | 0.72 | 0.73 | 0.69 | – | – | – | – | 1.00 |

All the values shown are significant ($P < 0.05$). Values shown in bold with an asterisk are significant at $P < 0.001$ ($n = 21$; – represents not significant)

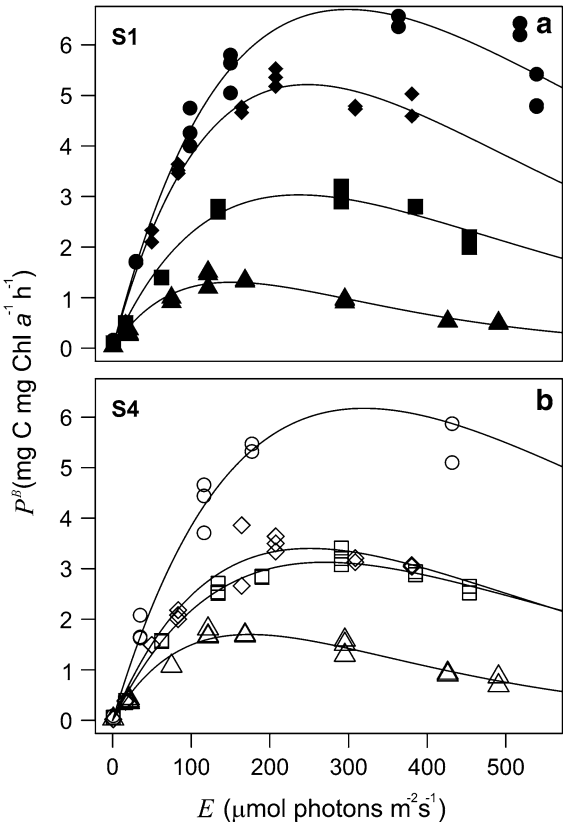


**Fig. 5** Examples of observed P–E curves and plotted with the adjusted Platt et al. (1980) model at site S1 (*black symbols*) (**a**) and S4 (*empty symbols*) (**b**): *filled circle*, *open circle*—summer (August); *filled square*, *open square*—autumn (October); *filled triangle*, *open triangle*—winter (January) and *filled diamond*, *open diamond*—spring (April)

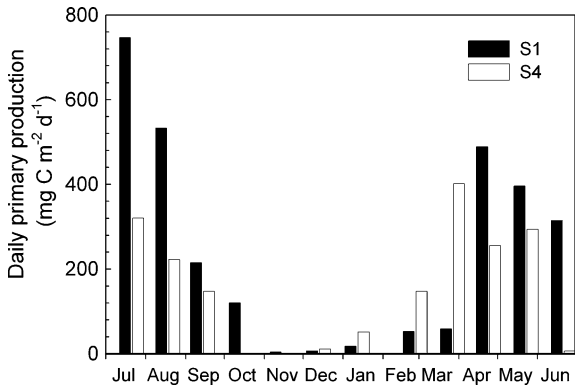


**Fig. 6** Sampling dates estimated mean daily primary production rate (mg C $m^{-2}$ $h^{-1}$) at S1 (*black bars*) and S4 (*white bars*)

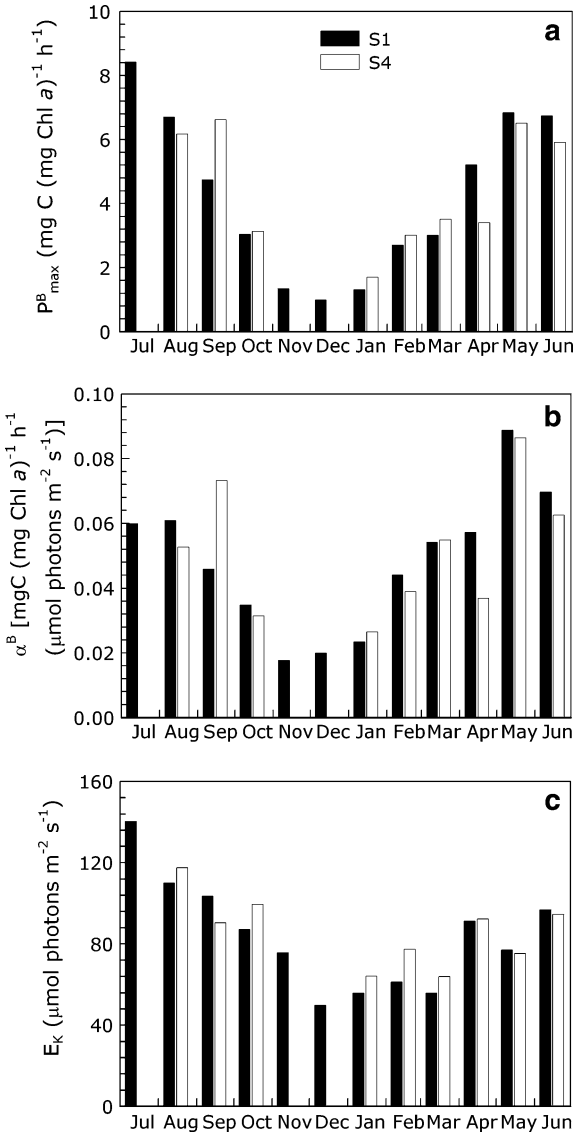


**Fig. 7** Annual cycle of P–E parameters at site S1 (*black bars*) and S4 (*white bars*) from July 2006 to June 2007. **a** Maximal rate of Chl *a*-specific photosynthesis ($P^B_{max}$); **b** initial P–E curve slope ($\alpha^B$) and **c** photo-adaptation parameter ($E_k$)

correlation between Chl *a* and both $P_{max}$ and $\alpha$ ($r = 0.95$ and $r = 0.96$ respectively, $P < 0.001$) at both sites. $E_m/E_{op}$ ratios were commonly lower than 1 throughout the sampling period, except during February (both sites) and March (S1) (Fig. 9).

**Table 3** Average ± standard deviation of photosynthetic parameters ($\alpha^B$ and $P^B_{max}$) and $E_k$ for the two sampling sites during this study

| | S1 | S4 |
|---|---|---|
| [a]$\alpha^B$ | 0.048 ± 0.021 | 0.051 ± 0.020 |
| [b]$P^B_{max}$ | 4.3 ± 2.5 | 4.4 ± 1.9 |
| [c]$E_k$ | 84 ± 27 | 86 ± 18 |

[a] Units mg C (mg Chl *a*)$^{-1}$ h$^{-1}$ µmol photons m$^{-2}$ s$^{-1}$

[b] Units mg C (mg Chl *a*)$^{-1}$ h$^{-1}$

[c] Units µmol photons m$^{-2}$ s$^{-1}$

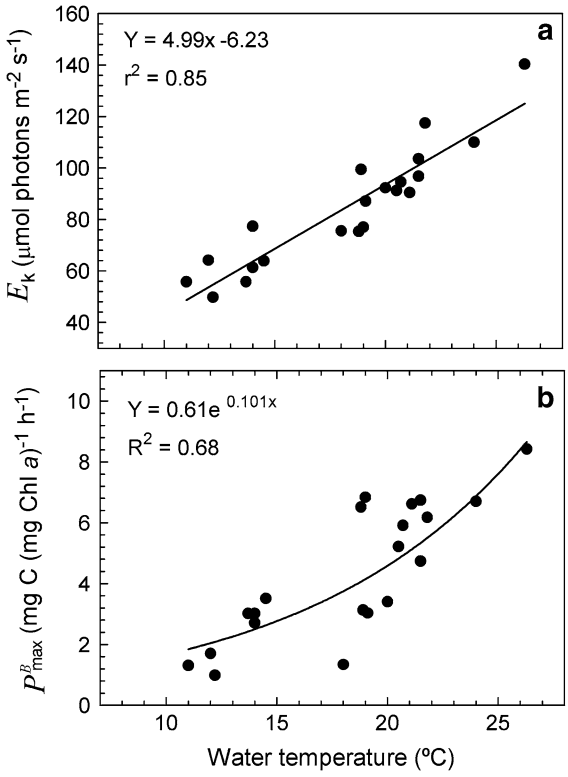


**Fig. 8** **a** Relationship between water temperature (°C) and photo-adaptation parameter ($E_k$, µmol photons m$^{-2}$ s$^{-1}$); **b** regression of water temperature versus maximum rates of primary production normalized to Chl *a* ($P^B_{max}$, mg C (mg Chl *a*)$^{-1}$ h$^{-1}$)

## Discussion

As in many estuarine systems, the seasonal pattern of nutrient concentration in the Tagus estuary was strongly linked to freshwater input, especially concerning DIN and silicates, as shown previously (Cabrita & Moita, 1995; Gameiro et al., 2007). The

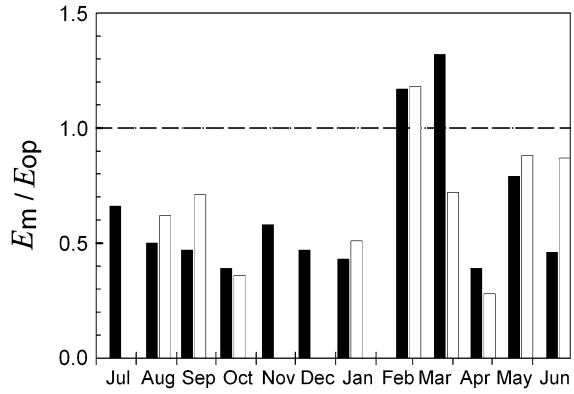


**Fig. 9** Ratio of daily mean water column irradiance ($E_m$) and saturating light intensities ($E_{op}$) during the sampling period at site S1 (*black bars*) and S4 (*white bars*). The *dotted line* ($E_m/E_{op} = 1$) is the upper limit to light limitation

opposite pattern for phosphate can be associated with the close relationship between the increase in water temperature during the summer and the phosphate sediment release (Nixon et al., 1980). Although, a dramatic decrease in DIN and silicate concentrations was found in summer when the river flow was particularly low, their concentrations were always higher than those considered limiting for phytoplankton growth (Fisher et al., 1988; Dortch & Whitledge, 1992). These results provide strong evidence that the nutrient dynamics is dominated by terrestrial inputs, which are in general high enough to not limit phytoplankton growth. The non-limitation by nutrients is furthermore confirmed by the lack of significant relationships between the latter and primary production or phytoplankton biomass (Chl *a*).

Primary production rates and phytoplankton biomass (as Chl *a* and cell number) showed considerable seasonal variation in the Tagus estuary. As in many turbid and shallow estuaries, primary production rates and phytoplankton biomass were low during most of the year, with maximum values observed during late spring and summer, when temperature and radiation peaked. This fact support the perception that in a nutrient-replete estuary, light availability and water column temperature are the key factors behind the primary production rates and phytoplankton biomass (Wofsy, 1983; Cole & Cloern, 1984; Cole et al., 1986). As a result, in Tagus estuary, the phytoplankton annual biomass is generally unimodal (Gameiro et al., 2007), with occasional high phytoplankton

growth events triggered by sporadic high temperature conditions (e.g. spring 2007—present study; winter 2005—Gameiro et al., 2007; autumn 1995—Cabrita, 1997). Overall, the phytoplankton biomass and production found in the Tagus estuary are relatively lower than the maximum values observed in other nutrient-rich estuaries in the world as cited in Monbet (1992) and Underwood & Kromkamp (1999). Previous studies have indicated that primary production can be very low in coastal plain and river-dominated estuaries, because of the high turbidity caused by river inputs of suspended particulate matter and by resuspension of bottom sediments (Cloern, 1987; Irigoien & Castel, 1997) which absorb and scatter light, limiting phytoplankton production (Kromkamp et al., 1995). Our results show that the mean euphotic zone irradiance ($E_m$) was lower than the saturating light intensities ($E_{op}$), which means that light within the water column was not sufficient to attain $P^B_{max}$. The ratio $E_m/E_{op}$ illustrate strong evidences that light have been one of the main limiting factor for photosynthesis in the water column throughout the study period. Furthermore, the percentage of total depth whereas mean water column irradiance was higher than $E_{op}$ ranged between 0 and 14%, revealing that photosynthesis was light-saturated at a very short water column layer. Therefore, the high attenuation of light creates a steep light gradient of which only a thin fraction is optimal or quasi-optimal for phytoplankton production: in the deeper section (with $E$ being less than 200 μmol photons $m^{-2}$ $s^{-1}$) photosynthetic rates are less than optimal, whereas in the upper water column (with $E$ being higher than 400 μmol photons $m^{-2}$ $s^{-1}$) photoinhibition starts to have a negative effect (cf. Fig. 5).

The photosynthetic characteristics of phytoplankton community at both sites show similar photosynthetic responses as evidenced by the P–E curves. Both photophysiological parameters varied seasonally, with higher values during summer. The obtained $P^B_{max}$ values (1.0–8.4 mg C (mg Chl $a$)$^{-1}$ $h^{-1}$) were within the range commonly found for other turbid estuaries (Table 4). The maximum $P^B_{max}$ found (8.4 mg C (mg Chl $a$)$^{-1}$ $h^{-1}$) was one-third of the theoretical $P^B_{max}$ upper limit value (25 mg C (mg Chl $a$)$^{-1}$ $h^{-1}$) established by Falkowski (1981) and relatively lower than the maximum values reported for other estuarine systems (Table 4). The positive relationship between $P^B_{max}$ and temperature is already well recognized as one of the most important factors for phytoplankton growth (Eppley, 1972), and has been used in remotely sensed global models as one of the most important predictor of the maximum production rates (Behrenfeld & Falkowski, 1997). In fact, this strong relationship has been observed in many temperate waters of the North Atlantic (Bouman et al., 2005). Temperature was expected to be a robust predictor of the maximum rate of photosynthesis because enzymes involved in the photosynthetic reactions are temperature controlled. In the Tagus estuary, temperature had an extremely significant correlation with maximum production rates, accounting for 83% of the variability of the $P^B_{max}$ (Table 2). However, the effect of temperature might be confounded, because temperature and irradiance were positively correlated, which in this case will result in an overstatement of its effect.

According to Behrenfeld et al. (2002), the improved light conditions during summer promote smaller-sized phytoplankton with higher rates of productivity per unit of Chl $a$ (Geider et al., 1986; Raven, 1998). This was probably not the case in the Tagus estuary because small phytoplankton cells (diatoms and cryptophycean flagellates) dominated the phytoplankton community throughout the year without a clear seasonality. A significant positive correlation was found between daily solar irradiation and $P^B_{max}$, suggesting a contribution of species composition in maximum production rates variance. On the other hand, the relatively low $P^B_{max}$ and an increase in $P^B_{max}$ when the water column light improves, as observed in Tagus estuary, points to shade-adapted phytoplankton community, according to light-shade adaptation[1] theory (Dubinski, 1980). This result is further emphasized by the dominance of cryptophycean flagellate cells, which are known to photosynthesize in low-light condition (Hammer et al., 2002).

From the analysis of the $\alpha^B$ values, the Tagus estuary phytoplankton assemblages appear to have higher photosynthetic efficiency when compared to other estuarine communities (Table 4). This result does not seems spurious as it has been previously detected (Cabeçadas, 1999) and may result from the

[1] The term "adaptation" is used here on a physiological level, as a phenotypic response of algae to changes in irradiance at the organism level (Falkowski & LaRoche, 1991).

**Table 4** Phytoplankton photosynthetic parameters ranges characteristic of some world estuaries and coastal areas: the maximum photosynthetic rate [$P^B_{max}$ (mg C (mg Chl *a*)$^{-1}$ h$^{-1}$)], *P–E* initial slope [$\alpha^B$, (mg C (mg Chl *a*)$^{-1}$ h$^{-1}$ µmol photons m$^{-2}$ s$^{-1}$)] and the light-saturation parameter [$E_k$ (µmol photons m$^{-2}$ s$^{-1}$)]

| | $P^B_{max}$ | $\alpha^B$ | $E_k$ | References |
|---|---|---|---|---|
| Europe | | | | |
| Marennes-Oléron Bay | 5.1–13.5 | 0.011–0.066 | 182–773 | Struski & Bacher (2006) |
| Scheld estuary | 0.5–18.8 | 0.002–0.080 | – | Kromkamp & Peene (1995) |
| Waddden Sea | 0.8–9.9 | 0.007–0.039 | 67–538 | Tillmann et al. (2000) |
| Tagus estuary | 2.4–4.0 | 0.100–0.200 | 14–60 | Cabeçadas (1999) |
| | 1.0–8.4 | 0.018–0.089 | 50–140 | This study |
| USA | | | | |
| Baja California | 0.5–11.3 | 0.004–0.028 | 102–917 | Aguirre-Hernández et al. (2004) |
| Delaware estuary | 1.0–22.7 | 0.020–0.064 | 61–218 | Pennock & Sharp (1986) |
| Gulf Mexico | 3.0–22.1 | 0.008–0.045 | 125–1268 | Lohrenz et al. (1994) |
| Neuse river estuary | 0.1–33.9 | 0.001–0.012 | <393 | Boyer et al. (1993) |

dominance of small cryptophytes, chlorophytes and diatoms (< 10 µm) which seem to be able to cope with low light conditions quite well, due to low respirations rates (Hammer et al., 2002). Thus, it is not surprising that they form the dominating part of phytoplankton assemblage. According to Sakshaug et al. (1997), living in low-light marine environment stimulates an increase in phytoplankton photosynthetic efficiency, while the maximum photosynthetic rate expressed per Chl *a* is reduced. Our results corroborate the earlier Sakshaug et al. (1997) findings. Furthermore, the photo-adaptation parameter ($E_k$), an indicator of photo-acclimation status (Talling, 1957), was consistently low (50–140 µmol photons m$^{-2}$ s$^{-1}$) throughout the sampling period, when compared with values obtained from other studies (Table 4). The relatively low $E_k$ values, which were positively correlated with surface irradiation, indicate that the Tagus estuary phytoplankton community has the capacity to adjust $E_k$ in order to maintain an optimum balance between light and dark reactions of photosynthesis (Table 2).

Daily integral production rates, estimated from P–E relationships, ranged from 2 to 780 mg C m$^{-1}$ day$^{-1}$ during the sampling period, mirroring the seasonal changes in chlorophyll concentrations. As a direct result, the productions rates were higher in the summer at upper site (S1). Thus, paradoxical is to observe the highest Chl *a* concentrations and primary production rates at the highest turbid site. As found by Van Spaedonk et al. (1993), probably the phytoplankton population at upper site, consisting of mainly freshwater green algae, died when entering more saline condition (S4). In average, the Tagus estuary daily production rates are similar to those mentioned for other European and North American estuarine systems (Table 5). An estimated annual rate of areal primary production was computed for each site, using information on light availability and photophysiological properties of the phytoplankton assemblages. An annual production rate of 92 and 78 g C m$^{-2}$ year$^{-1}$ was calculated for S1 and S4, respectively. The mean production (85 g C m$^{-2}$ year$^{-1}$, two sites average) was typical of a light limited estuary, placing this system within the low to moderate phytoplankton primary production ones (Table 5).

According to Nixon (1995) trophic status classification, Tagus estuary is considered as oligotrophic (< 100 g C m$^{-2}$ year$^{-1}$) based on annual primary production rates. Although this provides a very general guideline for evaluating trophic state, it is difficult to validate because it is based on only one variable, which may vary widely on an annual basis, in addition to being a variable that is not typically included in routine monitoring programmes. More realistic is the Trophic Index for Marine Systems (TRIX), based on nutrient concentrations, Chl *a* levels and dissolved oxygen saturation (Vollenweider et al., 1998), which classifies the Tagus estuary as mesotrophic. Nevertheless, preliminary attempts to use this approach for evaluation of nutrient over-enrichment in a number of northern European coastal systems indicated some difficulties in its use, largely related to normalizing data in a way

**Table 5** Mean annual phytoplankton primary production estimates (g C $m^{-2}$ $year^{-1}$) for some estuaries and bays worldwide

| | Annual production (g C $m^{-2}$ $year^{-1}$) | References |
|---|---|---|
| Europe | | |
| Arcachon Bay | 103 | Glé et al. (2008) |
| Bristol channel | 7–165 | Joint & Pomroy (1981) |
| Büsum Wadden Sea | 152 | Tillmann et al. (2000) |
| Colne estuary | 8.9 | Kocum et al. (2002) |
| Marennes-Oléron Bay | 185 | Struski & Bacher (2006) |
| Marsdiep | 150–385 | Cadée & Hegeman (1993) |
| Schelde estuary | 100–300 | Kromkamp & Peene (1995) |
| Tagus estuary | 26 | Cabeçadas (1999) |
| | 85 | This study |
| USA | | |
| Chesapeake Bay | 347–662 | Harding et al. (2002) |
| Delaware estuary | 307 | Pennock & Sharp (1986) |
| Hudson River estuary | 70–220 | Cole et al. (1992) |
| Narragansett Bay | 160–619 | Oviatt et al. (2002) |
| Neuse River estuary | 395–493 | Boyer et al. (1993) |
| San Francisco Bay | 95–150 | Cole & Cloern (1987) |
| San Joaquim River Delta | 70 | Jassby et al. (2002) |

that allows valid comparisons within and between systems.

Predicting phytoplankton production is a key process for coastal ecosystem modellers. Although phytoplankton growth involves several factors (e.g. temperature, light, turbidity, nutrient concentrations, species composition, species abundance, mixing), simple mathematical models describing phytoplankton production in coastal systems have been produced. Cloern (1987) develop an empirical composite parameter, $BE_0Z_{eup}$, based on biomass (B), daily irradiance ($E_0$) and photic depth ($Z_{eup} = 4.6/K_{par}$) and found that a simple linear regression accounted for more that 80% of the variance of the net primary production (mg C $m^{-1}$ $day^{-1}$) in San Francisco Bay. To test the generality of this relation, we applied it to our dataset and regressed daily primary production against the composite parameter $BE_0Z_{eup}$. As in the work of Cole & Cloern (1987), the model is highly significant, ($R^2 = 82\%$) and the production is described as $P_{(z,t)} = 30 + 0.03\ BE_0Z_{eup}$. Being aware that our estimates of daily primary production are close to gross primary production (2 h incubation), and not net production, our result implies that physiological variability and external controls such as grazing can be a secondary control on phytoplankton production in nutrient-rich estuaries, or at least not nutrient limited, and that one simple empirical function can be used to estimate seasonal variation in productivity. Besides, it should be noted that the accuracy of calculated net water column productivity is dependent upon accurate measures of phytoplankton respiration, a process that is not easily measured and is not constant over time (Cole & Cloern, 1984). In conclusion, our study shows that primary production in the Tagus estuary is mainly regulated by turbidity-dependent low light conditions, which, despite the shallow water column, limits phytoplankton growth even during the warmer and higher irradiance summer months.

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