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Phytoplankton production in the Tagus estuary (Portugal)

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Abstract – Biomass and phytoplankton photosynthetic response were studied in the lower Tagus estuary weekly, and related to environmental conditions in February, March and April 1994. The Photosynthesis–Irradiance (P^BI) relation was studied based on the light-saturated photosynthesis rate (P^Bm) and the light-limited initial slope (a^B). The nutrient concentrations observed were high enough to be considered as not limiting phytoplankton growth. Tagus estuary phytoplankton seems, to a certain extent, adapted to high turbid conditions, being able to utilize the low light levels more efficiently, which was translated by high values of a^B [0.10–0.20 mg C (mg Chl a)$^{-1}$ h^{-1} (W m^{-2})$^{-1}$]; however, light seems to limit phytoplankton production in the water column. © Elsevier, Paris / Ifremer / Cnrs / Ird

primary production / phytoplankton / estuary / biomass / turbidity

Résumé – Production phytoplanctonique dans l'estuaire du Tage (Portugal). Une étude de la biomasse et de la réponse photosynthétique du phytoplancton en fonction des facteurs environnementaux a été menée dans l'estuaire du Tage, de février à avril 1994, (prélèvements hebdomadaires). La relation photosynthèse–éclairement (P^BI) a été étudiée sur la capacité photosynthétique en lumière saturante (P^Bm) et le rendement photosynthétique en lumière limitante (a^B). La concentration de sels nutritifs, pendant les mois étudiés, a été suffisamment élevée pour être considérée comme non limitante pour la croissance du phytoplancton. Le phytoplancton de l'estuaire du Tage semble, par sa photosynthèse, bien adapté à des conditions de haute turbidité ; les cellules augmentent le rendement d'utilisation des bas niveaux d'éclairement, ce qui se traduit par les hautes valeurs de a^B [0,10–0,20 mg C (mg Chl a)$^{-1}$ h^{-1} (W m^{-2})$^{-1}$] bien que l'éclairement semble être le facteur limitant de la production phytoplanctonique sur la colonne d'eau. © Elsevier, Paris / Ifremer / Cnrs / Ird

production primaire / phytoplancton / estuaire / biomasse / turbidité

1. INTRODUCTION

The total surface area of the Tagus estuary is approximately 320 km^2 and the intertidal area occupies up to 40 %. It is a shallow, mesotidal estuary, and the tidal amplitude ranges between 1.3 m during neap tides and 3.5 m in spring tides. The Tagus river is rain-fed with an average flow rate of 300 m^3 s^{-1}, but in extreme conditions it can be over 2000 m^3 s^{-1} [8]. The currents in the estuary are highly influenced by the tides which are a very important factor since the mean tidal volume is 7.5 × 10^8 m^3 compared to the mean estuary volume, 19 × 10^8 m^3 [31, 32]. The Tagus estuary can be partially stratified or vertically mixed, depending on the interaction between tide and river flow [7], and supports intensive and multiple utilizations such as wastewater discharge from Lisbon, shipping, fishing, industrial and agricultural activities, recreation and the presence of an internationally important nature conservation area covering ca.146 km^2.

In Europe, river-influenced open estuaries in which primary production has been measured are rather limited [16] and the factors controlling phytoplankton estuarine primary production are still not well known [2]. Primary production of estuaries, as estimated by various authors, was averaged by Smith and Hollibaugh [26] as 190 g C m^{-2} yr^{-1} excluding macrophytes, which may produce about the same, and according to Heip et al. [16], annual

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pelagic primary production values lower than 160 g C m^{-2} yr^{-1} in nutrient-rich systems are the result of light limitation. Although several studies were realized in the Tagus estuary concerning microphytobenthos and macrophyte algae production [3, 12] and chlorophyll *a* distributions related to physico-chemical parameters [6, 11, 22], there is still a lack of information regarding the phytoplankton primary production process. The objective of this work is to contribute to the knowledge of factors which may control phytoplankton primary production in the Tagus estuary, during the spring period. For the present study, one station localized in the lower estuary zone (Seixal bay) was chosen both because of its sampling facilities and because of previous work there on phytoplankton and nutrient distribution [4]. Seixal bay is a peripheral embayment, localized in the southern part of the Tagus estuary and dependent on its general circulation [7]. This bay receives industrial effluents from the industrial complex Barreiro/Seixal and waste water from an area with dense population.

2. MATERIAL AND METHODS

Water samples for measuring primary production and chemical analysis were taken weekly from February to April during 1994 in Seixal bay, lower Tagus estuary, (*figure 1*). On all occasions, sampling was done during high tide conditions. Temperature and salinity profiles were determined with a CTD (Valeport) probe, and calibrated with a reversed thermometer and an AutoSal salinometer. Discrete water samples were collected with a Niskin bottle, at half-metre depth intervals.

The vertical light attenuation coefficient (k_{PAR}, m^{-1}) was calculated using a Licor quantum meter with cosine-corrected cell (PAR, Photosynthetically Active Radiation model LI-192SA). Daily irradiation, I_0 (PAR, J cm^{-2} h^{-1}) was measured at Lisbon (Institute Infante D. Luís) with a Kipp solarimeter. Data were integrated at 1 h intervals.

Samples for Suspended Particulated Matter (SPM) and Chlorophyll *a* (Chl *a*) were filtered (Whatmann GF/F, 45 mm diameter). SPM was determined gravimetrically (2 h drying at 70 °C). Chl *a* in 90 % acetone extracts was analysed by fluorometry with a Turner Designs fluorometer, as described by Strickland and Parsons [28].

Samples for dissolved nutrients [$(NO_3^- + NO_2^-)$, NH_4^+, PO_4^{3-} and $Si(OH)_4$] were filtered (MSI Acetate Plus) and the analyses were carried out on an Autoanalyser Alliance Integral-Plus, following Tréguer and Le Corre [30].

A subsample of 120 mL was taken for phytoplankton counts, fixed with concentrated Lugol's solution, and the counts performed using the inverted microscope technique, after allowing water to settle for 24 h in a 10 mL chamber.

Photosynthesis measurements using the ^{14}C-method were performed as described by Strickland and Parsons [28], with some modifications: for example, the samples were incubated at ST. Seixal at six light levels (obtained using neutral filters), under solar radiation and flowing sea water conditions in an incubator. To duplicates of 125 mL in Jena glass bottles was added 1 mL of ^{14}C-HCO_3^- solution (4 µCi activity). Photosynthesis measurement began

Definition of symbols and their units

Symbols	**Definition**	**Units**
a^B	Light-limited initial slope of the P^B-I curve. photosynthetic "efficiency".	mg C (mg Chl *a*)$^{-1}$ h^{-1} (W m^{-2})$^{-1}$
B	Biomass. Concentration of Chl *a*	mg m^{-3}
I_0	24h-hour mean PAR irradiance immediately beneath the sea surface	W m^{-2}
Iz	Downwelling irradiance at depth z	W m^{-2}
I_k	Photosynthetic saturation irradiation, $I_k = a^B/P^Bm$	W m^{-2}
k_{PAR}	Diffuse attenuation coefficient for PAR irradiance	m^{-1}
P^B	Photosynthetic rate normalised to biomass	mg C (mg Chl *a*)$^{-1}$ h^{-1}
P^Bm	Photosynthetic rate at saturating irradiation.	mg C (mg Chl *a*)$^{-1}$ h^{-1}
Z	Depth	m
E	Specific attenuation coefficient of Chl *a*	(mg Chl *a*)$^{-1}$ m^2
Zeu	Euphotic layer depth	m
Zm	Mixed layer depth	m
Psq	Daily water column gross production	mg C m^{-2} d^{-1}

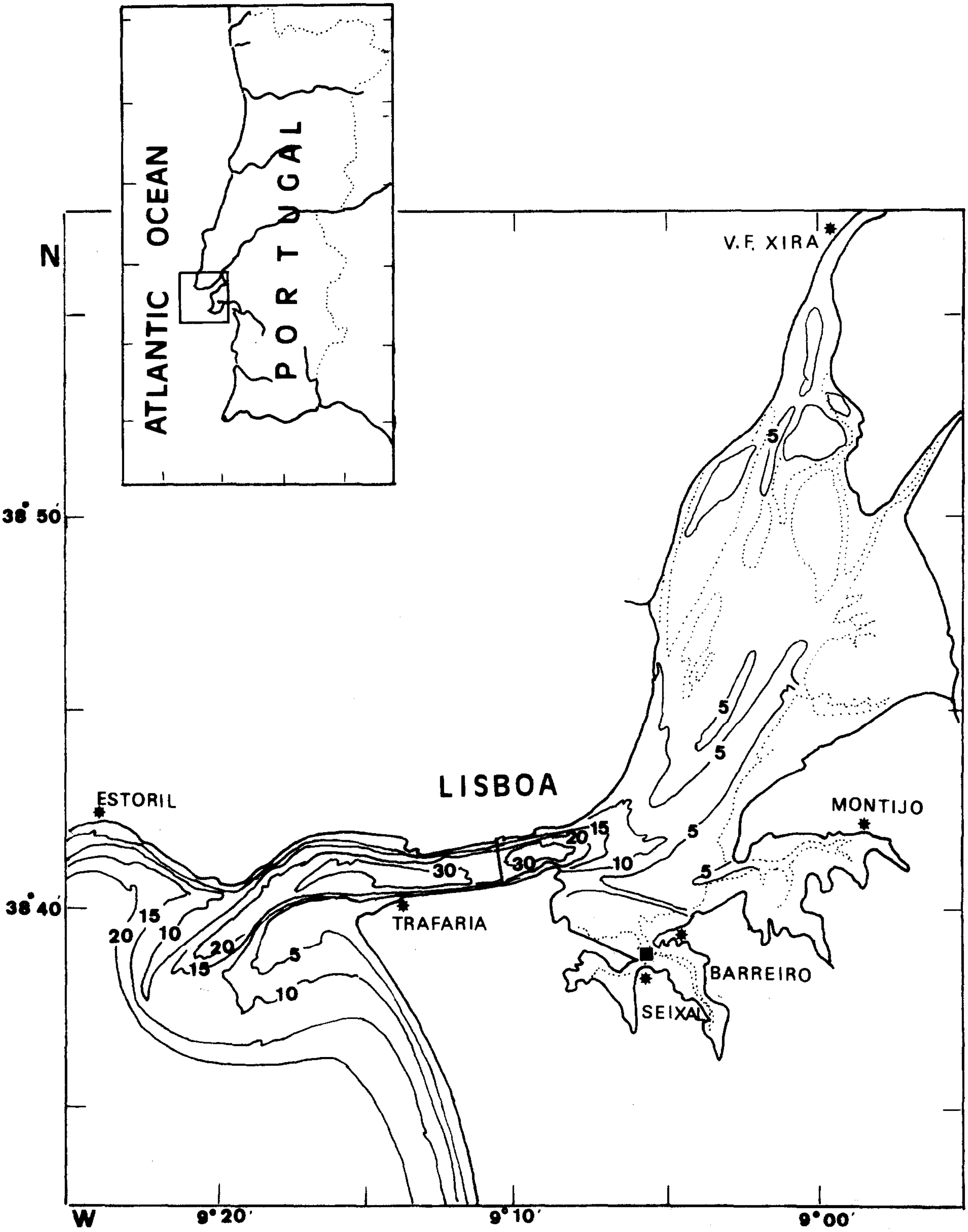

Figure 1. Location of the Tagus estuary, with the position of the sampling station at Seixal Bay, (■) lower estuary.

within 0.5 h of sampling and the incubation period took two hours, the samples being filtered (Whatmann GF/F, 25 mm diameter) afterwards. The filters were placed in HCl fume atmosphere for half an hour and then dried for 24 h. Scintillation counting was carried out after adding 10 mL of NE 216 solution in a Nuclear Enterprises scintillation counter (model: NE 6500). Quench correction was made using the internal standard method. Dissolved inorganic carbon was measured by potentiometric titration with 0.01 N HCl.

Gross photosynthesis data were fitted to the photosynthesis-light (P–I) curve by Talling's equation [29], nonlinearly, giving estimates of a^B (the initial slope of the chlorophyll *a*-normalized P–I curve) in mg C (mg Chl $a)^{-1}$ h^{-1} (W $m^{-2})^{-1}$ and $P^B{}_m$ (the photosynthetic rate at saturating irradiation) in mg C (mg Chl $a)^{-1}$ h^{-1}. The other parameter determined was: I_k (the photosynthetic saturation irradiation $I_k = P^B{}_m/a^B$) in W m^{-2}. Daily column gross production was calculated from the P–I curve and the daily irradiation.

To evaluate the relative importance of the variables determined over the period studied, a Correspondence Analysis (CA) was applied using the NTSYS-PC program, version 1.80.

The environmental, physico-chemical and biological variables selected for the analysis were: mean daily irradiance (I_0), daylength (Day), water column light vertical attenuation coefficient (K_{PAR}), water column photosynthetic parameters (a^B, P^Bm and I_k), daily production (Psq) and water column means (measurements each half-metre depth) of temperature (T), salinity (S), suspended particulate matter (SM), nitrate plus nitrite (NO_3), phosphate (PO_4), ammonium (NH_4), silicate [($Si(OH)_4$)] and Chlorophyll *a* (Chl *a*). The data were logarithmically transformed in order to reduce the proportion of variances explained by the different variables.

This study concerns a three-month period following a dry winter characterized by a low freshwater river input (INMG, 1993; IM, 1994). In February, the mean air temperature was 12.40 °C, total precipitation 114.4 mm, the wind was weak or moderate blowing predominantly from the southeast (18 km h^{-1}) and the sky was cloudy; in March, the air temperature was abnormally high (15.96 °C), the month extremely dry (total precipitation 6.3 mm) and the wind moderate from the northeast or southeast (7–15 km h^{-1}) the sky being in general clear; by April the temperature dropped to 15.40 °C, total precipitation reached 21.9 mm mostly from the 21st to the 25th.

3. RESULTS AND DISCUSSION

Water column mean temperature increased gradually during the period studied, ranging from 11.54 °C in February to 19.03 °C in the last week of April. A slight decrease compared to the last week of March (16.38 °C) was observed by the first and second weeks of April, with respectively 15.33 and 15.78 °C. Temperature was rather homogenous from surface to bottom. By contrast, water column salinity changed every week (*figure 2*). From the beginning of February to the beginning of March, mean salinity decreased from 29.07 to 17.99 ($-\delta S=11.1$). In the following week it increased to 26.74 ($+\delta S=8.75$); and again a slight decrease during the third week of March was observed. No vertical salinity stratification was observed throughout the water column except during the last week of April (vertical $\delta S=2.40$).

Figure 2 shows the mean concentration evolution during the spring period at ST. Seixal of Chl *a* in the water column. Chl *a* concentrations almost doubled from early to mid-March (*figure 2*). Chl *a* concentration decreased markedly at the beginning of April. The highest Chl *a* concentration reached values >2.5 mg m^{-3} and was observed during the last week of March and mid to late April. Ranges of phytoplankton biomass (Chl *a*) for February, March and April are given in *table I* and the highest are generally within the low ranges observed in other estuaries which are on the whole below 15 mg m^{-3} [2].

Suspended particulate matter (SPM) ranged from 5.4 to 25.0 mg L^{-1} with a mean value, over the study period, of 11.3. The vertical light attenuation coefficient (k_{PAR}) was always high, averaging 2.46 m^{-1}, which is a characteristic value for a turbid estuary (*figure 2*). During the period studied k_{PAR} was positively correlated with SPM through the water column ($r^2=0.80$). The high value of k_{PAR} (2.81 m^{-1}) observed on 15 March, could be related to some extent to the Cryptophyceae increase which occurred on that occasion (*figure 3*), whereas the highest value registered in mid-April (4.24 m^{-1}) was probably related to the amount of SPM of detrital origin.

Phytoplankton biomass (expressed as cells per L) increased from February to March (cell numbers doubled, see *figure 3*) and was uniformly distributed through the water column. The high biomass occurred during the first two weeks of March (0.6×10^6 and 1.2×10^6 cell L^{-1}), Cryptophyceae represented 66.7 % of the total phytoplankton biomass, on each occasion. In the third week of March, a phytoplankton decrease originating with the disappearance of the Cryptophyceae was observed. Phytoplankton increased slightly by the end of April, again with a dominance of Cryptophyceae. The Cryptophyceae increase expressed in cell numbers was not reflected in Chl *a* concentration. This was probably related to the fact that the accessory photosynthetic pigments of this group of algae are alloxanthin and phycobiliprotein. In fact, a high value of alloxanthin was found in the surface sediment, confirmed by HPLC measurements, at this site in previous years during the spring [3]. The rapid development of Cryptophyceae could have been the result of adaptation to rapid changes in salinity (from $-\delta S=11.1$ to $+\delta S=8.75$), temperature (from 13.95 to 15.88 °C), high nutrient uptake and growth rates and low light levels [27]. Cryptophyceae were indicated as an important group of the nanoplankton fraction in Seixal bay (Tagus estuary) and have been also reported in the Sado estuary (Portugal) for the spring period [5, 23] as well as in other shallow estuaries [20].

Nutrient concentrations were within normal ranges in mesotidal temperate estuaries for this time of the year, dissolved inorganic nitrogen exceeding 10 µM and phosphate approximately 2 µM, *table II* [13, 24, 33]. A clear decrease of PO_4^{3-} and NH_4^+ was observed in the third week of March (2.26 µM PO_4^{3-} and 6.00 µM NH_4^+), followed a week later by a clear decrease of NO_3^- (13.60 µM) to a certain extent associated with the increase in Cryptophyceae that occurred during the two first weeks of March. $Si(OH)_4$ did not seem related to phytoplankton growth in any occasion in agreement with the fact that diatoms were not the dominant group throughout this study. Nutrients never reached values that can be considered as limiting phytoplankton growth [13]. NH_4^+ concentrations were on some occasions above 30 µM, which may be considered an indication of organic pollution.

The evolution of photosynthetic "efficiency" a^B, expressed in mg C (mg Chl $a)^{-1}$ h^{-1} (W $m^{-2})^{-1}$ and photosynthetic rate at saturating irradiance P^Bm, in mg C (mg Chl $a)^{-1}$ h^{-1} at Seixal, can be followed, week-to-week, during the period studied (*figure 4*). The a^B results obtained for the phytoplankton, in which nanoplankton (<20 µm) is the dominant fraction, are in agreement with results previously published by Malone et al. [21] for an estuarine nanoplanktonic population. The P^Bm variation generally followed the phytoplankton biomass trend. P^Bm and a^B were not correlated, which is normal on a weekly time scale. The photosynthetic saturation irradiation I_k ranged from 13.5 to 59.7 W m^{-2} with a mean value of 23.2, which is found to be within the range of values for this type of estuary [16]. Therefore, these values of

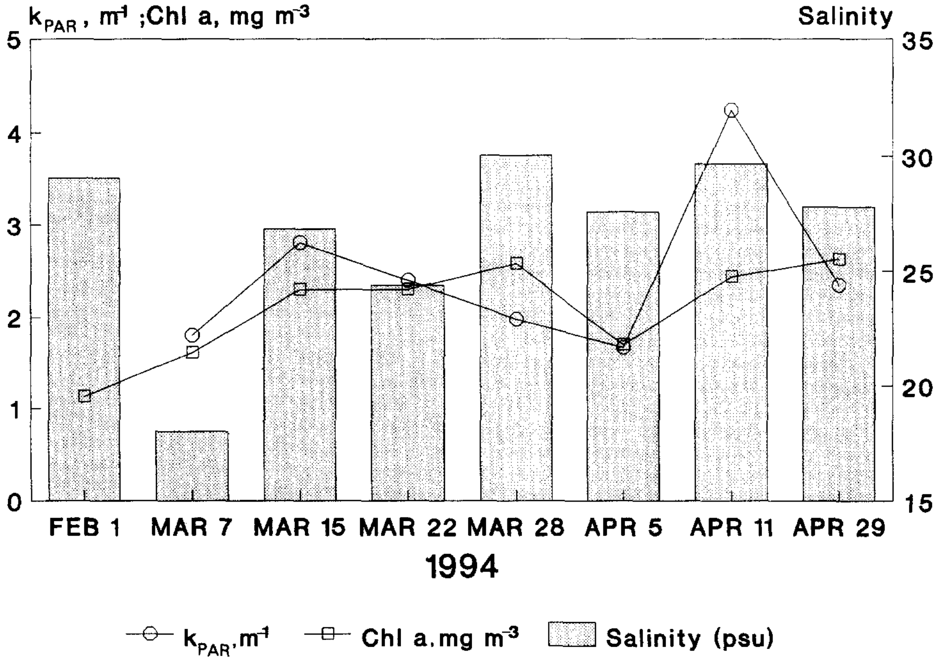

Figure 2. Week-to-week mean water column salinity (psu), Chl *a* (mg m^{-3}) and attenuation coefficient for downwelling PAR irradiance k_{PAR} (m^{-1}) evolution, at the lower Tagus estuary, from February to April 1994.

P^Bm and a^B characterize the photosynthetic activity of Tagus estuary phytoplankton, and are similar to those mentioned for marine phytoplankton [1, 9, 15, 25].

The specific attenuation coefficient of chlorophyll *a* (ε) depends on the size and shape of algae, the phytoplankton specific composition and the ratio of all photosynthetic pigments to Chl *a* [19, 10]. Actually, the in situ chlorophyll *a* (ε), was calculated at Seixal, for 29 April 1994, at different depths, from the regression of the water column light vertical attenuation coefficient and the Chl *a* concentration [10]. *Table III* shows the results obtained for the vertical light attenuation coefficient (k_{PAR}), Chl *a* concentration (Chl *a*) and the specific vertical in situ attenuation coefficient of Chl *a* (ε). Results obtained for ε ranged from 0.011 to 0.018 and are within the range of values obtained for waters of this type. The product of the Chl *a* by ε determines the absorbance fraction by phytoplankton, which was only 0.012 ± 0.004 of the total light absorption, in agreement with values found for highly turbid water. On the occasion referred to, the water column was well mixed, phytoplankton was homogeneously distributed and Cryptophyceae constituted the dominant group of phytoplankton biomass.

The evolution of daily gross production in the Tagus estuary (Seixal), during the spring period studied is shown in *figure 5*. Mean production, estimated by linear interpolation over the spring period and over the year, was respectively 71 mg C m^{-2} d^{-1} and 26 g C m^{-2} yr^{-1} which is a low estimation compared to other European estuaries (*table I*). However, there is a high degree of uncertainty in this type of interpolation, based on a single station and a short period of time (March and April 1994).

Turbidity may have an impact on losses of gross primary production through algal respiration. The mixing zone, Zm, reaches the total water depth and the euphotic zone, Zeu (defined as the depth of 1 % of irradiance at surface), is in general as deep as the mixing zone, except on two occasions (28 March and 11 April 1994), when Zeu/Zm ratios were <1, respectively 0.8 and 0.4. The average light

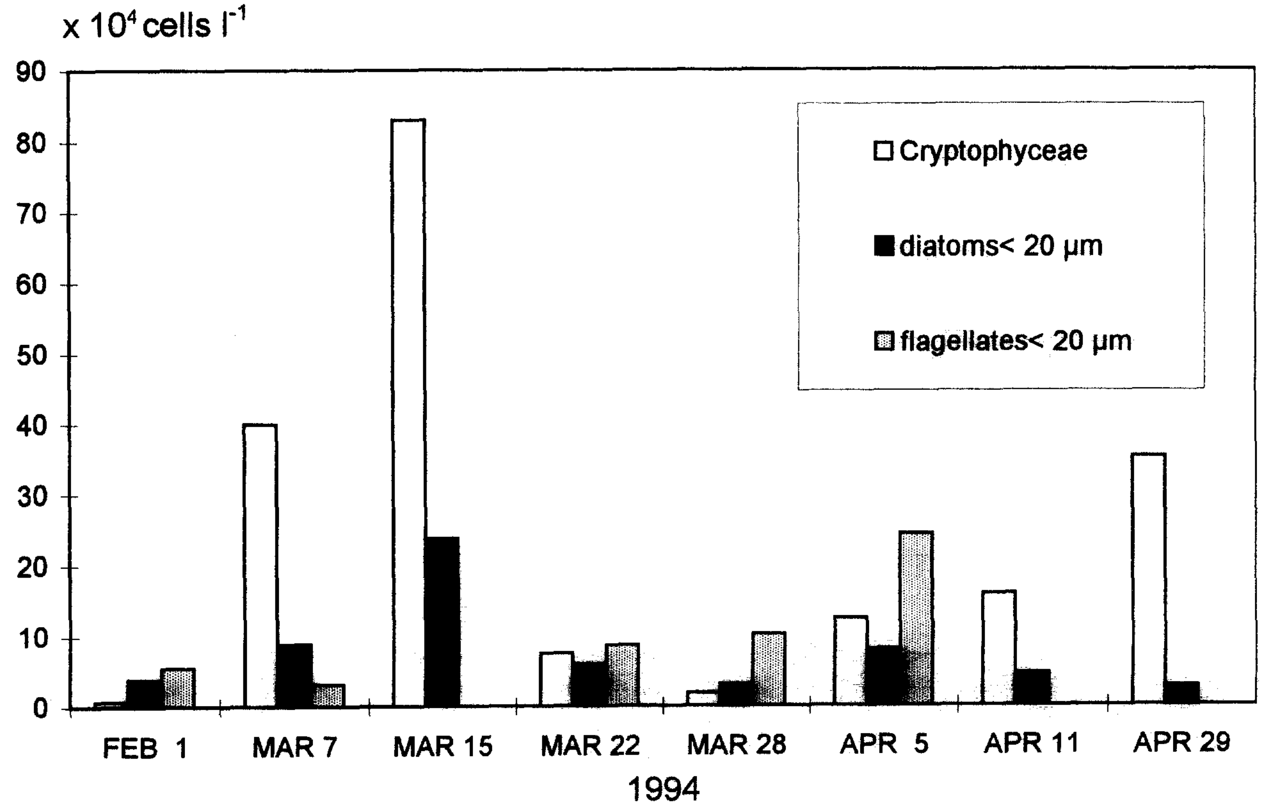

Figure 3. Week-to-week phytoplankton species composition evolution in cells L^{-1} from February to April 1994 at the lower Tagus estuary.

Table I. Primary production features of some European estuaries (lower zones)

Estuary	Chl *a* mg m^{-3}	P^Bm mg C (mg Chl *a*)$^{-1}$ h^{-1}	Daily Production mg C m^{-2} d^{-1}	Production g C m^{-2} yr^{-1}	Authors
Westerschelle	0.9–20.6 5.9 (mean)	1.7–9.4	9–2700	212	Van Spaendonk et al., 1993
Belfast Lough	2.0 – 28.0	4.9			Parker et al., 1988
Arousa "ria"	<1.0–3.0 (spring)	5.0–10.0	30–100 (spring)	250–260	Varela et al., 1984
Tagus estuary	0.5–4.3 2.3 (mean) (spring)	2.4–4.0 3.5 (mean) (spring)	30–135 71 (mean) (spring)	26	This study

Table II. Nutrient concentrations in the Tagus estuary (Seixal Bay) in spring 1994.

Nutrient	February	March	April
NO_3^-+NO_2^- (μM)			
min-max (mean)	20.60–21.00 (20.80)	10.00–34.20 (24.54)	17.30–28.80 (23.56)
NH_4^+ (μM)			
min-max (mean)	34.20–103.20 (76.8)	4.27–36.24 (14.86)	13.35–35.25 (19.40)
PO_4^{3-} (μM)			
min-max (mean)	1.57–2.04 (1.81)	1.57–6.41 (3.70)	2.23–4.95 (3.57)
$Si(OH)_4$ (μM)			
min-max (mean)	8.00–24.00 (15.30)	6.00–48.90 (13.52)	24.80–49.60 (34.50)

Table III. Results obtained for the specific attenuation coefficient of Chl *a*, (ε) in the Tagus estuary (Seixal Bay)

Depth (m)	ε (mg Chl *a*)$^{-1}$ m^2	k_{PAR} (m^{-1})	Chl *a* (mg m^{-3})	ε Chl *a* (m^{-1})	F = ε (Chl *a*)/k_{PAR}
0.10	0.015	6.04	3.30	0.0495	0.0082
0.25	0.014	3.63	3.32	0.0464	0.0127
0.50	0.018	3.34	3.33	0.0599	0.0179
0.75	0.014	3.14	2.87	0.0402	0.0128
1.00	0.018	2.80	2.40	0.0432	0.0154
1.25	0.011	2.28	1.95	0.0214	0.0094

to which cells were exposed decreased with a lower Zeu/Zm, and so respiratory losses would increase as cells remained in the dark for longer periods [14]. Therefore, in the lower Tagus estuary, light seems to be the limiting phytoplankton growth factor.

Results from the Correspondence Analysis showed that the first three axes extracted explained 92 % of the total variability within the data. The first axis alone accounted for 42 % of the total variability, showing photosynthetic "efficiency" (a^B) and daily column production (Psq) the highest positive loading in opposition to $Si(OH)_4$, suspended particulate matter and NH_4 (*figure 6a*). The projection of the variables can be interpreted in terms of temporal evolution. The first axis grouped the dates according to their relative variable relations into two main periods, 15 and 22 March being in contrast with 5 and 11 April, while 28 March and 29 April were not so well defined. Axis 2 accounted for 27 % of the total variability and again showed a^B with the most positive loading, in contrast with suspended particulate matter (SM). This axis highlighted the difference between 5 April and 11 April. Axis 3, which explained an additional 23 % of the variance (*figure 6b*), showed a^B and $Si(OH)_4$ with negative loading, in contrast with the other, positively loaded nutrients and separated 15 March from 22 March.

The main features that emerge from the multivariate analysis applied are as follows: the first period (15 and 22 March) was characterized in general by higher values of mean daily Irradiance, Daylength, Chlorophyll *a*, photosynthetic parameters (a^B, P^Bm) and daily production, compared to the second period (5 and 11 April); 22 March stands out in distinction from

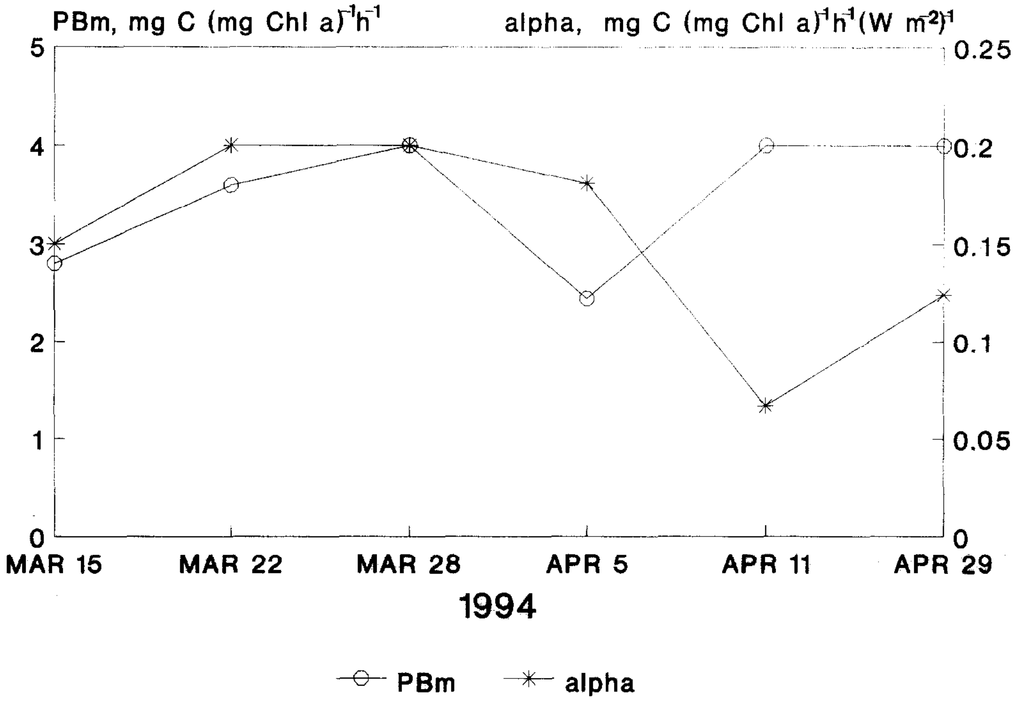

Figure 4. Week-to-week evolution of a^B, mg C (mg Chl a)$^{-1}$ h^{-1}(W m^{-2})$^{-1}$ and P^{B}m, mg C (mg Chl a)$^{-1}$ h^{-1} in the lower Tagus estuary during March and April 1994.

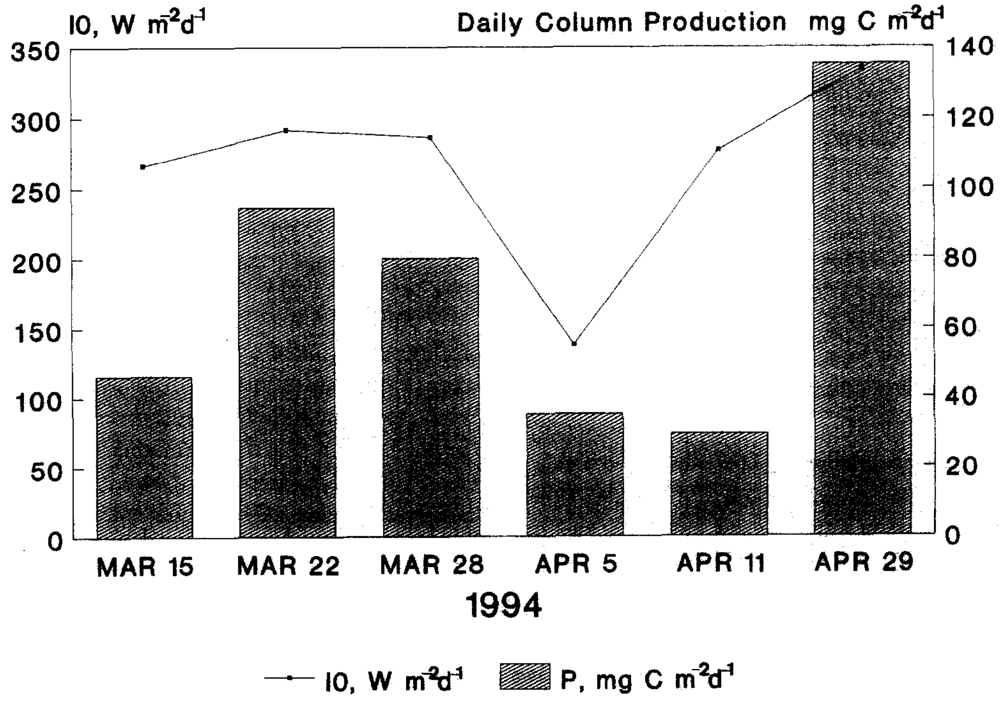

Figure 5. Spring evolution in 1994 of daily Tagus estuary production, Psq in mg C m^{-2} d^{-1} (bars) and the evolution of averages of surface irradiance, I0 in W m^{-2} d^{-1} (line). Average surface irradiances on a given day were calculated as the average of 7 previous days including the given day.

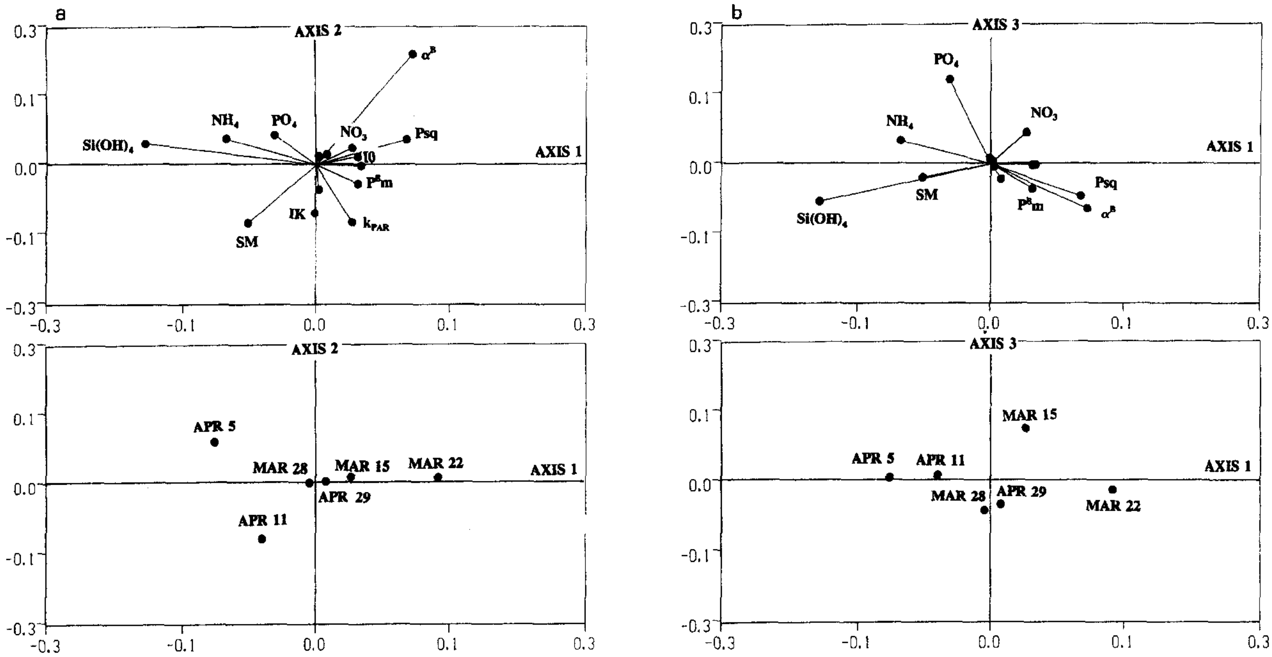

Figure 6. Projection of the environmental, physico-chemical and biological variables in the space defined by axis 1, axis 2 and axis 3, obtained from Correspondence Analysis. See text for variables codes: a) Axis 1 and axis 2, b) Axis 1 and axis 3.

15 March, on the basis of higher values of a^B, when the small flagellates (<20 μm) were the phytoplankton dominant group; A distinction between 5 April and 11 April was based on the lowest values of daily irradiance (I_0) and P^Bm recorded on the former date, in contrast with the highest k_{PAR}, the lowest a^B and Psq observed on the latter date.

4. CONCLUSIONS

As in many turbid temperate mesotidal estuaries, phytoplankton biomass in the Tagus estuary is relatively low and seems to be controlled by light, since nutrients are present in concentrations considered as not limiting phytoplankton growth.

Findings of a similar trend of irradiance and daily bay production as well as a high a^B and a relatively low P^Bm (*figures 4, 5*) point towards light-controlled photosynthesis, which results in a relatively low primary production in this turbid estuary.

The phytoplankton photosynthetic parameters show a rapid response to the environmental factors, and, more specifically, to daily irradiance and water column suspended particulate matter.

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