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# The influence of incubation periods on photosynthesis–irradiance curves

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## Abstract

In phytoplankton primary production studies, samples for the determination of photosynthesis versus irradiance relationship (*P–I*) are usually incubated at several irradiance levels during a fixed time period, commonly 2–4 h. However, it is not clear if the use of this fixed incubation time is appropriate to study the *P–I* relationship in any given ecosystem. The aim of this work was to study the influence of incubation time on the *P–I* relationship in natural phytoplankton populations from three different coastal ecosystems: an open coastal area, an estuary, and a coastal lagoon. Physical and chemical variables, phytoplankton biomass, species composition, and *P–I* curves were analysed. The results showed that, in the coastal area and in the estuary, *P–I* relationships were time dependent, whereas in the coastal lagoon different incubation periods produced the same *P–I* curve. An underestimation of daily primary production, ranging from 13% to 42.5%, was calculated when data from standard incubation times (2–4 h) were used in ecosystems where *P–I* curves present a dynamic time-dependent behaviour. This work suggests that the response of the *P–I* curves to the incubation time varies with the characteristics of the ecosystem and is related to the light regime to which phytoplankton cells are adapted. © 2002 Elsevier Science B.V. All rights reserved.

*Keywords:* Incubation periods; Modelling; Photoinhibition; Phytoplankton productivity

## 1. Introduction

The photosynthesis–irradiance (*P–I*) relationship is fundamental in primary productivity studies and has particular relevance in recent attempts to predict fluxes of carbon in

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the world's ocean. The parameters describing the $P–I$ curve contain information on the physiology of the algae and variations in the parameters can reflect changes in environmental conditions (Harrison and Platt, 1980; Falkowski, 1981; Falkowski and Raven, 1997; Sakshaug et al., 1997). To study the $P–I$ relationship, samples for primary productivity determination are usually incubated at several light intensities, under natural or artificial light during a fixed time period, commonly 2–4 h. The $P–I$ curve parameters calculated from the $P–I$ relationships obtained are then used in models to estimate daily or annual primary production. However, it is known that vertical water movements produced by surface waves, internal waves, convection, Langmuir circulation, etc., impose a fluctuating light environment for phytoplankton. Phytoplanktonic cells meet the demands of this unstable light climate by exhibiting extreme plasticity in their photosynthetic apparatus to optimise the photosynthetic yield (Flameling and Kromkamp, 1997). Understanding vertical mixing processes (Falkowski and Wirick, 1981; Ravens et al., 2000) and consequent phytoplankton photoacclimation represented a major challenge in marine biology in the recent years (Denman and Gargett, 1983; Falkowski, 1984; Iriarte and Purdie, 1993; Dusenberry et al., 1999).

Several workers studied the effect that fluctuations in the light field have on productivity (e.g., Gallegos and Platt, 1985; Mallin and Pearl, 1992; Franks and Marra, 1994; MacIntyre and Geider, 1996; Flameling and Kromkamp, 1997). One central issue related to the effect of light fluctuations on productivity is the occurrence of photoinhibition in response to high irradiance levels. There is abundant evidence that photoinhibition occurs in natural phytoplankton communities (Prasil et al., 1992; Long et al., 1994; Kirk, 1994; Falkowski and Raven, 1997; Han et al., 2000; Macedo et al., 2001). It is known that photoinhibition may depend not only on exposure to a critical light level, but also on the exposure time (Takahashi et al., 1971; Harris and Lott, 1973; Pahl-Wostl and Imboden, 1990). According to Pahl-Wostl and Imboden (1990), the time scale for full development of photoinhibition varies between 0.5 and 1.5 h. There is some evidence that phytoplankton can maintain high rates of photosynthesis during the first minutes after exposure to saturating or inhibiting irradiance (Harris and Lott, 1973; Harris and Piccinin, 1977; Marra, 1978b; Macedo et al., 1998). Therefore, static models based on $P–I$ curves obtained under constant light intensities and incubation periods between 2 and 4 h may clearly underestimate primary productivity under natural conditions, when vertical mixing prevents phytoplankton from long exposures to critical light levels (Goldman and Dennett, 1984; Gallegos and Platt, 1985; Duarte and Ferreira, 1997; Macedo et al., 1998). Moreover, the time taken by a phytoplanktonic cell to cross the photic zone and therefore the full gradient of irradiance in the mixed layer is highly dependent on the physical conditions of the ecosystem.

According to MacIntyre and Geider (1996), with a vertical diffusion coefficient of about 50 $cm^2 s^{-1}$, a phytoplanktonic cell may take 3 days to cross the photic layer in a continental shelf with a photic zone of 50 m, or 13 min in an estuary with a photic depth of 2 m. Even under stratified conditions, phytoplankton cells are not fixed at a specific depth or irradiance level. Considering a surface layer in a stratified water column, photoinhibition may be reduced if the layer depth is larger than the photoinhibition depth.

The nature of the vertical transport and consequently the phytoplankton photosynthetic response varies widely with the physical conditions. Therefore, it is crucial to know the response of the $P$–$I$ curves to exposure time and to verify if this response is the same in different ecosystems. Furthermore, it is important to understand the dynamics of the $P$–$I$ curve parameters for its incorporation in production models. Only a limited amount of work has been done regarding the response of the $P$–$I$ curves to exposure time. Harris and Piccinin (1977) monitored oxygen production in natural populations of phytoplankton, as a function of time in samples exposed to different light intensities. Marra (1978a,b) performed a set of similar experiments using laboratory conditions and more recently, Macedo et al. (1998) studied the dynamic behaviour of $P$–$I$ curves in a natural population of phytoplankton.

The specific objectives of this work are:

1. To study the influence of the incubation time on $P$–$I$ curve parameters in three different coastal ecosystems: a coastal area, an estuary, and a coastal lagoon.
2. To evaluate the importance of incubation time in the development of photoinhibition under different environmental conditions.
3. To examine for each ecosystem the main characteristics that can be responsible for the static or dynamic behaviour of the $P$–$I$ curve parameters.

## 2. Material and methods

### 2.1. Study areas

In this work, three marine ecosystems with different physical conditions, in the same geographic area, were chosen to represent the main coastal marine ecosystems: an open coastal area, an estuary, and a coastal lagoon (Fig. 1). The coastal area ecosystem is represented by the Arrábida coast (38°27′ N, 09°W), located south of Lisbon and in the vicinity of the Sado estuary (Portugal). This ecosystem presents a rocky coast with steep cliffs and high marine biodiversity (Almada et al., 1990; Santos, 1994; Henriques et al., 1999). Samples were collected from a station with a depth of 15 m. The estuarine ecosystem sampled is the Tagus estuary, located near Lisbon, with a surface area of 320 $km^2$ and a mean volume of $1900 \times 10^6$ $m^3$ (Ferreira and Ramos, 1989). The data presented and discussed here were obtained in a channel of the Tagus estuary called Cala do Norte (38°50′ N, 09°04′ W). Santo André lagoon (38°05′ N, 8°47′ W), located in Southwest of Portugal, represents a coastal lagoon ecosystem. This lagoon is a land-locked coastal ecosystem with a temporary connection with the sea. Santo André lagoon receives freshwater inputs from six small rivers forming a drainage basin of about 96 $km^2$, it has an average annual depth of about 1 m, with a maximum of 5 m in autumn. Table 1 shows the main characteristics of these coastal ecosystems.

### 2.2. Sampling and treatment

A total of eight experiments were performed: three in the Arrábida, one in the Tagus estuary, and four in Santo André lagoon. Physical and chemical variables, phytoplankton biomass, species composition, and $P$–$I$ curves were determined for each experiment.

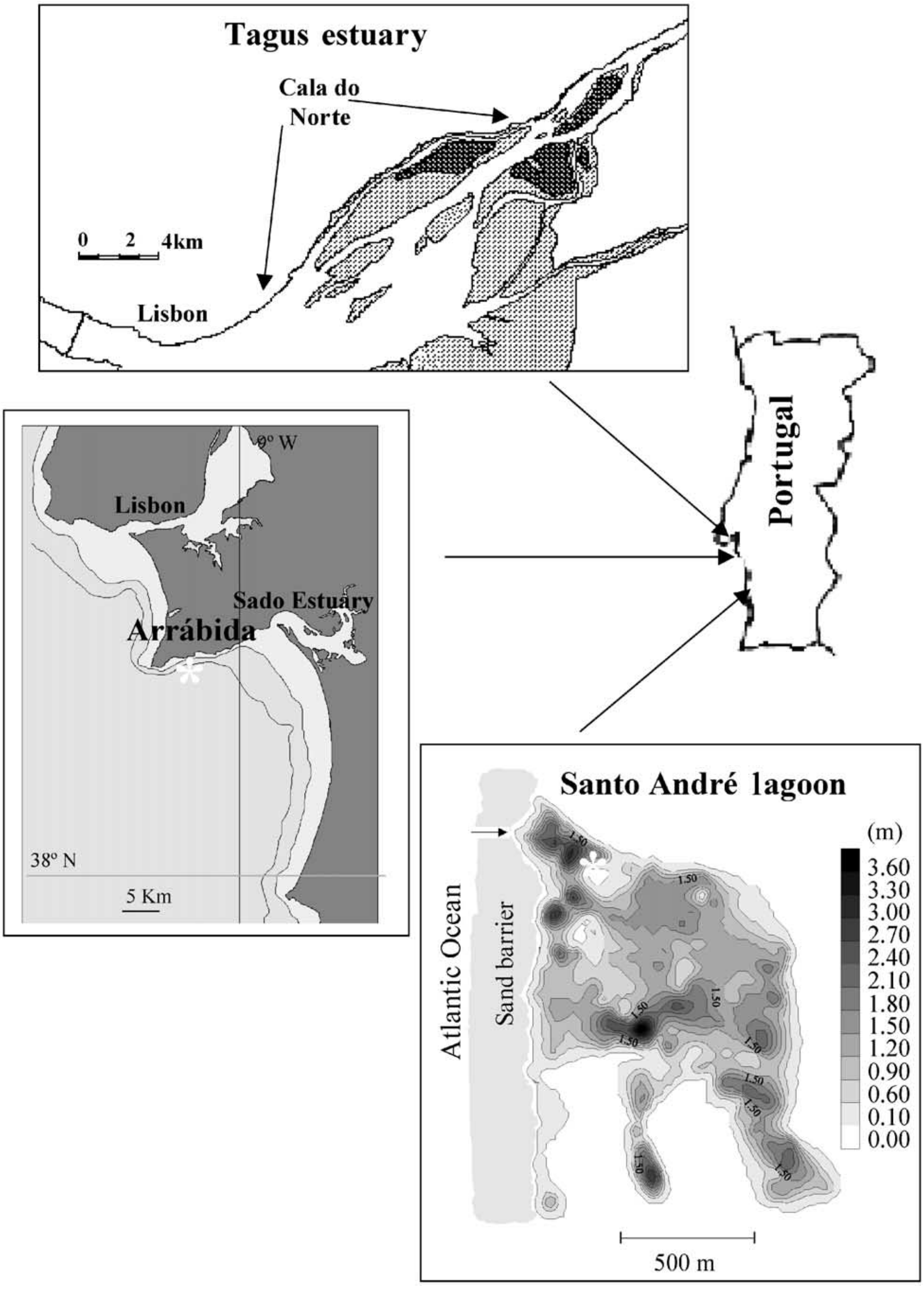

Fig. 1. Location of the three sampling areas: Tagus estuary, the Arrábida coast, and Santo André lagoon. The white asterisk marks the position of the sampling stations. The arrow on the upper left corner in the Santo André lagoon map shows the place where the artificial channel is opened between the lagoon and the sea.

Table 1
Main characteristics of the ecosystems considered in this work

| Ecosystem type | Name | Salinity range (psu) | Mean light extinction coefficient ($m^{-1}$) | Mean depth (m) |
|---|---|---|---|---|
| Open coastal area | Arrábida coast | 34–36 | 0.2 | 15.0 |
| Estuary | Tagus estuary | 0–32 | 3.4 | 2.3 |
| Coastal lagoon | Santo André lagoon | 2–30 | 2.0 | 1.0 |

Water samples for phytoplankton biomass, species composition, and primary productivity measurements were collected simultaneously at 0.5 m depth. All samples were sieved through a 200-μm mesh prior to filtration. Samples for primary production were always collected in the morning and kept in the dark before the incubations were carried out (see below). Temperature, salinity, and pH were determined in situ with an SCT Meter YSI model 33 and a pH Meter ESD model 69.

### 2.3. Species determination

Samples for species determination were preserved with Lugol's solution (Throndsen, 1978). Phytoplankton cells were counted by the Utermöhl technique using an Olympus IX70 light inverted microscope (Hasle, 1978). The classification scheme used followed Drebes (1974), Dogde (1975), and Hasle et al. (1996).

### 2.4. Chemical analyses

Samples for chlorophyll-*a* (Chl-*a*) were filtered through 0.45-μm membrane filters. Pigments were extracted in 90% acetone and analysed fluorometrically by the method of Yentsch and Menzel (1963) as modified by Holm-Hansen et al. (1965). Calibrations were made using a Sigma Chl-*a* standard. Total inorganic carbon was determined in the water samples prior to incubation from pH (pH Meter ESD model 69) and alkalinity measurements according to Parsons et al. (1984).

### 2.5. Incubation procedures

Samples were incubated in the laboratory with light provided by 1500-W tungsten halogen lamps. Irradiance (0–950 μmol quanta $m^{-2}$ $s^{-1}$) was measured using a LICOR spherical quantum sensor (LI-193SA). Attenuation was achieved with grey PVC nets. Preservation of the spectral characteristics was checked as in Macedo et al. (1998). Three replicate Winkler flasks were also incubated in the dark for the determination of the respiration rate. All the experiments were performed under controlled temperature, similar to that measured in the field.

### 2.6. Primary productivity determination

The photosynthetic parameters were evaluated from the *P–I* curves obtained by measuring primary productivity as a function of light by the oxygen or the $^{14}C$ incubation technique (Table 2). The former was used in all ecosystems, whereas the latter was applied only in Santo André lagoon in order to assure that the results obtained in this ecosystem were not due to the photosynthesis measurement method used. For the oxygen method three replicates were incubated and a photosynthetic quotient of 1.2 was used to convert oxygen to carbon according to Vollenweider (1974), Geider and Osborne (1989), and Laws (1991). Dissolved oxygen concentration was measured by titration with the azide modification of the Winkler method (Phillips, 1973). A microburette (Crison Micr. Bli. 2031) was used to titrate the whole contents of the Winkler bottles (Carritt and Carpenter, 1966; Strickland and Parsons, 1972). The primary productivity measurements with the $^{14}C$ technique (Steeman Nielsen, 1952) followed the standard ICES (1996) recommendations. Water samples were placed in 60-ml Winkler bottles and inoculated with 1 ml of $NaH^{14}CO_3$ with 10 μCi (Carbon 14 Centralen). A dark bottle was used for the blank determination. After incubation, samples were filtered onto 25-mm membrane filters (0.45 μm) using gentle vacuum, and filters were acidified with HCl to eliminate inorganic $^{14}C$. Filters were placed in 20-ml scintillation vials to which 10 ml of scintillation fluid (Insta-Gel Plus, Packard) was added. The samples were then counted in a liquid scintillation analyser (Tri-Carb Model 1600CA). Liquid scintillation counts were corrected for quenching by external standard. Primary productivity (mg C $m^{-3}$ $h^{-1}$) was calculated following ICES (1996) and then divided by the phytoplankton biomass to express productivity ($P$) in mg C (mg Chl-$a$)$^{-1}$ $h^{-1}$.

The Eilers and Peeters (1988) model, Eq. (1), was used to fit $P$–$I$ curves to the results obtained. This model was chosen since it is based on the physiology of photosynthesis.

$$P = I/(aI^2 + bI + c) \qquad (1)$$

where $P$ is the primary productivity (mg C (mg Chl-$a$)$^{-1}$ $h^{-1}$), $I$ is the irradiance (μmol quanta $m^{-2}$ $s^{-1}$), and $a$, $b$, and $c$ are the adjustment parameters.

The parameter $a$ is the photoinhibition parameter and at low intensities $bI$ and $aI^2$ can be neglected so the production rate increases approximately linearly with irradiance. By differentiating the Eilers and Peeters (1988) model, the initial slope ($S$ in mg C (mg Chl-$a$)$^{-1}$ $h^{-1}$ μE $m^2$ $s^1$), the maximum production rate ($P_{max}$ in mg C (mg Chl-$a$)$^{-1}$ $h^{-1}$), and

Table 2
Summary of the experimental incubation times, incubation temperatures, and methods used for primary productivity determination

| Experiment | Incubation temperature (°C) | Incubation period (min) | Method |
|---|---|---|---|
| I. Arrábida | 20.0 | 45 and 120 | oxygen |
| II. Arrábida | 20.0 | 45 and 120 | oxygen |
| III. Arrábida | 15.0 | 30 and 180 | oxygen |
| IV. Tagus estuary | 16.5 | 30 and 120 | oxygen |
| V. Santo André lagoon | 22.0 | 45 and 120 | $^{14}C$ |
| VI. Santo André lagoon | 25.0 | 30 and 120 | $^{14}C$ |
| VII. Santo André lagoon | 25.0 | 45 and 120 | $^{14}C$ and oxygen |
| VIII. Santo André lagoon | 12.5 | 120 and 210 | oxygen |

optimal irradiance ($I_{\text{opt}}$ in μmol quanta $\text{m}^{-2}\ \text{s}^{-1}$) can be expressed as a function of the *a*, *b*, and *c* parameters:

$$I_{\text{opt}} = (c/a)^{1/2} \tag{2}$$

$$S = 1/c \tag{3}$$

$$P_{\max} = 1/(b + 2(ac)^{1/2}) \tag{4}$$

and according to the previous authors, the reverse equations are:

$$a = 1/(SI_{\text{opt}}^2) \tag{5}$$

$$b = 1/P_{\max} - 2/(SI_{\text{opt}}) \tag{6}$$

$$c = 1/S \tag{7}$$

Some changes to the Eilers and Peeters (1988) model were introduced in order to account for the dynamic aspects of the *P–I* curves. The photoinhibition parameter *a* was recalculated as a function of exposure time to critical irradiance (above the optimal light level) according to the DYPHORA model described in Pahl-Wostl and Imboden (1990):

$$a(t) = (1 - \exp(-t/t_i))a \tag{8}$$

where $a(t)$ is the parameter *a* expressed as a function of time, *t* is the time exposure to a irradiance above $I_{\text{opt}}$, and $t_i$ is the light inhibition decay time.

The value of *a* in the second member of Eq. (8) corresponds to fully developed photoinhibition. Therefore, combining Eqs. (1) and (8)'s primary productivity can be described as a function of irradiance and incubation time, $P(I,t)$:

$$P(I,t) = I/((1 - \exp(-t/t_i))aI^2 + bI + c). \tag{9}$$

When the *P–I* curves showed a dynamic behaviour, Eq. (9) was used instead of Eq. (1).

Several *P–I* curves were obtained for each ecosystem, using different incubation times. The shorter incubation time used in the experiments (30 or 45 min) was chosen to be low enough to allow the photosynthetic rate determination before the full development of photoinhibition. The longer incubation time was chosen to allow the photosynthetic rate determination after the steady state was reached. In order to increase the sensitivity of the productivity measurements, under short incubation times, the phytoplankton concentration procedure described by Macedo et al. (1998) was used in the Arrábida experiments. Table 2 summarises all the experiments performed, incubation time, and methods employed.

Daily gross primary production (mg C $\text{m}^{-3}$ $\text{day}^{-1}$) was calculated using a simple mathematical model in which irradiance was determined according to formulations from Brock (1981) and Portela and Neves (1994). Phytoplankton primary production was calculated using Eqs. (1) and (9) and the equation parameters were obtained by a nonlinear regression method (see *Statistical Analysis*). In the Arrábida coast simulations, an average light extinction coefficient ($k$) of 0.2 $m^{-1}$ was assumed, and in the Tagus estuary model, a $k$ value of 3.4 $m^{-1}$, calculated from Secchi disc readings, was used. All the simulations were performed for the day of the experiments at a fixed depth of 0.5 m and using a time step of 1 h.

### 2.7. Statistical analysis

The $P$–$I$ curves parameters ($a$, $b$, and $c$) were derived after fitting Eq. (1) to the experimental data using the quasi-Newton nonlinear least-squares regression technique (Statistica software). Linear regressions (Type II) between the observed and the predicted values were used to verify the fitting quality according to Laws and Archie (1981) and following Sokal and Rohlf (1995). For each curve, the slope of the regression was checked for significant differences from unity and the $y$-intercept for significant differences from zero. The significance of these differences is an indication of a poor model fit to the observed data (Keller, 1989).

An $F$-test was performed according to Mead and Curnow (1983) and Potvin and Lechowicz (1990) to test if $P$–$I$ curves obtained by using two different incubation periods were significantly different. This test compares the increase in the mean square residual obtained by fitting the model to all data points, with the mean square residual obtained by fitting the model separately to results from each incubation period. Testing the null hypothesis, that the increase in the residual sum of squares obtained by grouping the data is not significant, is equivalent to testing the null hypothesis on the absence of a significant time effect on the $P$–$I$ curve parameters. When the $P$–$I$ curves obtained by two different incubation periods were significantly different, the parameter $t_i$ was derived after fitting Eq. (9) to the experimental data using the quasi-Newton nonlinear least-squares regression technique (Statistica software). Linear regressions (Type II) between the observed and the predicted values were used to verify the fitting quality according to Laws and Archie (1981) and following Sokal and Rohlf (1995).

## 3. Results

In order to verify the different physical conditions of the considered ecosystems, the euphotic depth, the photoinhibition depth, and the time spent by a phytoplanktonic cell in the light photoinhibiting layer were estimated (Table 3). For this calculation, a light inhibition above 300 μmol quanta $m^{-2}$ $s^{-1}$ (mean $I_{opt}$ value) and a irradiance of 2000 μmol quanta $m^{-2}$ $s^{-1}$ at noon were considered together with a range of vertical diffusivity coefficients from 0.005 to 0.1 $m^2$ $s^{-1}$.

It is interesting to notice that the time spent by a phytoplanktonic cell at the light photoinhibiting layer in the Arrábida coast varies from minutes to hours whereas in the other two ecosystems this time lies in the range of a few seconds to minutes (Table 3). However, in Santo André lagoon, the photoinhibition depth (0.95 m) almost reaches the total depth of the lagoon (1 m) and therefore the whole water column can be under photoinhibiting light for several hours.

Table 3
Estimated euphotic depth, photoinhibition depth, and time spent by a phytoplanktonic cell in the light photoinhibiting layer for each ecosystem (see text)

| Ecosystem | Depth (m) | Euphotic depth (m) | Photoinhibition depth (m) | Time remaining in the photoinhibiting layer (min) |
|---|---|---|---|---|
| Arrábida coast | 15.0 | 23.0 | 9.50 | 23.0–474.0 |
| Tagus estuary | 2.3 | 1.3 | 0.60 | 0.2–4.3 |
| Santo André lagoon | 1.0 | 2.3 | 0.95 | 0.2–3.2 |

A summary of the physical and chemical parameters and Chl-*a* concentration determined during sampling, for all the experiments performed, is presented in Table 4.

### 3.1. Arrábida coast

The Chl-*a* concentration in the Arrábida coast was consistently low (Table 4), which led to concentrated samples (cf. Material and Methods) being used in all the productivity measurements with the oxygen technique.

The percentage of the main phytoplanktonic taxonomic groups identified in the Arrábida samples showed a clear dominance of the diatoms *Chaetoceros* spp., *Leptocylindrus danicus* Cleve, *Pseudonitzchia* spp., and *Rhizosolenia stolterfothii* (Stolterfoth) Peragallo—more than 50% of the total number of cells. Data from a previous study (Cabeçadas et al., 1996) also reported the dominance of diatoms, during spring, in this coastal area.

The *P–I* curves obtained in the Arrábida experiments are shown in Fig. 2. From these results, it appears that productivity is dependent on the incubation time. Under light intensities below 150 μmol quanta $m^{-2}$ $s^{-1}$, the incubation period seems to have little effect on the *P–I* relationship, but at higher irradiance levels the time dependence is well developed, with the shorter incubation periods leading to higher productivity. The reduction of the photosynthetic rate due to long exposure time occurs at light levels lower than the optimal irradiance ($I_{opt}$). The initial slope of the *P–I* curves was not affected by the incubation time while $P_{max}$ and $I_{opt}$ changed remarkably.

Table 4
Chlorophyll-*a* concentration and physicochemical parameters measured in the samples collected in all the experiments (*T* is temperature and DO is dissolved oxygen)

| Experiment | Local | Season | Chl-*a* (mg $m^{-3}$) | Salinity (psu) | *T* (°C) | pH | DO (mg $O_2$ $l^{-1}$) |
|---|---|---|---|---|---|---|---|
| I | Arrábida | summer | 0.98 | 34.5 | 18.5 | 8.1 | 7.4 |
| II | Arrábida | autumn | 0.39 | 35.5 | 19.0 | 8.2 | 7.3 |
| III | Arrábida | spring | 1.33 | 36.1 | 14.0 | 8.2 | 7.8 |
| IV | Tagus | spring | 10.91 | 25.0 | 17.0 | 8.2 | 7.7 |
| V | Santo André | spring | 2.44 | 24.5 | 21.0 | 8.2 | 7.8 |
| VI | Santo André | summer | 4.40 | 27.6 | 24.5 | 8.6 | 7.7 |
| VII | Santo André | summer | 9.85 | 23.3 | 21.0 | 9.4 | 8.2 |
| VIII | Santo André | autumn | 8.17 | 16.4 | 11.2 | 8.9 | 9.7 |

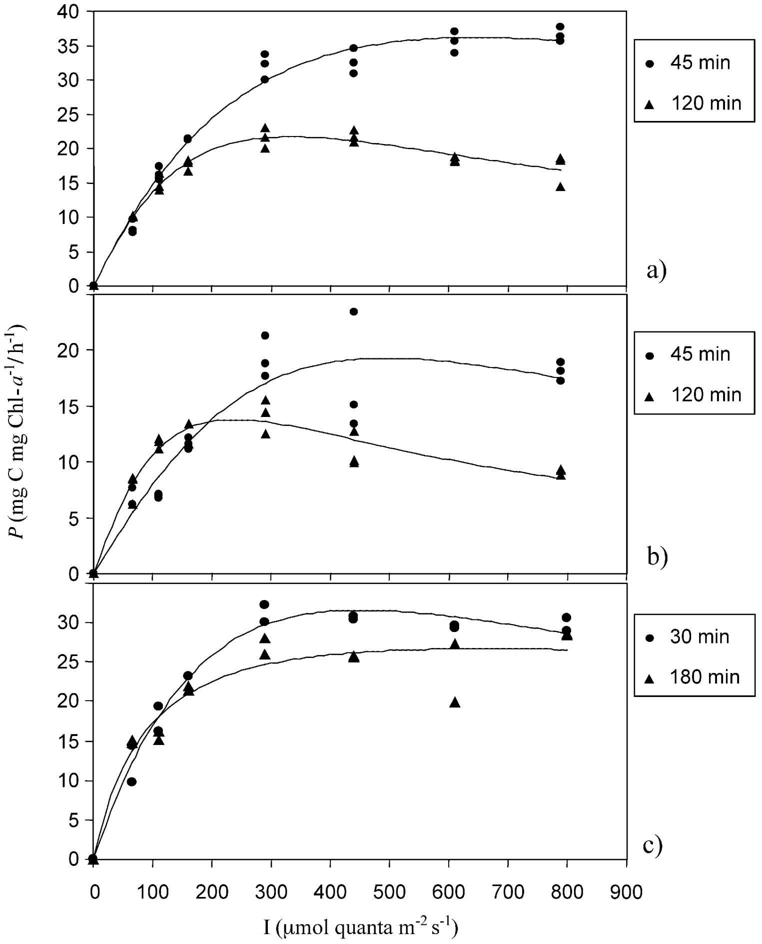

Fig. 2. *P–I* curves obtained with the Arrábida coast experiments: (a) Experiment I, (b) Experiment II, and (c) Experiment III.

The results of the *F* tests on the differences between the *P–I* curves obtained under different incubation times (cf. Material and Methods) showed that the null hypothesis on the absence of a significant time effect on the *P–I* curve parameters must be rejected in all cases ($p < 0.05$), i.e., the *P–I* curves obtained from each incubation period are significantly different. These results also confirm the time-dependent behaviour of the *P–I* curve parameters observed in Fig. 2.

Table 5 shows the differences between the daily gross primary production calculated using the *P–I* curve parameters obtained from the two incubation periods for each of the three Arrábida experiments. The larger values correspond to the shorter incubation periods. Therefore, a considerable underestimation of daily primary production, ranging from 13% to 38%, may occur if *P–I* curves are obtained from standard incubation times of 2–3 h.

Table 5
Daily gross primary production calculated from the Arrábida curve parameters

| Experiment | Incubation period (min) | Gross primary production (mg C $m^{-3}$ $day^{-1}$) |
|---|---|---|
| I | 45 | 358.7 |
| | 120 | 223.7 |
| II | 45 | 67.8 |
| | 120 | 50.4 |
| III | 30 | 464.8 |
| | 180 | 406.6 |

Primary productivity can be described as a function of irradiance and incubation time using Eq. (9). For Experiments I, II, and III, the following equations were obtained by nonlinear least-squares regression technique (see Statistical Analysis):

$$P(I,t) = I/(1.5 \times 10^{-4}(1 - \exp(-t/3.45))I^2 - 6.06 \times 10^{-4}I + 7.18) \qquad (10)$$

$$P(I,t) = I/(1.93 \times 10^{-4}(1 - \exp(-t/2.50))I^2 - 9.84 \times 10^{-3}I + 9.35) \qquad (11)$$

$$P(I,t) = I/(2.13 \times 10^{-5}(1 - \exp(-t/0.55))I^2 - 1.85 \times 10^{-2}I + 3.84) \qquad (12)$$

where $t$ is the time exposure to irradiance above $I_{\mathrm{opt}}$.

Eqs. (10)–(12) correspond to Experiments I, II, and III, respectively. The quality of the fit between the observed and the predicted values was verified using a Type II linear regression. In all three experiments, the regression slope was not significantly different from 1 and the $y$-intercept was not significantly different from 0 ($p < 0.05$). In the DYPHORA model, Pahl-Wostl and Imboden (1990) found a $t_i$ (light inhibition decay time) value that ranged between 0.5 and 1.5 h. For the Arrábida experiments, $t_i$ ranged from 0.5 to 3.5 h.

### 3.2. Tagus estuary

A summary of the physical and chemical parameters and Chl-*a* concentration determined for Experiment IV is presented in Table 4. Regarding the species composition, it is known from previous studies (Macedo et al., 1998) that phytoplankton populations in this area consist mainly of diatoms. Fig. 3 presents the $P$–$I$ curves determined in the Tagus estuary experiment.

From these results, it appears again that the $P$–$I$ curve parameters are time dependent. The initial slope of the $P$–$I$ curve was not affected by the incubation time while the changes in $P_{\max}$ and $I_{\mathrm{opt}}$ were notable. These results follow a similar pattern to the ones determined by Macedo et al. (1998) in the same location in the Tagus estuary.

The results of the $F$-tests to compare the curves obtained by using two different incubation periods in the Tagus estuary indicate that the null hypothesis on the absence of

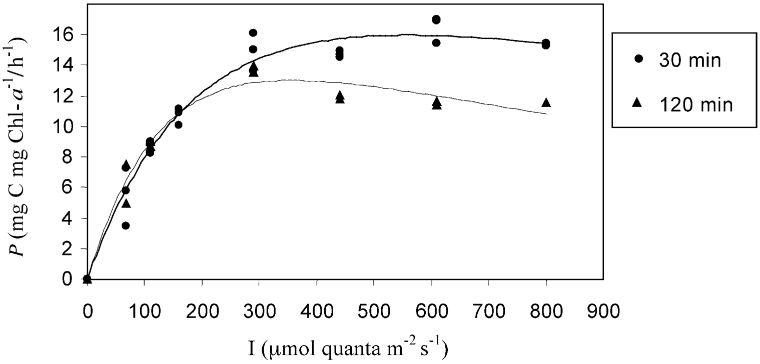

Fig. 3. *P–I* curves obtained with the Tagus estuary experiment.

a significant time effect on the *P–I* curve parameters must be rejected ($p < 0.05$). This confirms the time-dependent behaviour of the *P–I* curve parameters.

The results from Experiment IV can be described by the following equation:

$$P(I,t) = I/(7.05 \times 10^{-5}(1 - \exp(-t/1.00))I^2 - 3.15 \times 10^{-2}I + 8.79) \qquad (13)$$

For this experiment, a $t_i$ (light inhibition decay time) of 1 h was found. The daily gross primary production calculated from the 30 min curve parameters was 235 mg C m$^{-3}$ day$^{-1}$, while the one calculated from the 120-min curve was 135 mg C m$^{-3}$ day$^{-1}$. This implies an underestimation of 42.5% in the daily primary production when the latter curve is considered.

### *3.3. Santo André lagoon*

A summary of the physical and chemical parameters and Chl-*a* concentration determined for the Santo André lagoon experiments is presented in Table 4. The sample from Experiment V was constituted mainly by diatoms whereas in the other three experiments the most abundant specie was the dinoflagellate *Prorocentrum minimum* (Pavillard) Schiller, which accounted for more than 50% of the total number of cells.

Fig. 4 presents the *P–I* curves obtained from the Santo André lagoon experiments. These curves did not present any obvious change as a function of incubation time, unlike the results presented for the other two ecosystems. The results of the *F*-tests to compare the curves obtained by using two different incubation periods in the Santo André lagoon led to the acceptance of the null hypothesis on the absence of a significant incubation time effect on the *P–I* curve parameters ($p > 0.05$). This implies that it is possible to describe the data obtained from different incubation periods with the same *P–I* curve parameters.

In this ecosystem, phytoplankton primary production was determined by both the oxygen and $^{14}$C method (Table 2). In Experiment VII, both methods were used for the same sample to assure that the *P–I* time-independent results obtained in this ecosystem were not due to an experimental artifact. The $^{14}$C method has two principal advantages: first, it is easy to compare with other data, since most of the phytoplankton primary

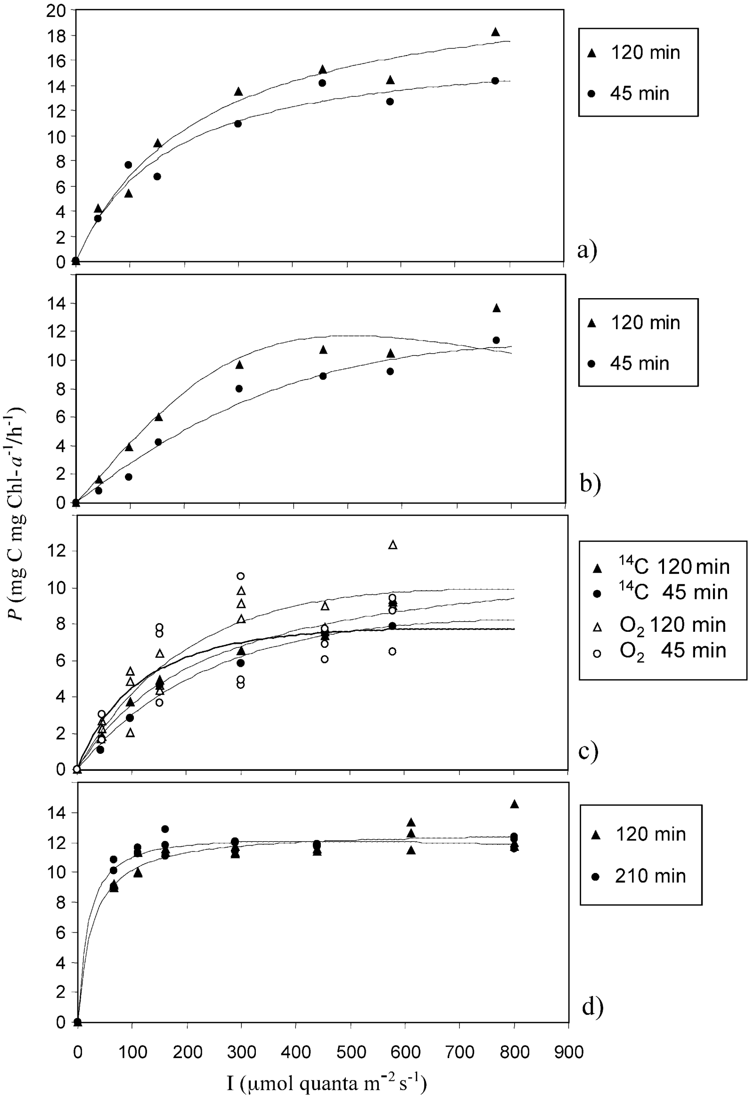

Fig. 4. *P–I* curves obtained with the Santo André lagoon experiments: (a) Experiment V, (b) Experiment VI, (c) Experiment VII, and (d) Experiment VIII.

productivity measurements are based on this technique; second, it allows the measurement of carbon fixation even at very low production rates, this can be a great advantage when measuring photosynthesis in a short-incubation period. However, the artifacts that may arise when using this method (measuring gross or net photosynthesis, recycling of the respiratory $CO_2$, differential uptake of $^{14}C/^{12}C$) are still a matter of controversy (Peterson, 1980; Williams and Lefèvre, 1996; Williams et al., 1996). These problems can become even more complex when different incubation times are used, since the coefficient *q* (the ratio of the respiration of "new" to "old" carbon, sensu Dring and Jewson, 1982) can be time dependent, according to Williams and Lefèvre (1996). When short incubation times are used, the cells' carbon may not have time to reach the isotopic equilibrium. Therefore, when longer incubation times are used these can result in higher $P$–$I$ parameters, in comparison to short incubation curves. This could explain the higher $P_{max}$ observed in long-time incubation curves in Experiments V, VI, and VII with the $^{14}C$ method, although these curves could not be considered significantly different from short incubated curves.

## 4. Discussion

The $P$–$I$ curves obtained in the Santo André lagoon experiments showed no time dependence contrasting with the Arrábida coast and Tagus estuary results. In the Arrábida coast and Tagus estuary (Figs. 2 and 3), the $P$–$I$ curves' time dependence is well developed, with the shorter incubation periods leading to higher productivity. The reduction of the photosynthetic rate due to long exposure time occurs at light levels lower than the optimal irradiance ($I_{opt}$). The initial slope of the $P$–$I$ curves was not affected by the incubation time while $P_{max}$ and $I_{opt}$ changed significantly. Lewis and Smith (1983), using natural phytoplankton samples, incubated for 20 and 180 min obtained $P$–$I$ curves with a similar initial slope but with a very different $P_{max}$ and $I_{opt}$. Neale and Marra (1985) pointed out that the variation of $P_{max}$ should be considered as the primary source of time dependence and Franks and Marra (1994) presented a nonlinear time-dependent $P_{max}$. The results presented for the Arrábida coast and Tagus estuary follow a similar pattern to the ones observed experimentally by Marra (1978b), predicted by the DYPHORA model (Pahl-Wostl and Imboden, 1990) and by the model of Duarte and Ferreira (1997).

$P_{max}$ values found for shorter incubation periods (45 and 30 min) determined by the oxygen method (Experiments I and III) are above the maximal theoretical assimilation number of 25 mg C (mg Chl-$a$)$^{-1}$ h$^{-1}$ calculated by Falkowski (1981). Although other authors (e.g., Riegman and Colijn, 1991) also present $P_{max}$ values higher than the above maximum assimilation number, it is important to consider the photosynthetic quotient (PQ). PQ varies as a function of the nitrogen source utilized by the algae. Laws (1991) suggested a PQ of $1.1 \pm 0.1$ for growth on ammonia and a PQ of $1.4 \pm 0.1$ for growth on nitrate. However, apparent PQ's substantially higher than expected from considerations of the N source assimilated by phytoplankton have been frequently measured, for instance, Oviatt et al. (1986) estimated PQ values as high as 5.0 and Iriarte et al. (1996) obtained a mean PQ of 2.2. In this study, a somewhat more standard PQ value of 1.2 was used according to Vollenweider (1974), Geider and Osborne (1989) and Laws (1991). If a PQ of about 1.8 had been considered, the $P_{max}$ values presented here would be lower than the abovementioned limit.

The $P$–$I$ curves obtained in the Arrábida coast and Tagus estuary exhibited photoinhibition, especially for longer incubations. Although there is abundant evidence that photoinhibition occurs in natural phytoplankton communities, the mechanisms of the phenomenon are complex and not fully understood (Prasil et al., 1992). However, it is known that photoinhibition is a time-dependent phenomenon (Takahashi et al., 1971; Harris and Lott, 1973) that may not occur when phytoplanktonic cells are exposed to very high irradiance levels for short periods. In contrast, cells exposed for longer periods show a decrease in photochemical energy conversion levels (Falkowski and Raven, 1997). It appears that photoinhibition needs time to develop and become measurable (Marra, 1978a,b; Belay, 1981; Whitelam and Codd, 1983). The response of the cells to light seems to be characterised by two time scales. An initial phase, of only a few minutes, when a rapid increase in the production rate should be expected until the cells reach their full rate of photosynthesis, and a second phase when the effect of photoinhibition sets in for strong light (Harris and Piccinin, 1977; Pahl-Wostl and Imboden, 1990). The time for photosynthesis to reach a steady state varies for different conditions. According to Harris and Piccinin (1977) it takes between 15 and 60 min for photosynthesis to reach a steady state. Marra (1978a,b) performed a set of similar experiments using laboratory conditions and it took a few hours of constant light intensities until steady state was achieved, and in Macedo et al. (1998), photosynthesis only reached a steady state after 90 min. Since in Santo André lagoon the shorter incubation period lasted only 30 min, it is not likely that in this ecosystem the phytoplanktonic cells had already reached steady state when the shorter $P–I$ curves were determined.

The absence of a $P–I$ time dependence observed in Santo André lagoon could not be attributed to seasonal effects since the same season, spring, was sampled in the three locations (see Table 4). Moreover, although in this paper only one experiment was conducted for the Tagus estuary, the $P–I$ time dependence was previously studied by Macedo et al. (1998) for the same location (Cala do Norte of the Tagus estuary), during the summer season. Those authors observed a time-dependent $P–I$ behaviour, using different incubation periods, from minutes to hours. In the present work, samples collected during late summer in the Arrábida coast showed a similar pattern, while samples collected for the same season in the lagoon (Experiments VI and VII) did not present $P–I$ time dependence, neither with the oxygen or $^{14}$C method). For the autumn, $P–I$ time behaviour was analysed for the Arrábida coast and lagoon ecosystems and the results (Experiments II and VIII) were consistent with the ones obtained in the previous experiments.

One possible explanation for the differences found in $P–I$ curves between Santo André lagoon and the other two ecosystems is that time-dependent $P–I$ curves are not a universal phenomenon and vary with the characteristics of the sampling area. Lizon and Lagadeuc (1998) observed that differences between daily production rates estimated from 40-min and 4-h incubation times depended on the sampling stations. Furthermore, it is not known how much of this phenomenon may be affected by other factors such as nutrient concentrations and floristic composition. A major source of variation could arise from the differences in phytoplankton composition. In Arrábida coast and Tagus estuary diatoms seem to predominate whereas in Santo André lagoon most of the samples were constituted by dinoflagellates. However, sample V from Santo André lagoon presented a species composition completely different from the other three, but the response of the $P–I$ curve to the incubation time was the same: no inhibition of photosynthesis was noted due to longer exposure time.

Another possible explanation for the observed differences is that the response of $P–I$ curves to exposure time varies with the characteristics of the ecosystem and is related to the light regime to which phytoplankton cells are adapted. Santo André lagoon is a shallow water ecosystem where phytoplankton cells are adapted to a more stable high light environment and probably less susceptible to photoinhibition. A decrease in the susceptibility to photoinhibition and enhanced photosynthetic capacity ($P_{max}$) in response to increasing irradiance are common phenomena in planktonic algae (Richardson et al., 1983; Sukenik et al., 1990; Flameling and Kromkamp, 1997). In fact, irradiance at the bottom of the lagoon may present saturation or inhibiting values under full sunlight, as may be seen from Table 3. Well-mixed conditions prevail in the Arrábida coast and in the Tagus estuary, due to wind stress and tidal mixing. Therefore, it may be hypothesised that in these two ecosystems phytoplankton is adapted to a more variable light regime and therefore more susceptible to photoinhibition when submitted to long exposures under strong light.

The results presented highlight the importance of the incubation time used in the phytoplankton primary production determinations. Most of these determinations are performed using long incubations (longer than 4 h). Behrenfeld and Falkowski (1997) estimated the global carbon fixation based on more than 11 000 $^{14}$C measurements of daily carbon fixation collected at about 1700 oceanographic stations, in both open-ocean and coastal waters, from 80°N to 70°S. These $^{14}$C measurements included both in situ and simulated incubations, with incubation time periods ranging from 2 to 24 h. However, only less then 3% of those data were derived from incubations shorter than 6 h (Behrenfeld and Falkowski, 1997).

The present work shows that the daily primary production can be underestimated by using the standard incubation time in ecosystems where $P$–$I$ curves present a dynamic behaviour. Therefore, it is likely that many primary production estimates, obtained through the assumption of a static behaviour of the $P$–$I$ curve parameters, may underestimate the real values. This can lead to an underestimation of the role of phytoplanktonic primary production as a carbon sink.

## 5. Conclusions

The experiments described in this work were conducted at different occasions, at different places. Although covering the same seasons, these were not replicated. Therefore, it is not possible to test statistically the differences among sites in terms of the $P$–$I$ curve time-dependent behaviour. However, the results obtained give a strong evidence for important differences among sites. This led to the hypothesis that the response of $P$–$I$ curves to time varies with the characteristics of the ecosystem. Therefore, in primary production studies, special care should be given to the incubation time used in the $P$–$I$ curve determination. The response of the $P$–$I$ relationship to time should be investigated in order to determine the appropriate incubation period(s).

The results presented suggest that, for a given ecosystem, the $P$–$I$ relationship should be investigated by testing the photosynthetic response to irradiance under short and long incubation periods. This would result in a dynamic photosynthesis versus irradiance and time function, $P(I,t)$, for the cases where phytoplankton cells presented a time-dependent behaviour to exposure, and in a static, $P(I)$, function for the cases where there is no time-dependent behaviour. Following this methodology, it would be possible to simulate the photosynthetic response of phytoplankton exposed to a fixed irradiance for long or short periods, according to the prevailing environmental conditions (intensity of vertical mixing, vertical mixing depth, and photoinhibition depth). This would allow a more reliable estimation of phytoplankton primary production and of its contribution to the global carbon cycle.

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