

PHYTOPLANKTON PHOTOSYNTHESIS-LIGHT RELATIONSHIP IN THE PAS ESTUARY, CANTABRIA, SPAIN

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ABSTRACT

The relationship between the photosynthesis-irradiance (P-I) curves of natural phytoplankton assemblages and environmental conditions was investigated in a partially mixed estuary, the Pas. During an annual cycle, photosynthesis was measured both by *in situ* and laboratory (light-saturation curve) incubation methods.

The initial slope (α^B) and the maximum photosynthetic rate (P^B_{max}) of P-I curves changed significantly over the year. The parameter (α^B) ranged from 0.001 to 0.04 mgC(mgChl a)⁻¹ h⁻¹ (μ E m⁻² s⁻¹) and P^B_{max} ranged from 0.6 to 15 mgC(mgChl a)⁻¹ h⁻¹. Maxima for both α^B and P^B_{max} occurred when the community of phytoplankton was dominated by small cell (small diatoms, *Cryptomonas* sp.) and the minima were obtained when the extinction coefficient was highest. In field (*in situ*) incubations, the production normalized to chlorophyll *a* (P^B_i) correlated positively with salinity and negatively with the nutrient concentration.

INTRODUCTION

Primary production is regulated spatially and temporally by chemical, physical, and biological factors. The study of this regulation is difficult and particularly so in estuarine ecosystems where conditions change rapidly and within a wide range.

The parameters of the photosynthesis-irradiance (P-I) curves (α^B = initial slope, P^B_{max} = maximum photosynthetic rate) and their relationship to environmental factors have been studied to achieve a better understanding of the control of photosynthesis in natural phytoplankton assemblages (COTE and PLATT, 1983). These parameters provide more physiological information than what is available from water column profiles (PLATT and JASSBY, 1976). HARRISON et al., (1985) suggested that P-I parameters chosen from a single depth stratum (upper mixed-layer) may be adequate in most cases to estimate total column production even under conditions of vertical stratification.

P^B_{max} is perhaps the easiest photosynthetic parameter to use to compare measurements between the laboratory and the field, because it is relatively insensitive to differences in light quality, its calculation does not rely on accurate measurement of irradiance, and it can reasonably be compared to the

maximum P^B measured in vertical profiles (CULLEN, 1991). The initial slope α^B , is the product of the specific absorption coefficient for chlorophyll *a*, and the quantum yield for photosynthesis ϕ_{max} . It is a measure of photosynthetic efficiency, but it is not equivalent to quantum yield. The magnitude is sensitive to the spectral quality of the light source and the absorption characteristics of the phytoplankton (CULLEN, 1991).

In this work we present the results obtained from the measurement of the assimilation number, P^B_i *in situ*, and the calculation of P-I parameter as well as their relationship to environmental factors in a small estuary.

STUDY AREA

The Pas Estuary (N. Spain) (Fig. 1) is an estuary which is typical for a flooded-valley. The depth increases towards the mouth, and average depth is 4 m. The average width as far as Mogro (Fig. 1) is 50-60 m. Beyond this area, the width of the river averages 50-60 m during low tide, and increases up to 200 m during medium tide.

The tides are semidiurnal with a fortnightly alternating cycle of spring and neap tides. The interval between two consecutive

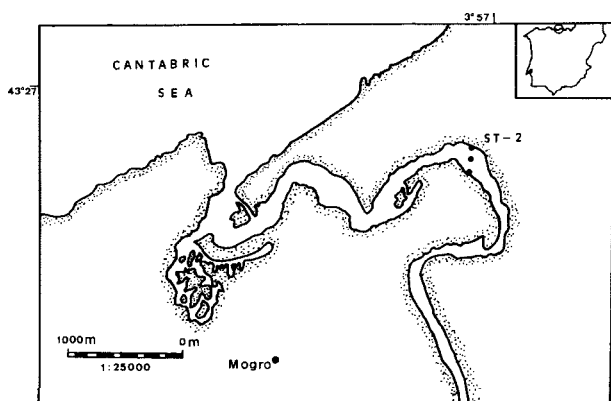


FIGURE 1. Map of the Pas estuary (N. Spain) showing the location of the station studied (Station 2).

FIGURA 1. Mapa del estuario del río Pas (N. España), mostrando la localización de la estación estudiada.

tides corresponds to the semilunar wave M, (12 h 25 min). The tidal amplitude is about 4 m in spring tides and approximately 2 m for neap tides. The maximum volume of the salt water wedge is 4,550,000 m³.

METHODS

Samples were collected over an annual cycle at Station 2 (Fig. 1) using 8 l Van Dorn bottles. Determination of P⁹¹ was done *in situ* while P-I experiments were conducted in the laboratory.

For the measurement of photosynthetic rate, 5 samples were collected (Fig. 2). The ¹⁴C method (STEEMAN-NIELSEN, 1952) was used to measure the photosynthesis. Transparent and opaque bottles (250 ml), inoculated with 5 μCi of NaH¹⁴CO₃ were incubated *in situ* for two hours. Following incubation, samples were filtered (0.45 μm pore size, 25 mm diameter), and placed in liquid scintillation counting vials. Each filter was fumed over HCl and 10 ml of scintillation cocktail was added. All samples were counted on a Liquid Scintillation Counter. The photosynthesis was estimated by the VOLLENWEIDER method (1969). Sampling alternated between high and low tide to examine tidal effects on photosynthesis.

For the P-I experiment, the water was taken at the surface, S (Fig. 2) during high tide. Samples were carried to the laboratory in a portable refrigerator. The time between the sampling and the incubation was half an hour. In the laboratory 5 μCi of NaH¹⁴CO₃ was added to each bottle (250 ml). The bottles were incubated at 18 °C, in a room with a temperature control system, for 4 h over a range of light intensity. The irradiance ranged from 0 to the estuary light intensity when the sampling was carried out (maximum values 1600 μE m⁻² s⁻¹).

All photosynthesis values were normalized to the chlorophyll "a" concentration. The light-limited and saturated parameters of the P-I curves ($\alpha^B = \text{mgC}(\text{mg Chla})^{-1}\text{h}^{-1} (\mu\text{Em}^{-2}\text{s}^{-1})$ and $P^B_{\text{max}} = \text{mgC}(\text{mgChla})^{-1}\text{h}^{-1}$) were estimated according to PLATT *et al.*, 1980.

All water samples were analyzed for salinity, temperature, nutrients (nitrate, nitrite, ammonium, soluble reactive phosphate (SRP) and silicate), chlorophyll a, and total number of cells. Methods described in STRICKLAND and PARSONS (1972) were used. Phytoplankton counts were done with an inverted microscope (UTERMOHL, 1958), and depending on the density of cells, 25 or 50 ml sedimentation chambers were used.

Incident (I₀) and subsurface (I_z) light intensity were measured *in situ* (Licor 850) and the diffuse attenuation coefficient (k:m⁻¹) calculated as follows: $k = (\ln I_0 - \ln I_z) / z$.

The statistical significance of differences in averages was obtained by means of an analysis of variance. This analysis was designed considering the three variation factors. Factor A: state of tides (low tide and high tide); Factor B: points where samples were taken (at the water column: surface (S); midway (M); and bottom (B); and the littoral right (LA) and littoral left

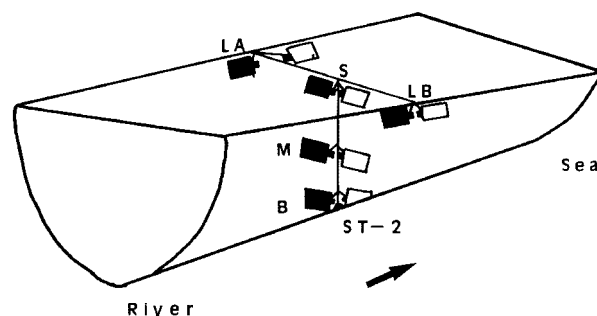


FIGURE 2. Station and sampling points (S = surface of central point, M = midway of central point, B = bottom of central point, LA = littoral right, LB = littoral left). Arrangement of the transparent and opaque bottles during incubation for measurement of the photosynthesis.

FIGURA 2. Estación y puntos de muestreo (S = superficie del punto central, M = punto medio, B = fondo del punto central, LA = litoral derecho, LB = litoral izquierdo). Disposición de las botellas transparentes y opacas durante la incubación para la medida de la fotosíntesis *in situ*.

(LB) (Fig. 2); Factor C: annual cycle. The analysis was performed using the SPSS program in a personal computer.

RESULTS and DISCUSSION

The annual cycle of hydrographical characteristics exhibits a period of high temperature and salinity in summer and early autumn, and low salinity in winter and during periods with

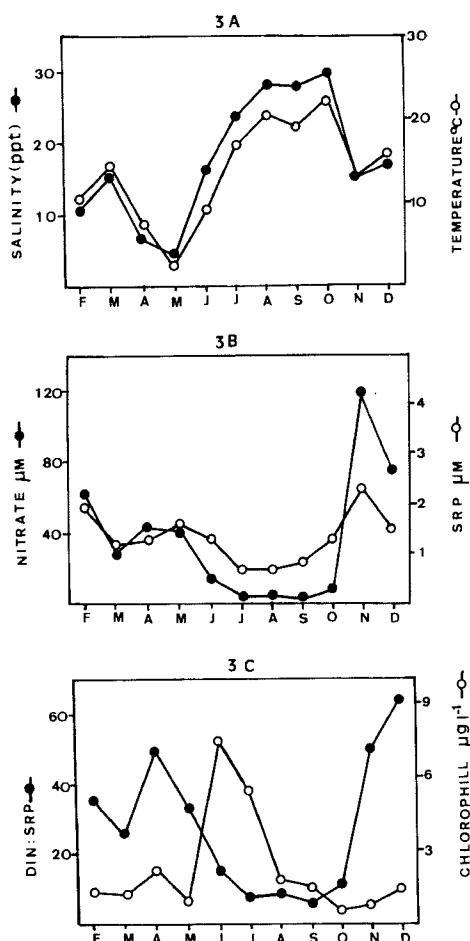


FIGURE 3. Seasonal variations of mean values ($n = 10$) of physical, chemical and biological characteristics at the Station 2: a. Salinity (ppt) and Temperature ($^{\circ}\text{C}$). b. Nitrate (μM) and SRP (μM). c. DIN: SRP ratios and Chlorophyll a ($\mu\text{g l}^{-1}$).
 FIGURA 3. Variación estacional de los valores medios ($n = 10$) de las características físicas, químicas y biológicas en la Estación 2: a. Salinidad (ppt) y Temperatura ($^{\circ}\text{C}$). b. nitrato (μM) y fósforo reactivo soluble (μM). c. Relación Nitrógeno inorgánico disuelto: fósforo reactivo soluble y clorofila a ($\mu\text{g l}^{-1}$).

high precipitation (April and May) (Fig. 3A). The pattern of nutrient concentrations (Fig. 3B) was as follows: continuous vertical stratification; maxima in winter and seasons with frequent rain and minima during summer; nutrient concentrations were lower at high tide than at low tide. Hydrographical characteristics and nutrients cycle have been described in a previous paper (PÉREZ and CANTERAS, 1990). The chlorophyll a concentrations (Fig. 3C) ranged from 8 to $0.5 \mu\text{L}$ with the lowest values in winter and at the end of spring. A small increase occurred in March, although maximum biomass was recorded in June.

With respect to abundance and specific composition of phytoplankton, three periods could be distinguished in the annual

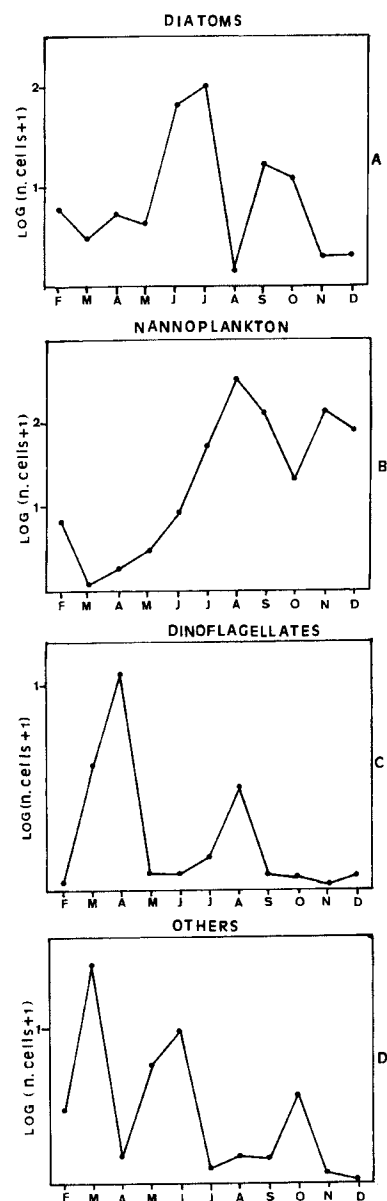


FIGURE 4. Seasonal variations in the cell abundance ($\log x+1$, $x = \text{cells/ml}$) in the surface central point (S) at high tide. The group others included Cryptophyceae, Euglenophyceae, Crysophyceae.
 FIGURA 4. Variación estacional de la abundancia celular ($\log x+1$, $x = \text{cells/ml}$) en la superficie del punto central (S) en pleamar. El grupo *otros* incluye Cryptophyceae, Euglenophyceae, Crysophyceae.

cycle: The first period, in which the number of cells was small, averaging $100 \text{ cells ml}^{-1}$ and during which there was a little phytoplankton bloom in March. During the second period, from June to October, there was a rise in the biomass, which reached its maximum in July. The third period, from October to December, showed a reduction in the biomass. Over the annual

cycle, the phytoplankton was predominantly composed of diatoms (Fig. 4A). Dinoflagellates appeared during August and April (Fig. 4C). Cryptophyceae were present almost all year round, with the largest numbers during June (Mean = 45 %) and March (M = 37%) (Fig. 4D). Nannoplankton appeared during

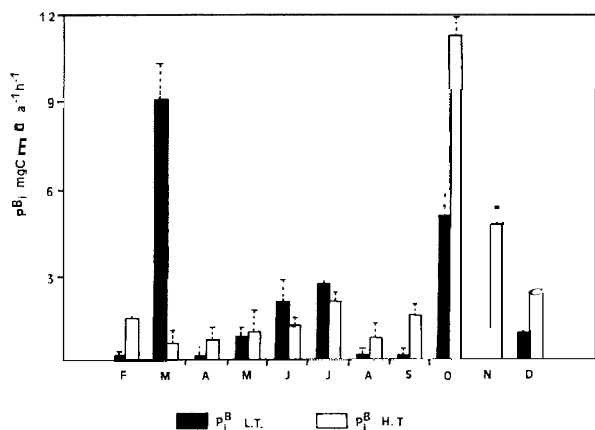


FIGURE 5. Temporal variations (n = 5) in assimilation numbers for the *m situ* experiment, P^B_i (mgC(mgChla)⁻¹ h⁻¹), at high (H.T.) and low (L.T.) tide. FIGURA 5. Variaciones temporales (n = 5) del número de asimilación en los experimentos realizados *in situ*, P^B_i (mgC(mgChla)⁻¹ h⁻¹), en pleamar (H.T.) y bajamar (L.T.)

the entire annual cycle and accounted for >50% of the cell number in the summer months and in the early autumn (Fig. 4B).

Highest assimilation numbers (P^B_i) (Fig. 5) were recorded in March (Mean = 9.09 mgC(mgChla)⁻¹ h⁻¹, Standard Deviation (S.D.) = 5.44) and October (M = 11.21 mgC(mgChla)⁻¹ h⁻¹, S.D. = 1.89). During the rest of the year they were < 5 mgC(mgChl a)⁻¹ h⁻¹. The results of variance analysis are shown in Table I. Only the annual cycle indicated statistically significant difference (p < 0.01) among different months.

Table II shows the correlation coefficients obtained with salinity (r = 0.32, p < 0.001), nutrients (silicate and nitrate) and dissolved oxygen. These correlations have been reported by

TABLE I. Results of variance analyses. TABLA I. Resultados del análisis de la varianza.

Source of variation	D.F.	Sum of squares	Mean squares	F	P _{0.05}	P _{0.01}
State tidal (A)	1	0.845	0.845	0.113	251	6282
Points of sampling(B)	4	17.089	4.272	0.574	5.71	13.74
Annual cycle (C)	10	14603	1460.03	19.62	2.67	4.17
Interactions (AB)	4	26.409	6.662	0.887	5.71	13.74
Interactions (AC)	10	287.864	28.786	3.867	2.67	4.17
Interactions (BC)	40	276.324	6.908	0.928	1.69	2.11
Interactions (ABC)	40	297.731	7.443			

D.F. Degrees of freedom

TABLE II. Significant correlation coefficients obtained between P^B_i and environmental parameters (d.f. = 108).

TABLA II. Coeficientes de correlación significativos obtenidos entre P^B_i y parámetros ambientales (g.l. = 108).

	Oxygen mg/l	Salinity ppt	Nitrate μM	Silicate μM
P ^B _i	-0.21*	0.32***	-0.20*	-0.31***

* p < 0.05

** p < 0.01

*** p < 0.001

other authors (HARRISON and PLATT, 1980) and could indicate a phytoplankton community adapted to neritic conditions, i.e. the community obtains a higher photosynthetic efficiency when salinity is high and nutrient concentrations low. Negative correlation between P^B_i dissolved oxygen could be due to characteristics of neritic waters which dissolve oxygen in a lesser amount. In the Pas estuary, the contribution of dissolved oxygen to the water is mainly due to physical processes (PÉREZ, 1987). Only occasionally, biological processes increase oxygen concentration.

P-I curves were fitted employing an exponential equation (PLATT et al., 1980). Fig. 6 shows curves obtained from light-gradient incubations, corresponding to samples incubated in January, June and November.

The parameters of the photosynthesis-irradiance curves showed low values in Spring (April α^B = 0.001 mgC(mgChla)⁻¹ h⁻¹ (μEm⁻²s⁻¹); P^B_{max} = 0.5 mgC(mgChla)⁻¹h⁻¹) and in August α^B = 0.002 mgC(mgChla)⁻¹h⁻¹ (μEm⁻²s⁻¹); P^B_{max} = 2.6 mgC(mgChla)⁻¹h⁻¹). The maxima were obtained in March (α^B = 0.04 mgC(mgChla)⁻¹h⁻¹ (μEm⁻²s⁻¹); P^B_{max} = 15 mgC(mgChla)⁻¹h⁻¹) (Fig. 7), when *Cryptomonas* sp. (size < 5 μm) was predominant (80 %). The second peak was obtained in October (α^B = 0.03 mgC(mgChla)⁻¹h⁻¹ (μEm⁻²s⁻¹); P^B_{max} = 13 mgC(mgChla)⁻¹h⁻¹), when the community was dominated by small diatoms, *Thalassiosira* sp. (diameter = 3 μm) and *Skeletonema* costatum.

The annual average values for α^B were low during the study (α^B = 0.011 mgC(mgChla)⁻¹h⁻¹ (μEm⁻²s⁻¹). The low values of α^B could suggest limitation of photosynthesis. PLATT and JASSBY (1976) gave values of α^B = 0.05 mgC(mgChla)⁻¹h⁻¹ (μEm⁻²s⁻¹) (0.02 to 0.16) in Nova Scotia; 0.05 mgC(mgChla)⁻¹h⁻¹ (μEm⁻²s⁻¹) (0.02 to 0.16) in Bedford Basin and 0.01 mgC(mgChla)⁻¹h⁻¹ (μEm⁻²s⁻¹) (0.003 to 0.015) in Scott Inlet.

Table III shows the significant correlations (p < 0.05) between environmental factors and P-I curves parameters. α^B was negatively correlated to k and nitrate and P^B_{max} positively with incident light (I₀) and negatively with k. Light, probably, was a limiting factor in some of the time of the annual cycle.

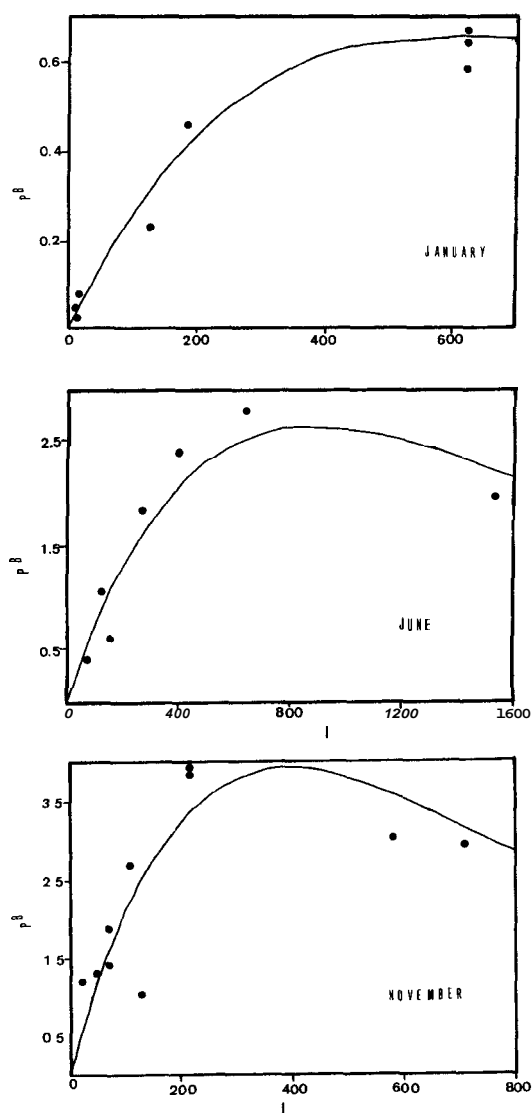


FIGURE 6. Examples of Photosynthesis-Irradiance (P-I) curves in estuarine phytoplankton assemblages from January, June and November.
 FIGURA 6. Ejemplos de las curvas Fotosíntesis-Irradiancia obtenidas con muestras de fitoplancton de estuario de Enero, Junio y Noviembre.

The effect of nutrient availability on α^B and P^B_{max} is not well documented. COTE and PLATT (1983) suggested that if nutrient stress decreases the size of photosynthetic units (PSU) (FALKOWSKI, 1981; PERRY *et al.*, 1980), α^B would be expected to increase with nutrient limitation. Nitrogen concentration in the Pas estuary was high during the main part of year (Fig. 3B) the same as DIN:SRP ratios (Fig. 3C); only in summer (June-September) the nitrate concentration was low ($5 \mu\text{M}$) and DIN:SRP ratios less than 10:1. We think that had no limitation for nitrogen. PEREZ and CANTERAS (1991),

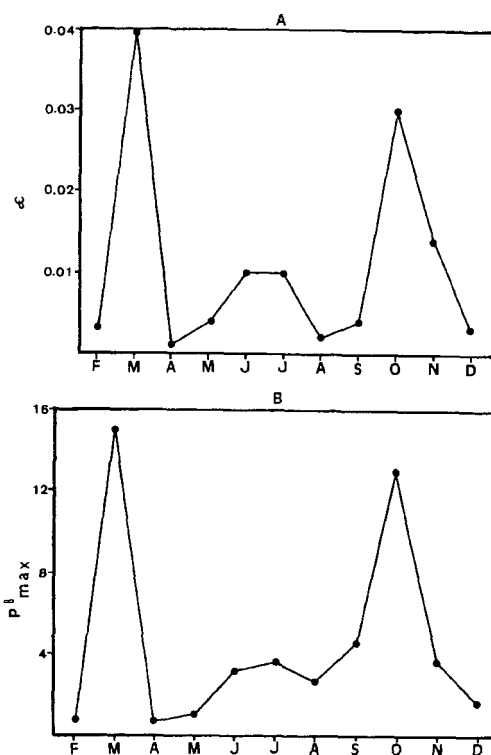


FIGURE 7. Temporal variations of α^B (a) ($\text{mgC}(\text{mgChla})^{-1} \text{h}^{-1} (\mu\text{Em}^{-2}\text{s}^{-1})$) and P^B_{max} (b) ($\text{mgC}(\text{mgChla})^{-1} \text{h}^{-1}$).
 FIGURA 7. Variaciones temporales de α^B (a) ($\text{mgC}(\text{mgChla})^{-1} \text{h}^{-1} (\mu\text{Em}^{-2}\text{s}^{-1})$) and (b) P^B_{max} ($\text{mgC}(\text{mgChla})^{-1} \text{h}^{-1}$).

through bioassay experiments and the study of DIN:SRP relations, verified the phosphate limitation in the Pas estuary. We believe it is difficult to think that α^B is negatively correlated to nitrate concentrations through the stress created in the size of PSU, by phosphorus limitation. CULLEN (1991), suggests that in the bibliography there are different results, obtaining high photosynthetic parameters when the nutrients are low and reciprocally. He thinks it is necessary to leave these conclusions.

The results obtained, in this paper, suggest α^B and P^B_{max} are related to the specific composition and cell size of phytoplankton community. The highest values of α^B and P^B_{max} belong to samples in which community was dominated by

TABLE III. Significant correlations ($P < 0.05$) between environmental factors and P-I curve parameters.
 TABLA III. Correlaciones significativas ($P < 0.05$) entre factores ambientales y los parámetros de las curvas P-I.

	10	k	Nitrate
α^B	n.s.	-0.49	-0.53
P^B_{max}	0.60	0.50	n.s.

opportunistic and typical estuarine species (*Cryptomonas sp.*, *Skeletonema costatum*, *Thalassiosira sp.*, *Gymnodinium minus*). These species are small-sized, have an elevated growth rate and are able to survive in superficial waters and well-illuminated areas. The lowest values correspond to communities dominated by fresh water species (*Rhoicosphenia curvata*, *Asterionella formosa*). Intermediate values are related to marine communities (*Rhizosolenia stolterforthii*, *Bacteriastrium hyalinum*, *B delicatulum*). This paper shows that

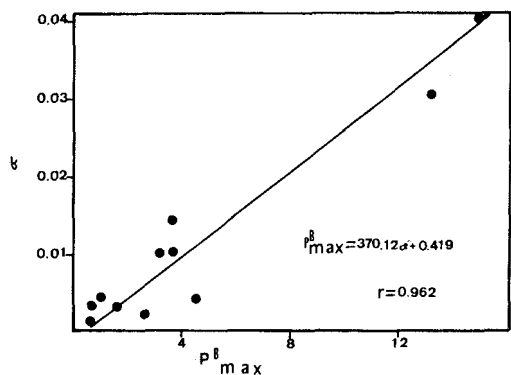


FIGURE 8. Plots of α^B versus P^B_{max} . Data were analyzed by least-squares linear regression. Regression equation and correlation coefficient are presented.
FIGURA 8. Regresión entre α^B y P^B_{max} . Se presenta la ecuación de regresión y el coeficiente de correlación.

α^B and P^B_{max} increase when dominant species are small-sized and are related to the marine and estuarine waters.

The initial slope (α^B) has been plotted versus P^B_{max} (Fig. 8). There is a strong dependence between both parameters ($r = 0.962$, $P < 0.001$). A correlation between α^B and P^B_{max} has been observed before for laboratory cultures and natural assemblages of phytoplankton (MACCAULL and PLATT 1977; HARDING *et al.*, 1982). The dependence between α^B and P^B_{max} indicates that these processes are coupled dynamically within the cell, so that the action of one provides the energy to drive the other.

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