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Pelagic metabolism of the Douro estuary (Portugal) – Factors controlling primary production

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Abstract

The pelagic metabolism of the Douro estuary (Portugal) and the factors influencing primary production (PP) and community respiration (CR) in this system were studied during an annual cycle (December 2002–December 2003). Sampling surveys were conducted twice a month during ebb and flood spring tides and water samples were collected for PP and CR assessments at three stations along the estuary (lower, middle and upper stretches). During the study period, PP values were in the range of 4.7–1878.5 mg C m⁻² d⁻¹ (average, 319.9 mg C m⁻² d⁻¹). River discharge controlled phytoplankton biomass inputs into the estuary as well as residence time. A decreasing trend in water column PP from the upper to the lower estuary related to higher nitrogen concentrations and phytoplankton biomass from riverine origin was observed. An inverse trend was found for CR, i.e., higher values were found in the lower, more urbanized stretch. During the study period, averaged CR values reached 1154 mg C m⁻² d⁻¹. In general, heterotrophy dominated the entire estuary, except in the upper stretch from May through July, when increased PP, but also lower CR values were recorded. A positive correlation between chlorophyll *a* and P_{\max} was found which is unusual in coastal ecosystems, where a decreasing trend of the *P/B* ratio as a function of net primary production is generally observed. This could be explained by the relatively low phytoplankton biomass, preventing intraspecific competition from lowering photosynthetic capacity, on one hand, and the physiology of phytoplankton related to their origin in a semi-lotic (reservoir) ecosystem. No significant differences between tides were observed for all variables, except for the water light extinction coefficient (*k*) values, reflecting higher turbidity during the ebb.

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1. Introduction

The metabolic balance of a given system depends upon its primary production and community respiration. Primary production is dependent on physical (light availability and temperature), chemical (nutrients) and biological factors, like phytoplankton biomass, species composition, size structure and grazing (Stearns et al., 1987; Cloern, 1991; Landry et al., 1995; Gallegos and

Jordan, 1997; Calbet and Landry, 2004; Cermeno et al., 2006), as well as viral control (Proctor and Fuhrman, 1991).

In estuaries, these factors may be influenced by freshwater inflow, since it carries nutrients, phytoplankton and suspended matter, which determines light availability in the water column. River flow magnitude also controls residence time and, hence, the susceptibility of ecosystems to algal blooms, with effects propagating throughout the food web to higher trophic levels (Kimmerer, 2002). Seasonal shifts from auto- to heterotrophy according to river flow variations have also been reported. For example, heterotrophy occurs during monsoon periods in tropical estuaries, when increased allochthonous organic input leads to enhanced respiration (Ram et al., 2003).

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Phytoplankton dynamics may also be influenced by tides. Episodes of biomass increase during neap tides and decline during spring tides have been reported (Cloern, 1991), as well as differences between ebb and flood tide phytoplankton biomass (Roegner, 1998). Aubry and Aciri (2004) found higher phytoplankton abundance at flood than at ebb for most of the year in the Lagoon of Venice, due to the presence of neritic species. Tidal turbulence can also influence vertical distribution of different phytoplankton species, e.g., slack water periods enhance aggregation of dinoflagellates while diatoms rely on periods of turbulence to ensure entrainment into the upper water column and to prevent sinking from the photic zone (Lauria et al., 1999).

Another important factor affecting estuarine metabolism is human pressure, for example, through the construction of dams since they alter the timing and quantity of freshwater, sediment, inorganic and organic matters delivered to estuaries and adjacent coastal zones (Hopkinson and Vallino, 1995). The discharge of treated or untreated wastewater into estuaries also affects metabolism by increasing allochthonous nutrient or organic matter inputs, respectively. The former may increase production and the latter bacterial respiration. If bacterial respiration exceeds net primary production due to utilization of external sources of organic matter, heterotrophy dominates (delGiorgio et al., 1997).

Thus, in estuaries, a transient environment, the understanding of the trophic status is crucial in order to evaluate the role of such systems as a potential source of autochthonous organic matter for the coastal environment. The autotrophic–heterotrophic nature of an estuary is determined by three primary factors as follows: the ratio of inorganic to organic matter inputs, water residence time and the overall degradability of allochthonous organic matter inputs (Hopkinson and Vallino, 1995). Data on metabolic balance of European estuaries are scarce, namely due to lack of studies concerning pelagic depth-integrated community respiration (Gazeau et al., 2004).

The river Douro originates from the largest watershed in the Iberian Peninsula. Its 98,000 km² are unequally shared between Portugal (20%) and Spain (80%). Over 50 large dams have been constructed especially in the last 50 years for irrigation and electric power generation purposes, resulting in flow regulation.

The Douro estuary is limited upstream by the last dam, located 21.6 km from the mouth. This dam determines the freshwater flow into the estuary, ranging from 0 to 13,000 m³ s⁻¹ (Vieira and Bordalo, 2000) with an average of 501 m³ s⁻¹. River flows present a large inter-annual variability, with considerable differences between wet and dry years. A decrease of annual flow has been reported, due to climate phenomena (Trigo et al., 2004) but also to an increase of water storage for hydroelectric power generation, agriculture and domestic consumptions (Bordalo and Vieira, 2005). The fact that freshwater flowing into the estuary originates in a reservoir, a semi-lotic ecosystem, may also influence estuarine metabolism due to specific phytoplankton characteristics. Moreover, in the case of the Douro, the last dam is the main source of phytoplankton biomass (Bordalo and Vieira, 2005).

The objective of this work is to analyse estuarine environmental conditions and photosynthetic parameters in relation to its metabolism, in order to answer the following questions:

- Is the Douro estuary auto- or heterotrophic?
- Which factors control primary production (PP) on a spatial and temporal basis?
- Are there significant differences in estuarine PP and metabolism between ebb and flood tides?

2. Materials and methods

2.1. Study area

The Douro is a granitic drowned valley river, draining to the north-western shore of Portugal. Its estuary is mesotidal, characterized by semidiurnal tides and a mean tidal range of 2.8 m. During the flood and under low river flow, sea water creates a salt wedge that eventually reaches the head of the estuary (at 21.6 km from the mouth), where the tidal excursion is halted by the Crestuma dam (Fig. 1), and remains within the estuary during the next ebb. In this situation, residence time can reach 14 days, whereas during high discharge events, the estuary is flushed completely during one tidal cycle and seawater intrusion is prevented during the flood (Vieira and Bordalo, 2000).

The last 8 km stretch of the river is heavily modified and over 700,000 inhabitants live within the estuarine area. A total of eight wastewater treatment plants (WTPs) drain into the estuary, without nutrient removal.

2.2. Sampling

Data presented in this study were obtained within a larger sampling program under a contract with Oporto Water Authority, designed to evaluate the influence of WTPs on estuarine water quality. Sampling surveys were conducted monthly, during ebb and flood spring tides, in order to sample the most extreme situations, namely concerning seawater intrusion.

From December 2002 to December 2003, three stations were visited, in the lower, middle and upper estuary, at approximately 0.7, 5.0 and 21.6 km, respectively, from river mouth (Fig. 1). The boundaries of these three estuarine stretches have been defined based on the seasonal salt water intrusion (Vieira and Bordalo, 2000). Each survey lasted between 90 and 120 min according to flow conditions.

Vertical profiles of temperature, conductivity, salinity, dissolved oxygen, pH and turbidity were performed with a CTD (YSI, 6600). Salinity was measured using the Practical Salinity Scale. Photosynthetic active radiation (PAR) profiles were obtained with a spherical quantum sensor light meter, at 0.5 m depth steps (LI-COR, LI-250).

Simultaneously, samples were collected at three depths (surface, middle and near bottom) with a Van Dorn bottle for chlorophyll *a*, nutrients (nitrate, nitrite, ammonium, phosphate and silicate), total particulate matter (TPM) and particulate organic matter (POM) assessments.

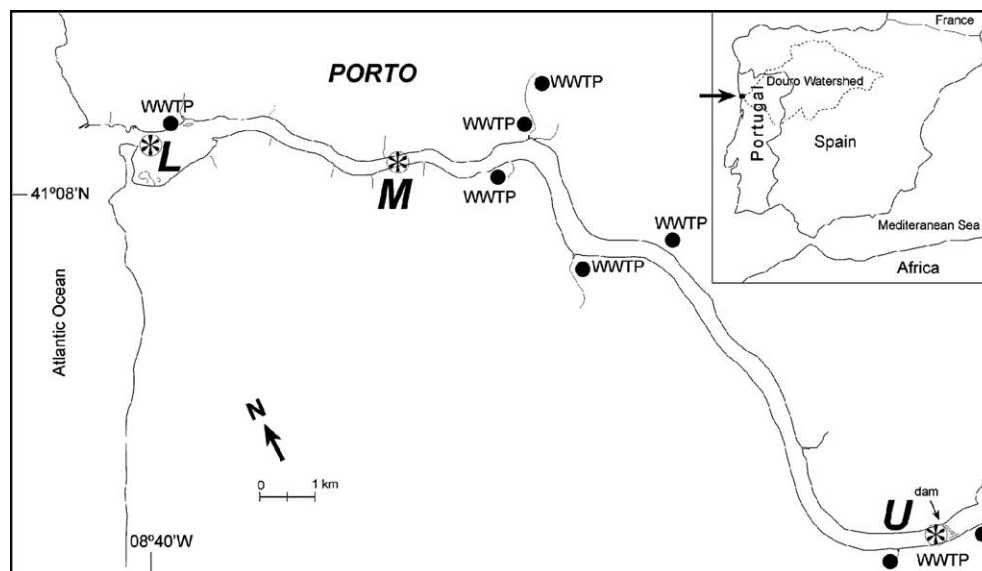


Fig. 1. The river Douro estuary and location of sampling stations (L – lower, M – middle and U – upper estuary, and WWTP – wastewater treatment plant).

Mean water depth during sampling is presented in Table 1. During the survey period, tidal height ranged between 0.13 and 1.29 m, at ebb surveys, whereas at flood surveys, was within the 2.65–3.52 m range.

For PP assessments, subsurface samples were used, after testing their adequacy to depth-integrated PP assessments in the present estuarine system (c.f. – Section 2.3). Community respiration (CR) was initially estimated from samples collected at three depths and from surface samples only, after confirmation that no significant ($p > 0.05$) differences were found with depth.

Water samples were kept refrigerated in ice chests and processed in the laboratory within 1 h from collection of the last sample.

2.3. Experimental and analytical procedures

PP was assayed by the ^{14}C method (Steeman Neilsen, 1952), following the standard ICES (1996) recommendations.

Briefly, water samples were placed in 125-ml Pyrex glass flasks and 2 mCi of aqueous solution of sodium bicarbonate was added (Carbon 14 Centralen). Duplicate samples were incubated for 1–2 h in a water bath, at 5 light levels (100%, 75%, 50%, 25%, and 1%), to mimic light attenuation of different depths within the euphotic zone, at in situ temperature. An artificial light source providing a PAR of $920 \mu\text{E m}^{-2} \text{s}^{-1}$ was used. Attenuation was achieved by means of a neutral screen. One additional dark bottle was also incubated. After incubation, samples were filtered through $0.45 \mu\text{m}$ membranes, washed, placed in 20-ml scintillation vials and 10-ml scintillation cocktail (Beckman Instagel Packard) was added. Countings, in disintegrations per minute (DPM), were performed in a Beckman LS3801 liquid scintillation analyser using internal standards for the automatic establishment of the quenching curve. Dark DPM values were subtracted from light DPM values and results were expressed in $\text{mg C m}^{-3} \text{h}^{-1}$.

In order to validate the sampling strategy for PP assessments, simultaneous incubations, at 5 light intensity levels,

Table 1
Averages and standard errors for selected variables at each location during flood and ebb surveys for the December 2002–December 2003 period

Parameter	Units	Lower		Middle		Upper	
		Flood	Ebb	Flood	Ebb	Flood	Ebb
Salinity	–	20.4 ± 2.6	11.0 ± 1.6	12.1 ± 2.3	5.8 ± 1.3	1.7 ± 1.1	2.0 ± 1.1
Temperature	$^{\circ}\text{C}$	14.2 ± 0.5	14.2 ± 0.7	14.5 ± 0.7	15.3 ± 0.8	17.0 ± 1.1	16.0 ± 1.0
$\text{NO}_3 + \text{NO}_2$	μM	57.1 ± 8.9	71.5 ± 6.7	86.2 ± 8.9	85.5 ± 6.6	103.9 ± 8.0	87.3 ± 6.2
Ammonium	μM	8.8 ± 0.7	12.5 ± 2.7	9.3 ± 0.5	7.8 ± 0.5	6.6 ± 0.8	4.0 ± 0.4
Phosphate	μM	1.1 ± 0.1	1.4 ± 0.1	1.5 ± 0.2	1.4 ± 0.1	1.4 ± 0.1	1.6 ± 0.2
Silica	μM	39.8 ± 6.0	59.7 ± 6.2	53.3 ± 6.0	58.4 ± 5.9	56.3 ± 5.0	68.6 ± 6.6
N:P ratio		53.8 ± 4.7	63.4 ± 3.5	67.4 ± 5.9	72.7 ± 5.2	109.7 ± 19.6	69.4 ± 6.9
TPM	mg l^{-1}	24.0 ± 3.6	19.5 ± 1.7	11.5 ± 1.2	16.7 ± 1.9	10.9 ± 1.1	11.1 ± 1.0
POM	mg l^{-1}	4.8 ± 0.5	4.4 ± 0.4	3.1 ± 0.2	3.4 ± 0.3	2.7 ± 0.2	2.7 ± 0.2
Chlorophyll <i>a</i>	mg m^{-3}	2.4 ± 0.2	3.6 ± 0.3	3.1 ± 0.4	4.7 ± 0.5	4.4 ± 0.6	4.6 ± 0.6
PP daily	$\text{mg C m}^{-2} \text{d}^{-1}$	312.2 ± 118.7	205.5 ± 76.5	328.2 ± 109.1	252.9 ± 93.7	455.8 ± 163.1	411.1 ± 150.0
CR daily	$\text{mg C m}^{-2} \text{d}^{-1}$	1810.1 ± 407.1	1355.2 ± 587.3	1170.2 ± 196.9	1487.2 ± 342.0	474.2 ± 84.2	629.7 ± 126.3
Water column depth	m	6.8 ± 0.2	5.5 ± 0.3	9.7 ± 0.4	7.7 ± 0.3	8.1 ± 0.5	6.0 ± 0.4

of surface samples and samples collected at depths correspondent to those light levels, were performed. These experiments yielded similar results, thus only surface water samples were used for PP measurements.

Inorganic carbon was assayed from pH and alkalinity measurements by direct titration according to Parsons et al. (1984).

CR was estimated as the difference in dissolved oxygen at the beginning and after 24 h incubation of samples in the dark at in situ temperature. Oxygen was assayed by a modification of the Winkler method (Carpenter, 1965). Oxygen values were converted into carbon units using a conversion factor of 0.375 (Uthicke and Klumpp, 1998).

Chlorophyll *a* was assayed spectrophotometrically after extraction with 90% acetone (Parsons et al., 1984) with cell homogenisation, using the SCOR–UNESCO (1966) trichromatic equation. Dissolved orthophosphate, nitrite, ammonium and silicate were analysed following the methods described in Grasshoff et al. (1983). Nitrate was quantified by an adaptation of the spongy cadmium reduction technique (Jones, 1984), subtracting nitrite value from the total. All the analyses were performed in triplicate. Samples were filtered through glass fibre filters which were dried at 105 °C for TPM assessment and then incinerated at 500 °C for POM assessment (APHA et al., 1992).

2.4. Data analysis

Bi-dimensional plots, generated by “Surfer” software, were used to represent variation with depth of measured variables along the sampling period. Data were interpolated using the “kriging” gridding method.

In order to perform statistical and multivariate analyses, data were depth averaged.

2.4.1. PP calculations

Steele’s production-light function (P/E) Eq. (1) (Steele, 1962) was fitted to experimental data from incubation experiments, using the Gauss–Newton non-linear regression method with Statistica software, since photoinhibition was apparent. The photosynthetic parameters obtained, maximum production rate (P_{\max}) and optimal light intensity (E_{opt}), were used to fit Eq. (1). The normalisation to chlorophyll *a* was made using the values obtained for each sample analysed. Depth-integrated primary production (\bar{P}) was then calculated by integrating Steele’s equation over depth, Eq. (2).

$$P = P_{\max} \left[\frac{1}{E_{\text{opt}}} \exp \left(1 - \frac{E}{E_{\text{opt}}} \right) \right]^n \quad (1)$$

$n = 1$ (n , empirical integer)

$$\bar{P} = P_{\max} \frac{\exp(1)}{kz} \left[\exp \left(-\frac{E_0 \exp(-kz)}{E_{\text{opt}}} \right) - \exp \left(-\frac{E_0}{E_{\text{opt}}} \right) \right] \quad (2)$$

where P_{\max} – maximum production rate ($\text{mg C mg Chl } a^{-1} \text{ h}^{-1}$); E_0 – surface light intensity ($\mu\text{E m}^{-2} \text{ s}^{-1}$); k – light extinction coefficient (m^{-1}); and z – depth (m).

Light extinction coefficients (k) were estimated from the vertical profiles of PAR measured during each sampling survey, using the Lambert–Beer equation, Eq. (3).

$$E = E_0 \exp(-kz) \quad (3)$$

Time integrated PP estimates were obtained as follows:

- (i) Hourly E_0 values were estimated by means of a model implemented with Stella software using standard formulations described in Brock (1981) and Portela and Neves (1994) for periods of 24 h and adjusted to values measured during the sampling surveys. This adjustment was made by changing cloud cover values, to make sure that predicted light intensities at the hours when sampling took place were similar to those measured.
- (ii) From these surface light intensity estimates, the k values measured during the surveys and the P/E curves obtained in the incubation experiments, daily and depth-integrated PP was estimated separately for ebb and flood surveys. In these estimates, vertically averaged chlorophyll values (measured at three depths, c.f. – Section 2.2) were considered.

Compensation depth (z_c) – the depth at which CR equals PP (net production equal to zero) – was calculated solving Eq. (4) by the Lambertw function ($w.e^w = x$) using the Matlab 6.5 software.

$$P_{\max} \frac{E_0 \exp(-kz_c)}{E_{\text{opt}}} \exp \left(1 - \frac{E_0 \exp(-kz_c)}{E_{\text{opt}}} \right) - \text{CR} = 0 \quad (4)$$

where CR – community respiration (mg l^{-1}).

Initial slope was estimated by deriving the Steele’s function in relation to E and calculating the limit of the derivative when E approaches zero.

2.4.2. Statistical and multivariate analyses

Spearman rank correlation analysis was performed to evaluate relations between environmental and biological variables and metabolic processes. Regression analysis was also carried out between some specific variables.

MANOVA was used to investigate differences between results obtained at different times, tides and stations. To analyse the factors “time” and “tides” a Two-way factorial MANOVA was carried out, using as surrogates for replicates the values measured at the three sampling stations at each month and each tide. To analyse the factor “station”, a One-way MANOVA was carried out. In this case, all values (26) measured at each station over the 13-month sampling were used as surrogates for replicates. In the absence of true replicates, the assumptions here are that each of the mentioned “surrogates” was a representative sample of real conditions, although not based on a random sampling, since time, tides and stations were sampled on a systematic way. Wilks test was computed for the multivariate analysis as well as the univariate tests. Calculations were carried out with the Statistica software. MANOVA was computed with raw and log transformed data, after analysing the homogeneity of variances

and the relationship between means and variances. Newman–Keuls test was applied a posteriori.

Principal components analysis (PCA) was performed using Primer Software in order to investigate patterns of similarity between samples (Q-mode analysis) based on the values of environmental and biological variables. The data matrix was organized with samples as rows and observations (variables) as columns. Data were log transformed to account for non-normal distribution of variables and standardized to account for the different units in which the variables were expressed.

In this work, seasons are defined as: winter (December–February); spring (March–May); summer (June–August) and fall (September–November).

3. Results

3.1. Environmental conditions

In Table 1, averages and standard errors for the major environmental parameters measured at each sampling station, at ebb and flood tide, are presented. River flow values during each survey, at sampling time, and monthly averaged values are shown in Fig. 2. As expected, higher values were found in winter and lower values in summer, with relatively small differences between actual survey values and monthly averaged values, except for December 2002. The highest river discharge was registered during the January ebb survey when flow reached $2700 \text{ m}^3 \text{ s}^{-1}$, and the lowest in August during both surveys, when river discharge was zero. Average river flow during the sampling period was $935 \text{ m}^3 \text{ s}^{-1}$.

Average estuarine salinity during the study period was 8.5 with values ranging between 0 and 35. Salinity was significantly correlated to river discharge, regardless of the tide ($p < 0.01$). During high discharge periods, mostly in winter, estuarine water was completely flushed out at low tide, and even during the flood, salinity values remained low at the mouth (Fig. 3). On the other hand, during the summer low discharge period, salinity values increased throughout the estuary

and, in the upper estuary, salinity higher than 18 could be found, independently of the tide.

Noticeable stratification of the water column occurred in some occasions, and a halocline was present at the lower station in the March ebb, April flood, May flood, July ebb and November flood surveys; and at the middle station in the May flood, June ebb, July flood and November flood surveys.

Water temperature followed the expected seasonal trend from a minimum of $7.2 \text{ }^\circ\text{C}$ during January and February freshet to a maximum of $25.4 \text{ }^\circ\text{C}$ in the upper estuary, during the high salinity summer period.

In general, nutrient concentrations showed a seasonal pattern, increasing during the fall–winter period and decreasing during summer. Significant ($p < 0.01$) correlations with salinity (negative) and river flow (positive) were observed. The exception was ammonium, which exhibited no clear seasonal trend and was positively correlated with salinity.

During the August flood survey, high values of $\text{NO}_3 + \text{NO}_2$ were observed in the upper estuary along the water column and in the middle estuary only at surface. Averaged values decreased from the dam to the mouth independently of the tide (Table 1). Considering the study period, $\text{NO}_3 + \text{NO}_2$ ranged between 1.4 and $227.4 \text{ } \mu\text{M}$.

Ammonium concentration ranged between 0.3 and $108.5 \text{ } \mu\text{M}$. The averaged values (Table 1) increased downstream during the ebb, while during the flood the middle station presented the highest value. Silicate values decreased downstream (Table 1) both during the ebb and the flood.

Concerning phosphate variability, values ranged from 0.2 to $4.7 \text{ } \mu\text{M}$. In general, concentration values were under $3 \text{ } \mu\text{M}$ throughout the sampling period, except in December. Only at the lower station during the flood, a significant linear relationship ($p < 0.001$) was found between phosphate and salinity, i.e. salinity did not control the dynamics of phosphate and average values throughout the estuary were rather similar.

N:P ratio ranged between 493, at the upper station during the August flood survey, and 8, at the middle station during the September flood survey. Nitrate was generally the predominant form of nitrogen. N:P values were always above the

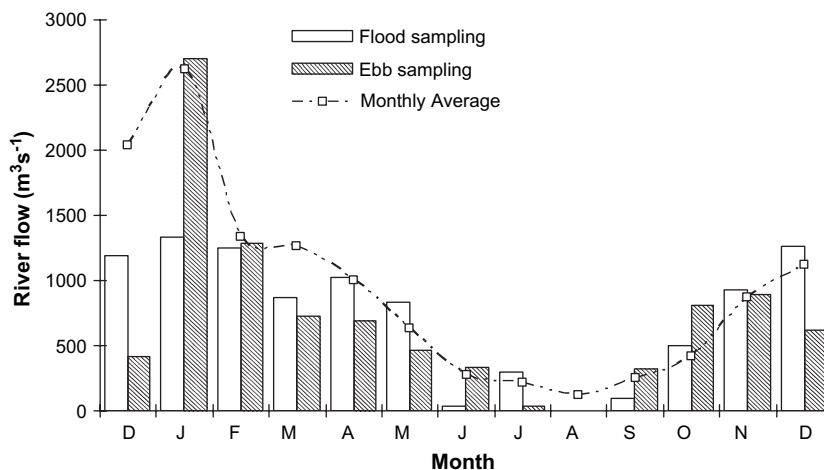


Fig. 2. River flow ($\text{m}^3 \text{ s}^{-1}$) during sampling surveys (vertical bars) and monthly averages (dotted line), from December 2002 to December 2003.

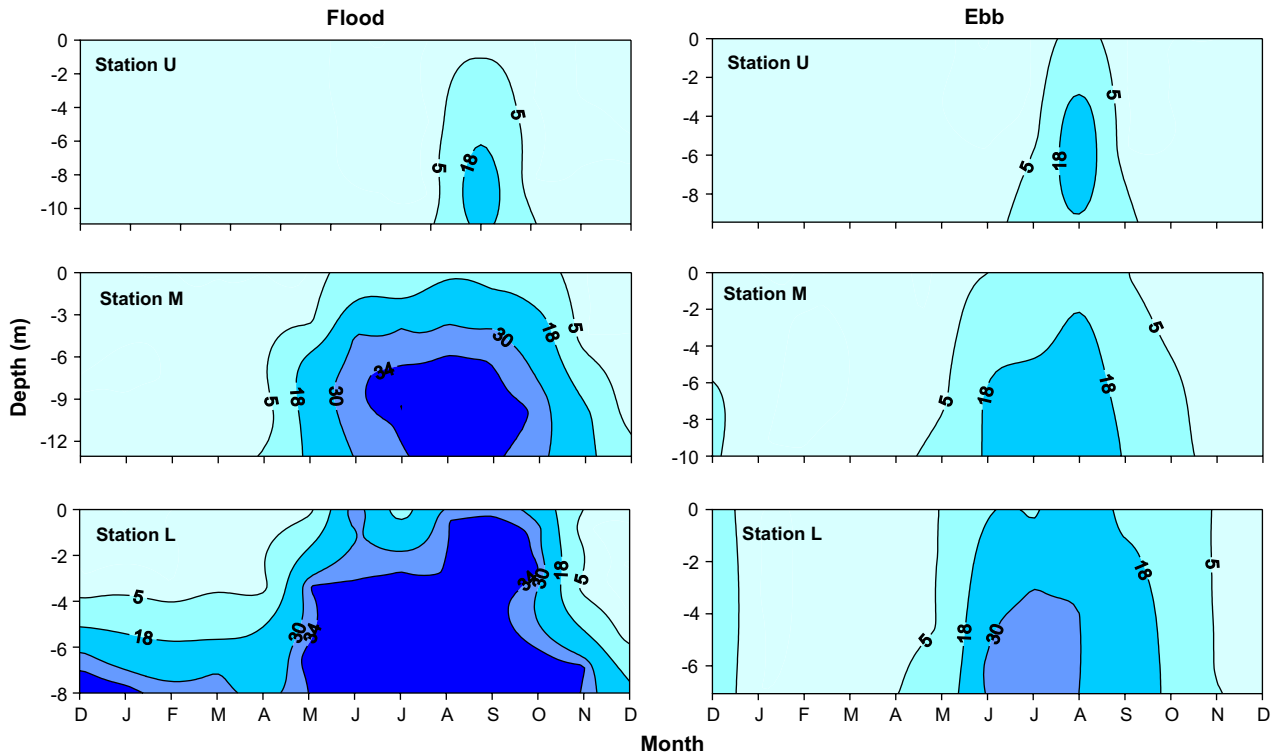


Fig. 3. Water column salinity levels at the upper (U), middle (M) and lower (L) estuarine stations during the ebb and flood surveys from December 2002 to December 2003.

Redfield ratio of 16, except for the bottom and middle depth samples collected during the September flood survey at the lower and middle stations. A general decreasing trend downstream was observed (Table 1), consistent with the salinity increase.

TPM values ranged between 2.2 and 69.2 mg l⁻¹ and POM ranged between 0.2 and 14.4 mg l⁻¹. In general, no seasonal trend was observed and maxima occurred in winter but also in summer. On an average, the highest values were measured in the lower estuary during the flood (Table 1). A significant positive linear relationship ($p < 0.01$) between TPM and the light extinction coefficient was found, independently of tide and location, as well as between TPM and POM. k Values ranged between 0.3 and 4.1, with an average of 1.3 m⁻¹.

3.2. Chlorophyll *a* and photosynthetic parameters

Phytoplankton biomass, expressed in terms of chlorophyll *a* contents, showed a clear seasonal trend (Fig. 4). Values ranged from 0.3 mg m⁻³, during the December flood survey, to 14.9 mg m⁻³, during the June flood survey, with higher biomass occurring generally in the upper estuary, particularly during the mid-spring early-summer phytoplankton bloom originated from the river. During this bloom period, biomass steadily decreased from the upper to the lower estuary. A second, more modest bloom was observed in most stations in late-summer early-fall, especially during the ebb. It should be noticed that maximal concentrations were observed in the upper station, during both the flood and the ebb. Variation

with depth occurred only during the bloom period (June), with concentration of biomass at the surface and in August at the upper station with a reduction of biomass over depth (Fig. 4). Phytoplankton biomass was positively correlated with temperature ($p < 0.01$) and negatively correlated with river flow, phosphate and silicate ($p < 0.01$). No statistical relationship was found between chlorophyll *a* and NO₃ + NO₂ or ammonium.

Table 2 summarizes the results obtained by fitting Steele's equation to P/E data (see Section 2). A general good fit was obtained between model predicted values and observations as shown by the r^2 values. P_{\max} values were higher in spring and summer, with the highest value during the August ebb survey at the upper station. During the ebb, higher values of P_{\max} were found in June, at the lower and middle estuary, whereas during the flood, the highest values were found in July–August at the lower and middle estuary and June and August at the upper estuary. Globally, photosynthetic parameters P_{\max} and E_{opt} were significantly ($p < 0.01$) correlated with chlorophyll *a* and temperature (positively) and with river flow and k (negatively). The initial slope was correlated significantly and positively with temperature. The relationships between P_{\max} and the initial slope versus temperature were also analysed by regression analysis. A relatively good fit was obtained with linear regression (respectively, $r^2 = 0.4$; ANOVA $p < 0.0001$ and $r^2 = 0.16$; ANOVA $p < 0.001$).

Compensation depth values are presented in Table 3. These values were compared with sampling stations' mean depth and the depth of the halocline when present. In the majority of

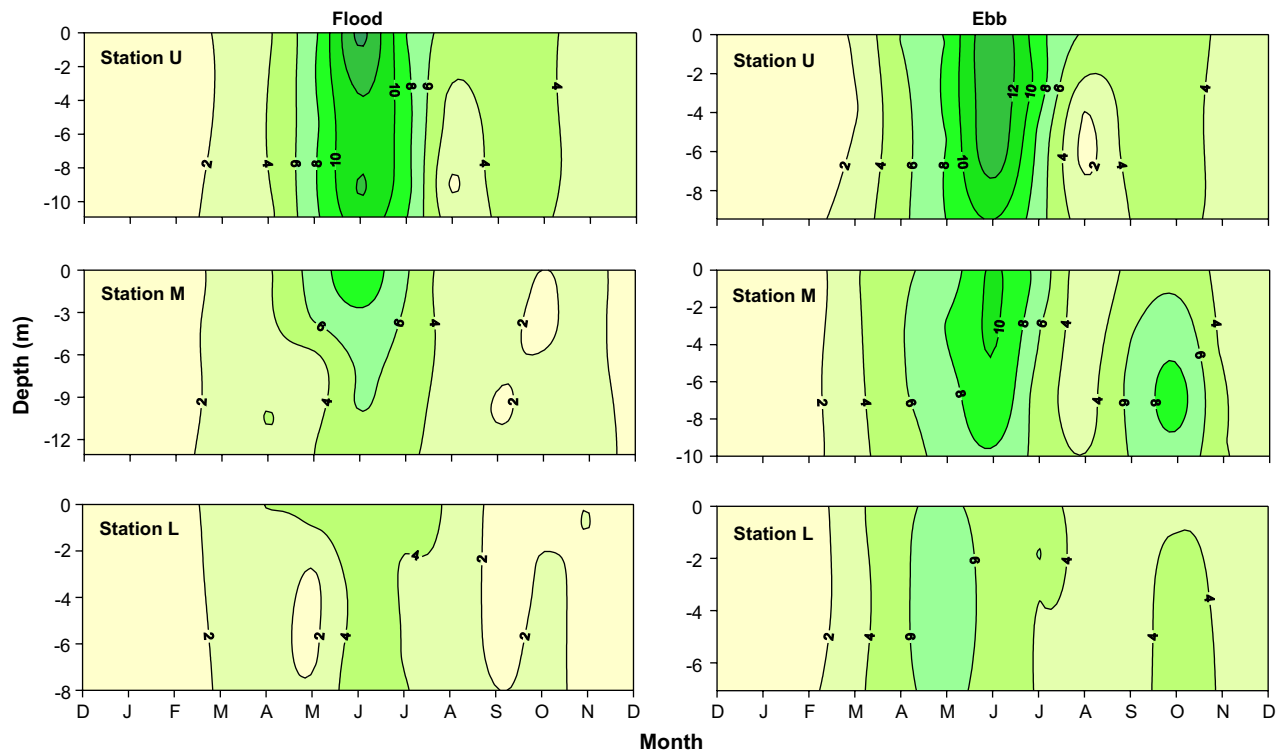


Fig. 4. Water column chlorophyll *a* values at the lower (L), middle (M) and upper (U) estuarine stations, during the ebb and flood surveys, from December 2002 to December 2003.

situations analysed, compensation depths were less than half of water column depth. During spring and summer, however, a fully euphotic water column occurred in some stations, namely at the upper station in May, during the flood, and in May through August, during the ebb; at the middle station in June, during the flood, and at the lower station in June. Only in two situations the compensation depth was higher than the halocline: at the lower station, during the July ebb survey and at the middle station, during the July flood survey, when the halocline was present at 2.8 and 4.4 m, respectively.

3.3. Estuarine metabolism

Except for the May–July period in the upper estuary, corresponding to the phytoplankton bloom, CR was always higher than photosynthetic production, i.e. heterotrophy was the dominant process in the estuarine water column (Fig. 5).

A decreasing trend in integrated PP from the upper to the lower estuary was found, independently of the tide. Integrated daily values, however, were higher during the flood. Water column PP was significantly ($p < 0.01$) and positively correlated with temperature and salinity but negatively correlated with river flow, k , nutrients (except ammonium), TPM and POM. No relationship was found between PP and water column CR. From the data obtained during the study period, water column PP annual averages were calculated. Values of 95, 106 and 160 $\text{g C m}^{-2} \text{y}^{-1}$ were obtained for the lower, middle and upper estuary, respectively. It is noteworthy that these PP annual values are estimates from spring tides only, since

there are no data available for neap tides, that would allow a more accurate estimate of water column annual PP.

Regarding CR, values increased steadily from the upper to the lower estuary, reaching its maximum expression during the summer months. Annual averages of 570, 478 and 199 g C m^{-2} were obtained for the lower, middle and upper estuary, respectively. Depth-integrated hourly CR showed a positive significant correlation with salinity and ammonium and negative correlation with $\text{NO}_3 + \text{NO}_2$, silicate and river flow ($p < 0.01$).

3.4. Effect of time, tide and stations

After testing the MANOVA assumptions, it was concluded that not all variances were homogeneous, even after standardization and log transformation. The effect “time” was significant ($p < 0.05$) for P_{max} , k , salinity, phosphate and ammonium. The effect “tide” was significant ($p < 0.05$) for k only.

The effect “Station” was significant for salinity, $\text{NO}_3 + \text{NO}_2$, ammonium and POM.

A posteriori comparisons with the Newman–Keuls test indicated significant differences ($p < 0.05$) between the upstream station and the other two stations. Regarding the variable CR, there was a significant “station” effect ($p < 0.01$), unlike the variable PP. However, both results must be considered with caution, because of variance heterogeneity.

Table 2
Monthly photosynthetic parameters obtained for the PI curves for the three sampling stations along the year during the ebb and flood surveys. P_{\max} in mg C mg Chl $a^{-1} h^{-1}$; E_{opt} in $\mu E m^{-2} s^{-1}$; initial slope of the P/E curve in mg C mg Chl $a^{-1} h^{-1} (\mu E m^{-2} s^{-1})^{-1}$

Date	Station L				Station M				Station U			
	P_{\max}	E_{opt}	r^2	Slope	P_{\max}	E_{opt}	r^2	Slope	P_{\max}	E_{opt}	r^2	Slope
<i>(A) Ebb</i>												
Dec-02	0.87	446.58	0.42	0.0053	1.25	508.02	0.83	0.0067	2.38	398.24	0.99	0.0162
Jan-03	1.46	450.84	0.86	0.0088	1.83	418.97	0.88	0.0119	2.91	507.30	0.94	0.0156
Feb-03	1.35	423.07	0.68	0.0086	1.66	408.89	0.72	0.0110	1.51	379.93	0.71	0.0108
Mar-03	1.76	469.45	0.83	0.0102	2.86	610.88	1.00	0.0127	2.59	535.93	0.84	0.0131
Apr-03	1.95	554.82	0.88	0.0096	2.97	540.28	0.92	0.0149	2.73	608.54	0.90	0.0122
May-03	2.29	466.31	0.88	0.0133	3.99	612.00	0.99	0.0177	3.44	631.26	0.98	0.0148
Jun-03	3.27	455.66	0.76	0.0194	5.56	1882.83	0.86	0.0080	4.09	641.63	0.96	0.0173
Jul-03	2.67	553.08	0.95	0.0131	2.57	560.27	1.00	0.0124	3.87	595.88	0.99	0.0176
Aug-03	3.21	623.17	0.84	0.0140	3.18	687.98	0.92	0.0125	9.24	626.14	0.92	0.0401
Sep-03	1.90	603.28	0.96	0.0086	2.40	684.95	0.96	0.0095	2.73	670.71	0.86	0.0110
Oct-03	2.57	522.12	0.93	0.0134	2.99	675.28	0.96	0.0120	3.79	618.67	0.95	0.0166
Nov-03	2.02	498.35	0.79	0.0110	2.32	524.11	0.80	0.0120	2.89	544.53	0.83	0.0144
Dec-03	1.00	592.37	0.98	0.0046	1.44	925.37	0.88	0.0042	1.02	440.12	0.90	0.0063
<i>(B) Flood</i>												
Dec-02	2.51	374.46	0.44	0.0182	3.76	460.18	0.50	0.0222	1.18	718.43	0.84	0.0045
Jan-03	1.45	373.77	0.46	0.0105	1.42	417.60	0.90	0.0092	2.86	380.81	0.89	0.0203
Feb-03	1.74	509.36	0.91	0.0093	1.96	473.66	1.00	0.0112	1.90	505.96	0.97	0.0102
Mar-03	1.73	487.23	0.97	0.0096	2.41	427.91	0.98	0.0153	3.45	812.50	0.80	0.0115
Apr-03	2.43	508.38	0.95	0.0130	2.65	565.28	0.91	0.0127	1.93	587.68	0.93	0.0089
May-03	2.30	587.68	0.93	0.0106	4.43	516.55	0.98	0.0232	3.56	540.42	0.97	0.0179
Jun-03	2.64	582.00	0.89	0.0123	3.08	663.97	0.99	0.0126	4.66	616.00	0.96	0.0205
Jul-03	5.06	722.95	0.92	0.0190	4.52	825.55	0.96	0.0149	3.81	657.61	0.91	0.0157
Aug-03	4.40	675.82	0.94	0.0177	5.45	676.56	0.94	0.0218	4.65	606.66	0.92	0.0208
Sep-03	1.49	698.70	0.84	0.0058	3.66	657.70	0.91	0.0151	3.25	643.49	0.96	0.0137
Oct-03	1.68	489.07	0.81	0.0093	2.58	685.56	0.95	0.0102	3.84	801.13	0.99	0.0130
Nov-03	3.68	552.63	0.98	0.0181	3.38	587.42	1.00	0.0156	3.17	591.01	0.99	0.0145
Dec-03	1.44	551.88	0.90	0.0071	1.30	531.66	0.97	0.0066	1.13	539.04	0.85	0.0057

3.5. Patterns of similarity between samples

The eigenvalues corresponding to the five principal components (PCs) considered for analysis are presented in Table 4. These were chosen because all were greater than the unity and together explained 77.7% of the total variance contained

Table 3
Compensation depths calculated for the three sampling stations along the year during the ebb and flood surveys. Compensation depth values represented in bold type are close to or higher than water column depth

Date	Ebb			Flood		
	Station L	Station M	Station U	Station L	Station M	Station U
Dec-02	^a	^a	^a	^a	1.1	^a
Jan-03	^a	^a	^a	^a	^a	1.2
Feb-03	^a	^a	^a	^a	^a	5.6
Mar-03	1.7	1.5	1.2	^a	0.7	1.7
Apr-03	2.8	2.7	3.1	3.2	2.2	^a
May-03	2.5	1.3	4.6	6.0	3.0	6.7
Jun-03	7.3	2.3	3.6	3.5	10.3	5.6
Jul-03	2.8	3.2	4.8	^a	4.4	5.3
Aug-03	^a	0.7	6.5	2.7	3.2	5.8
Sep-03	^a	0.5	0.4	^a	3.7	3.3
Oct-03	3.0	1.7	4.2	^a	^a	3.0
Nov-03	0.7	0.6	3.1	0.8	1.4	2.8
Dec-03	^a	^a	^a	^a	^a	^a

^a The solution of Eq. (4) resulted in an imaginary number (see Section 2).

in the original data set. The correlation coefficients between PCs and variables are presented in Table 5. The variables that contributed the most to PC1 were temperature and PP (positively), k and silica (negatively), suggesting a dominant influence of physical factors on primary production. PC2 was positively participated by POM, TPM, and salinity, and negatively participated by $NO_3 + NO_2$ and the N:P ratio, mostly chemical factors. PC3 was negatively participated by N:P ratio, TPM, POM and initial slope. PC4 was highly and negatively participated by ammonium, possibly indicating an anthropogenic influence. PC5 was positively participated by chlorophyll a and E_{opt} and negatively by the initial slope. However, these last two PCs explained only a small portion of the total variance (Table 4).

In Fig. 6 a representation of the first two PCs is shown. Samples have been labelled based on season. Winter and summer samples were arranged at opposite extremes of PC1 while spring and fall were spread across the middle, showing a clear seasonal pattern. This is in agreement with the contribution of physical variables for PC1. On the other hand, Fig. 7 represents the same projection but with the samples labelled by sampling station, highlighting spatial trends. In this case, stations were spread along the PC2 axis. The lower estuarine station samples were spread over the positive side whereas the upper station samples were located at the negative side. This agrees with the positive contribution of salinity (higher in

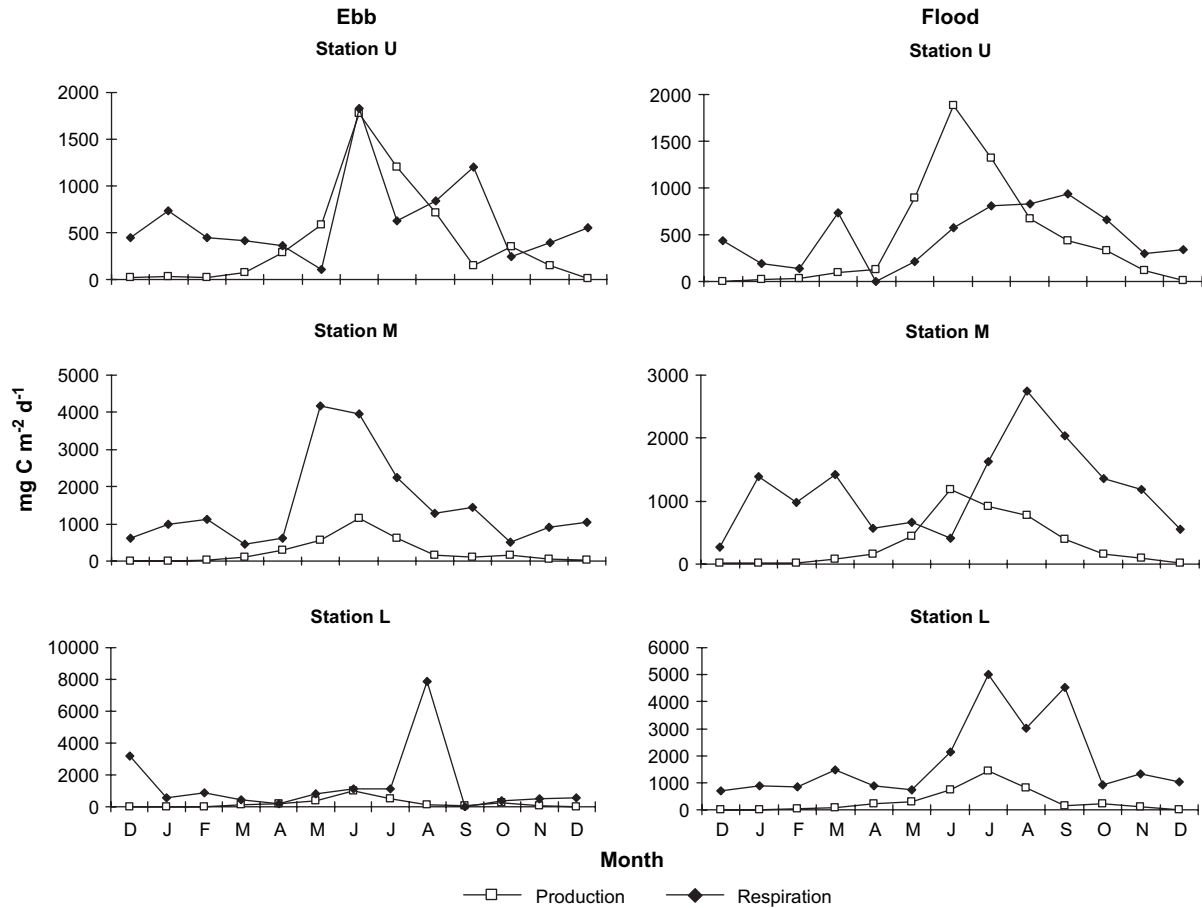


Fig. 5. Daily integrated phytoplanktonic PP and water column CR in the lower (L), middle (M) and upper (U) estuarine stations, during the ebb and flood surveys, from December 2002 to December 2003.

the lower estuary) and the negative contribution of $\text{NO}_3 + \text{NO}_2$ (higher in the upper estuary). No association between samples based on tide was observed. Fig. 8 shows graphically the weight of variables PP and extinction coefficient superimposed on the projection of samples, labelled by season, in the two-dimensional space defined by the first two principal components. The larger the circle, the greater the value of the superimposed variable. The samples with the highest values of PP are located in the positive side while the highest values of k are located on the negative side of the PC1 axis, reflecting the negative correlation found between these two variables.

4. Discussion

The Douro estuary is a highly dynamic system like most estuaries and very dependent on river flow variations which are due to seasonal changes and dominate water circulation (Vieira and Bordalo, 2000). River inflow determines the extent of salt water intrusion, residence time, levels of nutrients and phytoplankton biomass. During winter, high flows often prevent coastal water from entering into the estuary, even during the flood, raise nutrient levels, lower phytoplankton biomass and reduce water residence time. In this estuarine system, river

flow rather than tides controls water residence time (Bordalo and Vieira, 2005). The surveys were carried out during a wet year, since the average river discharge was almost twice the long-term average inflow of freshwater.

Considering nutrient limitation of phytoplankton production, oceanic systems are considered to be nitrogen limited (Eppley et al., 1973) while freshwater systems are generally viewed as phosphorus limited (Schindler, 1977). In estuaries, where seasonal and spatial variations of freshwater and seawater mixtures occur, this concept is not so clear (Bernhard and Peele, 1997). Seasonal alternation of nitrogen and phosphorus limitation has been reported (Fisher et al., 1992; Mallin et al., 1999) as well as a spatial shift from phosphorus to nitrogen limitation in some estuaries (Yin et al., 2001). In the present study, N:P ratio was usually high and always above the Redfield ratio except for the bottom and middle depth samples collected at the lower and middle stations during the September flood tide survey. These were high salinity low nutrient samples, characteristic of seawater where nitrogen limitation is common. Hence, generally, in the Douro estuary phosphate, rather than nitrate, was the potentially limiting nutrient. Nevertheless, nitrate limitation can occur in low river flow situations when scarcely diluted seawater is present within the estuary.

Table 4
Eigenvalues, percent variation and cumulative percent variation of the first five principal components

PC	Eigenvalues	%Variation	Cum.%variation
1	7.05	39.1	39.1
2	2.8	15.6	54.7
3	1.68	9.3	64
4	1.3	7.2	71.2
5	1.17	6.5	77.7

The PP obtained in the Douro estuary is within the productivity range for other temperate and subtropical estuaries (Table 6). The results clearly show that the Douro estuary is predominantly heterotrophic. This is an expected result, considering the shallowness (around 23%) of the euphotic layer/compensation depth compared to the depth of the estuary (Table 3) and it is also a normal feature in temperate tidal estuaries (Heip et al., 1995). The deficit in PP may be compensated by external sources of organic matter, such as untreated sewage discharge or treated effluent disposal by the eight WTPs (Fig. 1). The contribution of benthic PP is limited owing to the small intertidal area of the estuary (Magalhaes et al., 2003) and, due to land reclamation, saltmarsh areas were dramatically reduced to less than 0.1 km² (Bordalo, unpublished data).

The analysis of Figs. 6 and 7, and the MANOVA results indicate significant ($p < 0.01$) differences in CR between sampling stations and suggest different metabolic patterns over time and space in the Douro estuary. The gap between PP and CR increases towards the river mouth, with some positive values in summer in the upper and middle estuary. It is noteworthy that heterotrophy is much more evident towards the sea. The explanation for this fact lies probably in the higher TPM and POM loads at this end of the estuary, a very uncommon phenomenon that may be explained by the dam effect at the upstream end of the estuary, retaining large amounts of

sediments. Also, the human-induced contamination of the lower and middle estuary (Bordalo, 2003), contributed to an increased organic loading and hence of respiration, leading to the heterotrophy situation observed all year round.

The results show that different factors may control PP over space and time. From the PCA analysis, it is clear that spatial differences arose mostly along PC2, contrasting mostly larger nitrate + nitrite concentrations and N:P ratios (upper station) to higher salinities, TPM and POM concentrations (lower station). This suggests that the upper estuary is more productive than the lower estuary as a result of higher nitrogen concentrations from riverine origin (Mallin et al., 1993; Malej et al., 1995). Also, higher chlorophyll biomass from the reservoir (Bordalo and Vieira, 2005) may help to explain these trends, but to a lesser extent (Table 5). Regarding temporal differences, higher PP values were associated to higher temperatures, lower light extinction coefficients (k) and higher P_{\max} , coinciding with the summer period. According to Heip et al. (1995), annual PP values lower than 160 g C m⁻² y⁻¹ result from light limitation in nutrient-rich or heterotrophic systems, which is the case of Douro estuary, with an annual estimate of 120 g C m⁻² y⁻¹. This is partly confirmed from the results obtained in this work, regarding temporal variability in PP, statistically related to k values.

Probably, one of the most interesting aspects of this work is the positive correlation of chlorophyll a with P_{\max} . In marine ecosystems, there seems to be a general decreasing trend of the P/B ratio as a function of net primary production (NPP). In one extreme there are oligotrophic ecosystems, with high P/B , low biomass and NPP, such as open ocean pelagic systems, and in another extreme there are ecosystems with low P/B , high NPP and biomass, such as algal reefs and beds (Duarte et al., 2006). These trends suggest that low biomass and NPP ecosystems are more efficient in using limiting resources. Furthermore, low biomass standing stocks may also leave more resources per unit of biomass, helping to explain

Table 5
Eigenvectors or coefficients in the linear combinations of variables making up PC's

Variable	PC1	PC2	PC3	PC4	PC5
P_{\max}	0.282	-0.211	-0.256	0.046	-0.199
E_{opt}	0.217	-0.097	0.208	0.144	0.468
k	-0.3	-0.073	-0.274	-0.002	0.152
Slope	0.191	-0.168	-0.363	-0.094	-0.545
PP	0.327	-0.149	-0.125	0.193	0.093
CR	0.13	0.213	-0.013	-0.209	0.197
Chlorophyll a	0.241	-0.201	-0.12	0.278	0.36
Temperature	0.325	-0.049	0.038	0.206	0.05
Salinity	0.217	0.364	-0.052	-0.14	0.071
NO ₃ + NO ₂	-0.238	-0.384	-0.064	-0.207	0.115
Phosphate	-0.258	-0.066	0.314	-0.108	-0.071
Silica	-0.32	-0.232	0.002	-0.036	0.004
Ammonium	0.062	0.203	-0.141	-0.595	0.332
N:P ratio	0.001	-0.365	-0.449	-0.246	0.263
Turbidity	-0.262	0.009	-0.256	0.212	0.142
TPM	-0.174	0.342	-0.385	0.27	0.056
POM	-0.125	0.411	-0.33	0.15	0.002
River flow	-0.261	-0.06	0.058	0.371	0.126

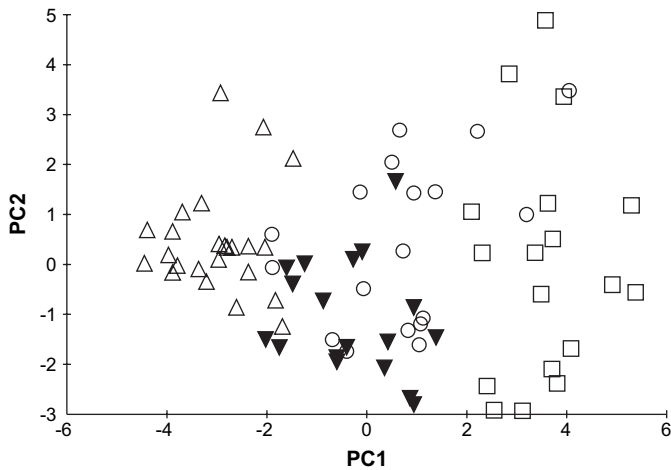


Fig. 6. Projection of samples in two dimensions, defined by the first two principal components, labelled by season – winter (Δ); spring (\blacktriangledown); summer (\square); and fall (\circ).

higher P/B ratios. P_{\max} , expressed as $\text{mg C mg Chl } a^{-1} \text{ h}^{-1}$, may be viewed as a potential P/B ratio. Therefore, from the results obtained, it seems that the Douro estuary has a different pattern regarding the usual relationship between P/B and biomass standing stock – higher biomass situations coincide with higher phytoplankton photosynthetic capacity. This positive feedback is reinforced by higher initial slopes (Table 2). The significant linear relationships between those parameters and temperature highlighted the importance of temperature in contributing to higher P_{\max} and photosynthetic efficiency. Madariaga (1995) in the Urdaibai estuary (Bay of Biscay) and van Spaendonk et al. (1993) in the Westerschelde (The Netherlands) obtained higher P_{\max} values downstream than upstream, whereas the contrary was true for PP and chlorophyll concentrations: exactly the opposite trends obtained in this work for P_{\max} and in line with previous comments on the P/B ratios. P_{\max} values reported here are well within those measured by

Madariaga (1995), 2.03 and $15.21 \text{ mg C mg Chl } a^{-1} \text{ h}^{-1}$, and van Spaendonk et al. (1993), 0.08 and $16 \text{ mg C mg Chl } a^{-1} \text{ h}^{-1}$.

The mentioned patterns may result from the specific characteristics of the Douro river and its estuary. The relatively low chlorophyll concentrations in the Crestuma-Lever reservoir, within $8\text{--}12 \text{ mg Chl } a \text{ m}^{-3}$ (Bordalo, unpublished data), discharging directly into the estuary, may explain the low phytoplankton biomass within the estuary.

The river is dammed all over its course and this may justify the relatively low TPM loads to the estuary as compared to other European estuarine systems, such as the Sado estuary (Portugal), the Gironde (France) and the Scheldt (Belgium and The Netherlands). In such systems TPM concentrations in the upper estuarine area were 600 mg l^{-1} , $>200 \text{ mg l}^{-1}$ and c.a. 40 mg l^{-1} (Cabecadas et al., 1999). Moreover, in the Douro estuary an increasing trend of TPM with salinity was observed (Table 1), whereas in the above-mentioned systems an opposite trend was found. Chlorophyll concentrations were in the range of those measured in the Douro, except for the Scheldt, with maximal values above $200 \text{ mg Chl } a \text{ m}^{-3}$. For both the Sado and the Gironde, chlorophyll maxima occurred at intermediate salinities, whereas for the Scheldt, upstream maximum was reported. This was also the case of Douro, where the highest chlorophyll concentration was observed at the estuary head, indicating a riverine origin of phytoplankton.

A possible explanation for the positive correlation between photosynthetic capacity and chlorophyll concentration of the Douro phytoplankton may be the relatively low phytoplankton biomass, preventing intraspecific competition from lowering photosynthetic capacity, on one hand, and the physiologic characteristics of phytoplankton itself, originated from a semi-lotic (Crestuma reservoir) rather than from a true lotic ecosystem. Probably, this last hypothesis deserves further investigation, since it may clarify some impacts of river damming over estuarine metabolism in accordance with the Water Framework Directive (EC, 2000).

The absence of historical data to compare the obtained results with similar studies carried out before dam construction prevents any definite conclusion about the dam effect on estuarine production and metabolism. However, considering the important differences between the Douro estuary and other European estuaries, it may be acceptable that the river Douro dams lead to a reduction in TPM and chlorophyll loads into the estuary and a decrease in estuarine metabolism at its upstream end. Whilst it is generally accepted that TPM loads decrease as a result of dam retention, the same is not so clear regarding chlorophyll. However, considering that the Crestuma-Lever reservoir has an average depth of above 13 m, that only about one-third of the water column is euphotic and that, for most of the year, the water column is well mixed (Bordalo et al., unpublished data), it may be speculated that PP in the reservoir is not very high and therefore relatively low chlorophyll values may be expected as an input to the estuary.

Finally, significant differences between tides were not observed, as shown by MANOVA and multivariate analyses

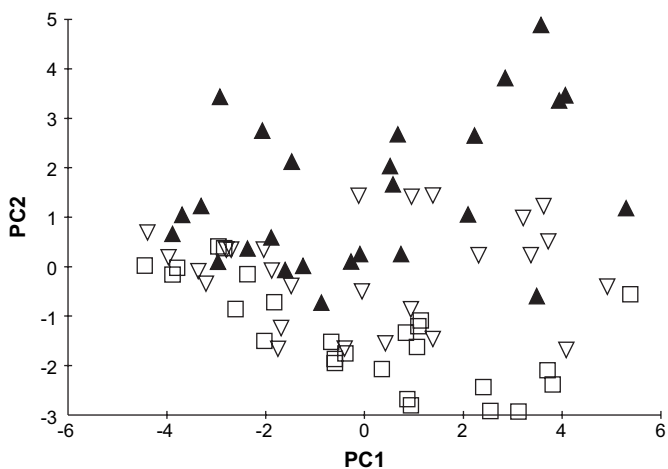


Fig. 7. Projection of samples in two dimensions, defined by the first two principal components, labelled by station – Lower (\blacktriangle); Middle (∇); and Upper (\square).

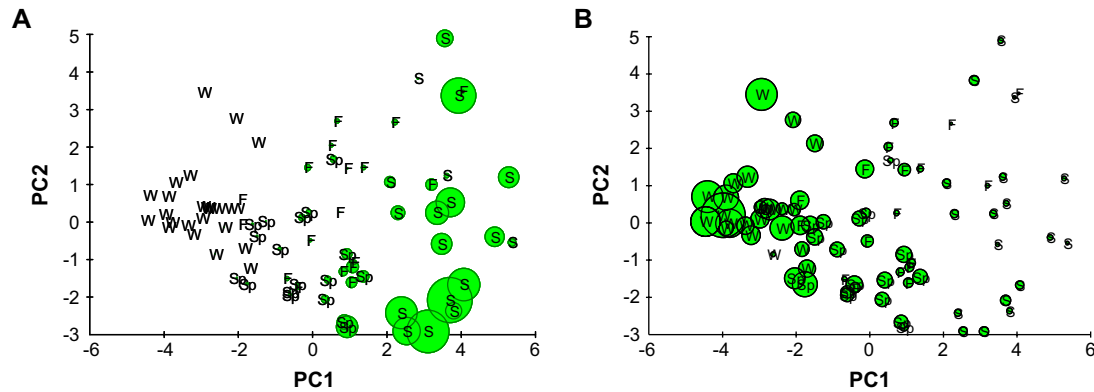


Fig. 8. Projection of samples in two dimensions defined by the two first principal components. Bubble size is related to the magnitude of the variable represented. A – depth-integrated hourly PP; B – extinction coefficient (k). Samples are labelled by season: winter (W); spring (Sp); summer (S); and fall (F).

results, except for k , reflecting higher water turbidity during the ebb. Considering spatial variability, higher turbidity in the lower estuary is rather derived from sewage discharge within that estuarine stretch or from oceanic outfalls than from sediment resuspension, which traditionally originates the turbidity maximum. Differences between tides concerning phytoplankton biomass reported in other studies are related to a much higher difference between chlorophyll a coming from the river and the adjacent coastal waters (Roegner, 1998); or to seasonal phytoplankton cycles (Aubry and Acri, 2004).

5. Conclusions

The results obtained from this study suggest that:

- (1) The Douro estuary is predominantly heterotrophic, with some exceptions in the lower salinity upstream area in summer.
- (2) PP seems to be mostly controlled by temperature, being also influenced by a positive feedback from photosynthetic capacity and chlorophyll biomass.

- (3) No significant differences between ebb and flood were observed in what PP is concerned.
- (4) The reservoir located upstream may have a dominant influence on TPM concentration gradients in the estuary, which exhibit low TPM and chlorophyll a comparing to other systems.
- (5) Further studies addressing the impact of the upstream dam on the characteristics of phytoplankton arriving at the estuary, as well as its influence on estuary metabolism are needed.

Acknowledgements

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Table 6

Summary of pelagic primary production (PP) measurements (mean values or ranges) in temperate estuaries

Site	Pelagic PP (mg C m ⁻² d ⁻¹)	Author
Bristol Channel	204	Joint (1978)
Chesapeake Bay	500–3500	Malone et al. (1996)
Colne	24	Kocum et al. (2002)
Danube	200–4400	Humborg (1997)
Douro	4.7–1879 (mean = 320)	This work
Eastern Scheldt	908	Wetsteyn and Kromkamp (1994)
Ems-Dollard	20.4	Van Es FB (1977)
Ems-Dollard	36	Cadée and Hegeman (1974)
Lynher	222	Joint and Pomeroy (1981)
St. Lawrence	10–800	Sinclair (1978)
Swan river	2192–2740	Thompson (1998)
Western Scheldt	485	van Spaendonk et al. (1993)
Western Scheldt	632	Kromkamp et al. (1995)
Apalachicola Bay	90–1800	Mortazavi et al. (2000)
Mississippi	50–1000	Thomas and Simmons (1960)

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