A MICROCOSM APPROACH ON THE POTENTIAL EFFECTS OF THE VERTICAL MIXING OF WATER MASSES OVER THE PRIMARY PRODUCTIVITY AND PHYTOPLANKTON BIOMASS IN THE SOUTHERN BRAZILIAN COASTAL REGION

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Abstract

The vertical mixing between South Atlantic Central Water (SACW) and Coastal Water (CW) was simulated through microcosm experiments using the autochthonous phytoplankton community (fraction < 150 μm), without nutrient enrichments. SACW is cold (T< 18°C) and nutrient rich, while CW is warmer (T> 20°C) and oligotrophic. The phytoplankton growth potential of SACW, CW and an equivalent mixture of both (SACW+CW) was compared, under 100, 30 and 10% of sunlight, at surface seawater temperature, in winter and summer conditions. Results demonstrate the importance of SACW as a natural eutrophication agent for the mixing layer, allowing the occurrence of new production by nutrient input, and also as a biological seeder through the development of its autochthonous phytoplankton community when it reaches the euphotic zone. The time lag for phytoplankton development during winter was around 4-5 days, against 1-2 days in summer. The hypothesis of physiological differences between surface and bottom phytoplankton populations from a deep (80 m) and thermally homogeneous water column (common winter feature) was also tested through the microcosm experiments. Results obtained clearly demonstrate that bottom water presented higher phytoplankton growth potential than the surface one.

Resumo

A mistura vertical entre a Água Central do Atlântico Sul (ACAS) e a Água Costeira (AC) foi simulada através de experimentos tipo microcosmos, com o fitoplâncton autóctone (fração < 150 μm) e sem enriquecimentos nutricionais. A ACAS é fria (T< 18°C) e rica em nutrientes, enquanto a AC é mais quente (T> 20°C) e oligotrófica. O potencial trófico dessas águas e de uma mistura equivalente de ambas (ACAS+AC) foi comparado a 100, 30 e 10% da luz solar, sob temperatura da água do mar na superfície, em condições de inverno e verão. Os resultados demonstram a importância da ACAS como agente fertilizador da camada de mistura tanto por introduzir nutrientes, favorecendo a ocorrência de produção nova, como pelo incremento da biomassa fitoplanctônica autóctone ao atingir a zona eufótica. A fase de adaptação observada no inverno foi de 4-5 dias enquanto no verão foi de 1-2 dias. A hipótese da existência de diferenças fisiológicas entre as populações fitoplanctônicas de superfície e fundo em uma coluna de água profunda (80 m) e termicamente homogênea (padrão comum de inverno) foi também testada utilizando microcosmos. Os resultados mostraram que potencial de crescimento fitoplanctônico nas águas de fundo foi significativamente maior que nas de superfície.

Descriptors: Vertical mixing, Phytoplankton, Primary production, SACW, Nutrients, Microcosms, Physiological stratification, Southwest Atlantic.

Descritores: Mistura vertical, Fitoplâncton, Produção primária, ACAS, Nutrientes, Microcosmos, Estratificação fisiológica, Atlântico Sudoeste.

Introduction

The Southeastern Brazilian coastal region is dominated by three water masses: Coastal Water (CW), South Atlantic Central Water (SACW) and Tropical

Water (TW). CW and TW are present in the upper layers and SACW remains close to the continental slope during autumn until the early spring. In late spring and summer, SACW floods the bottom layers of the continental shelf, favored by NE winds, and sometimes reaches the euphotic zone (Emílsson, 1961; Matsuura, 1986; Castro Filho *et al.,* 1987; Pires-Vanin *et al.*, 1993). Thermohaline indexes from these water

masses are: CW 24°C, 34.9; SACW 13°C, 35.4 and TW 24°C, 37.0 (Castro Filho *et al. op. cit.*). SACW possess high nutrient availability followed, in decreasing order, by CW and TW (Aidar *et al.,* 1993).

Several studies carried out at the Southwest Atlantic report the importance of SACW seasonal advection in introducing new nutrients into the mixing layer, thus, promoting phytoplankton biomass increase (Matsuura, 1986; Valentin *et al.*, 1986; Brandini, 1988a,b; Ciotti *et al.*, 1995; Moser & Gianesella, 1997; Rocha *et al.*, 1998; Gaeta *et al.*, 1999; Saldanha-Corrêa, 1999) and potentially supporting higher fish stocks (Moreira da Silva, 1971; Pires-Vanin *et al.*, 1993). The mixing layer off Ubatuba is generally nutrient depleted and regenerative processes maintain the low phytoplankton biomass, represented almost exclusively by pico- and nanoflagellates (Aidar *et al.*, 1993). The episodic occurrence of physical phenomena such as cyclonic eddies (Mascarenhas *et al.,* 1971; Castro Filho *et al.*, 1987), coastal upwelling (Émilsson, 1961; Maglioca *et al.*, 1979) and shelf break fronts (Mesquita *et al.*, 1983 & Brandini, 1990) reported in this area, should have a great impact on the phytoplankton standing stock in the euphotic layer.

Although the importance of SACW as a natural fertilizer has been well recognized, no studies had yet quantified its contribution. The present work proposes to evaluate the chemical and biological potential of SACW in increasing phytoplankton biomass and primary productivity rates, based on microcosms experiments. The SACW vertical advection and its mixture with CW (which dominates the upper layers of the inner continental shelf) were simulated in winter and summer conditions.

As a complementary study, microcosms filled with water taken from surface and bottom layers of a deep and physically homogeneous water column, common winter condition, were followed aiming to verify possible differences in phytoplankton community development from both depths.

MATERIAL AND METHODS

Natural seawater was collected off Ubatuba region (Lat. 23°30´S - Long. 45°06´W), Northern coast of São Paulo State, Brazil, from subsurface (CW) and bottom layers (SACW), according to the temperature profile. In the winter experiment (July/88) the sampling point was on the 135 m isobath, in the outer shelf, and in summer (January/89), with the SACW proximity to the coast, water samples were taken at the inner shelf, over the 45 m isobath. In the microcosms conducted in July/ 89, referred from this point as c-winter (common winter), the water was collected over the 80 m isobath, in a physically homogeneous water column, from 5 and 75 m depth.

In all periods, 45 *l* of water were taken from both depths using Van Dorn bottles, and transferred to two 50 *l* plastic carboys after being filtered through a 150 μm mesh nylon net, to remove large zooplankters. From these carboys, samples for inorganic nutrients, Chl and phytoplankton counts were taken immediately to characterize initial conditions for each water mass. The carboys were kept in the dark at room temperature until the beginning of the experiment, which occurred no longer than 20h later. Every material used for sampling and incubations was previously washed according to Miller *et al.* (1978).

Experimental design for winter and summer included 9 microcosms, disposed in 10 *l* borosilicate bottles (Table 1a). These bottles were filled with nine liters of seawater in the following manner: 3 with SACW, 3 with CW and 3 with a mixture of equal parts of both (SACW+CW). To match the vertical advection of the deep SACW and its mixture with CW, one bottle of each water type (CW, SACW and CW+SACW) was submitted to 100, 30 and 10 % of total sunlight radiation (attenuation levels obtained by putting the bottles inside neutral filter bags). Aeration inside the microcosms was provided by bubbling filtered air through a pipette in the middle portion of the bottle. The microcosm-bottles received cotton stoppers, which were covered by plastic hoods. No nutrient was added to any microcosm and they were kept in an outdoor tank placed at the pier in the coastal laboratory of Instituto Oceanográfico da Universidade de São Paulo, in Ubatuba.

Table 1a. Experimental design used in winter and summer microcosms.

Water type / Light level	SACW	SACW+CW	CW
100%	SACW100	SACW+CW100	CW100
30%	SACW30	SACW+CW30	CW30
10%	SACW10	SACW+CW10	CW10

In the c-winter, as CW dominates the whole water column, experimental design comprised only 6 microcosms (3 with surface and 3 with bottom water). No mixture between surface and bottom water was performed, since this experiment aimed to compare the development of the phytoplankton community from these two positions in the same water mass. The

light attenuation levels were the same from the previous experiments (Table 1b). The incubation periods were 8 days in winter, 15 days in summer and 14 days in the c-winter.

Table 1b. Experimental design used in c-winter microcosms.

Water type Light level	Surface	Bottom
100%	S100	B100
30%	S30	B30
10%	S10	B10

In the three experimental series, the outdoor incubation tank was maintained under a continuous surface seawater circulation and the water temperature was monitored through 5 daily systematic readings of a thermometer kept inside the tank. The daily registers from an R-Fuess actinograph, which data were converted to μE m^{-2} s^{-1} units, monitored the photosynthetic active radiation (PAR).

Microcosms were sampled daily at 9 a.m. when aliquots of 300 ml were taken from each one and transported in the dark to the lab, where primary production incubations by ^{14}C method (Steemann-Nielsen, 1952) were immediately conducted. Also, samples for total chlorophyll-a (Chl) and its active fraction (acChl), phytoplankton counts and inorganic nutrients were obtained. For primary production measurements, a sub-sample of 100 ml of each microcosm was inoculated with 1 ml of $NaH^{14}CO_3$ (20 μCi ml^{-1}) and, from this mixture, aliquots of 30 ml were transferred to 3 borosilicate incubation bottles (2 light and one dark) and put in a BOD-like chamber under 400 μE m^{-2} s^{-1} halogen lamp light. Temperature was maintained close to the mean values registered in the outdoor tank. After 4h incubation, carbon uptake was ended by filtering the samples onto Millipore® filters (0.45 μm pore), which were disposed in scintillation vials containing 7 ml of scintillation liquid (Bray, 1960). DPM was measured with a scintillation spectrometer Packard 1600. Carbon uptake (mgC m^{-3} h^{-1}) was computed according to Strickland & Parsons (1968).

Samples for Chl determination were filtered onto GF/F Whatman® and the filtrate water was used to nutrient determination. These samples were stored at -20°C in the dark until the processing. Pigment analyses from extracts in acetone 90% were done in a Turner 111 by the fluorescence method of Lorenzen (1966), which discriminates the Chl-a

fraction able to photosynthesize, referred as "active Chl" (acChl), from phaeophytin-a, fraction that lost the photosynthetic capability. The sum of these two fractions (acChl + phaeophytin) indicates the total Chl-a (Chl). Photosynthetic carbon assimilation was determined by the ratio PP/acChl expressed in mgC (mgChl h) $^{-1}$.

Dissolved inorganic nitrogen (DIN) represents the sum of nitrate, nitrite and ammonium, which were analyzed according to Aminot & Chaussepied (1983). Phosphate and silicate analyses were conducted according to Grasshoff et al. (1983), but silicate results were not presented since its concentration tended to increase with the time, probably due to the washout from the borosilicate glass walls of the incubation bottles.

Phytoplankton samples were fixed in buffered formalin (0.4% final concentration) and counts were done according to Uthemöhl (1958), in an inverted microscope Zeiss at 625X amplification. At least 400 cells were counted per sample in order to keep the error between 10 and 20% (Lund et al., 1958). Taxa identification was based on Cupp (1943), Wood (1968) and Chrétiennot-Dinet (1977).

To compare the results, time series of Chl, acChl, cell density (cell) and primary productivity (PP) were normalized by the time zero (t_0) value of the respective microcosms growth curve. The non-dimensional variables derived from these computations were reported as Chl/Chl_0, $acChl/acChl_0$, $cell/cell_0$ and PP/PP_0,

Specific growth rates (μ), were computed as:

$$\mu = \frac{1}{t_{t+1} - t_t} \ln \frac{B_{t+1}}{B_t}$$

where B represents the biomass related variables: Chl_t/Chl_0, $acChl_t/acChl_0$, $cell_t/cell_0$, and t the day of incubation.

Nutrient assimilation rates were computed according to Aidar et al. (1991) as:

$$V_{(t)} = -\frac{dN}{dt}\frac{1}{X} + \mu\left(\frac{N}{X}\right)$$

where dN/dt is the temporal variation of nutrient concentration (in μM d^{-1}), X is acChl concentration (μg l^{-1}), μ is growth rate determined by changes in acChl (d^{-1}), N nutrient concentration (μM). Thus, vDIN and vPO_4^{-3} are given in μmol (μg acChl)$^{-1}$ d^{-1},

indicating the daily assimilated nutrient mass per 1μg of acChl.

Several parameters derived from every microcosm growth curves were grouped in a matrix, in order to compare the results obtained in each experimental period. These parameters were: maximum value obtained for Chl/Chl_0, $acChl/acChl_0$, PP/PP_0, $PP/acChl$, $cell/cell_0$, vDIN and vPO_4^{-3} ($v_{max}DIN$ and $v_{max}PO_4^{-3}$, respectively); time integrated values (represented by the brackets) of $[Chl/Chl_0]$, $[acChl/acChl_0]$ and $[PP/PP_0]$; maximum growth rates (μ_{max}) in terms of Chl/Chl_0, $acChl/acChl_0$ and $cell/cell_0$ and mean growth rates in the exponential phase (μ_{mean}) for Chl/Chl_0 and $acChl/acChl_0$. Those data were standardized and normalized before running cluster and discriminant analysis (using Statigraphics 5.1), aiming to point out the significant differences among the microcosms. Also, Pearson correlation was computed between pairs of those variables to verify significant linear relationships, denoted by P-values lower than 0.05 at 95% confidence level.

RESULTS

Winter

The water representative of CW presented low concentrations of nutrients and phytoplankton biomass (Table 2). In the SACW, abundant DIN and low phosphate led to a N:P imbalance. Chl was extremely low (0.05 mg m^{-3}) as the water was collected from the aphotic zone. In both waters, the phytoplankton community was represented mainly by picophytoflagellates (more than 80% in number of cells).

The temperature of flowing seawater in the outdoor incubation tank stayed around 18.3 ± 1.1° C, close to the *in situ* temperature of the CW. PAR daily average was 761.5 μE m^{-2} s^{-1} with higher variations occurring in days 4 and 5 (Table 3). In this first experimental series, the incubation period was planned to be 8 days, based on bibliographic references of similar experiments developed in Ubatuba, specially in the work of Teixeira *et al.* (1986) which reports a decline in natural phytoplankton population after 6 days of incubation, in microcosms without nutrient addition and at the same temperature range.

Phytoplankton development was monitored daily by *in vivo* fluorescence. However, this method failed to detect the small growth rates verified along the incubation period. When Chl analyses were done, results revealed that the experiment was ended when the microcosms were entering at the exponential growth phase (Fig. 1). Consequently, complete phytoplankton development was not observed and certainly the maximum growth yield was not attained. So, the SACW fertilizer potential during winter conditions could not be properly evaluated. The most remarkable feature, independently of the water type or light attenuation level, was the long lag-phase observed: a minimum of 4 days.

Although the maximum growth had not been attained in any of the nine microcosms, some differences could be observed in the curves of $acChl/acChl_0$ and PP/PP_0. Microcosms at 10% light level displayed the largest lag-phase. At 30% light, lag-phase is one day shorter than at 100% light level, varying between 4-5 days. SACW30 and SACW10 presented peaks of Chl and PP in the first day of incubation. Also, the initial slopes of the log-phase varied according to the water type: SACW100 and SACW30 microcosms displayed the highest slopes compared to the SACW+CW and CW at the same light levels, indicating that the SACW phytoplankton community was able to respond faster to favorable growth conditions, represented in this case to light availability. PP/PP_0 curves followed closely the Chl/Chl_0 ones, indicating constancy in the assimilation rates per biomass units in all microcosms (Fig. 1). Inorganic nutrient concentrations in the nine microcosms did not show a common pattern (Fig. 2). Phosphate concentrations seemed to vary with small Chl increases in the second day and also at the end of the incubation, preceding the log-phase. Nitrate and DIN seemed to vary in some cases also preceding the log-phase. But, in all cases, no nutrient depletion was verified, indicating that the assayed waters still had chemical potential to develop a larger phytoplankton biomass.

Phytoplankton community was dominated by the picophytoflagellates (< 2 μm), which represented more than 80% of the total number of cells along the entire incubation period. It is interesting to note that, in the day 8 in the microcosms SACW100 and SACW30, diatoms became more abundant, representing respectively 22 and 10% of the total cell density, suggesting a possible diatom bloom development from this day. The main genera observed were *Thalassiosira*, *Thalassionema* and *Pseudo-nitzschia*.

No statistical analysis was applied to this data set, since the maximum growth rates were not observed.

Table 2. Physical, chemical and biological characteristics of the water samples collected as representatives of CW and SACW for microcosm experiments, at the sampling time. (CWs and CWb indicate respectively the CW taken from surface and bottom layers).

Period Water mass	Day	Sampling depth (m)	Zeu (m)	Light (I_z/I_0) %	T (C)	Salin.	NO_3^- (μM)	NO_2^- (μM)	NH_4^+ (μM)	DIN (μM)	PO_4^{-3} (μM)	N : P	Si (μM)	Chl-a (mg m^{-3})	active Chl-a (%)
Winter															
CW	07/19/88	15	30	10	18.98	33.36	0.04	0.04	0.04	0.12	0.41	0.29	0.73	0.25	12.0
SACW		133		0	16.06	35.63	7.72	0.06	0.08	7.86	0.21	37.43	8.18	0.05	40.0
Summer															
CW	1/17/89	2	35	> 50	23.85	36.08	0.16	0.03	0.79	0.98	0.19	5.16	6.02	0.29	93.1
SACW		42		< 1	14.57	36.18	6.01	0.49	0.61	7.11	0.84	8.7	10.31	1.35	80.7
c-Winter															
CWs	07/11/89	5	27	< 50	21.40	35.79	0.22	0.31	1.96	2.49	0.20	12.5	5.59	0.60	100.0
CWb		75		< 1	21.40	35.88	0.23	0.33	0.46	1.02	0.27	3.78	5.97	0.80	100.0

Table 3. Environmental variables monitored along the three microcosm series: daily average of seawater temperature in the incubation tank (°C), PAR (μE m^{-2}s^{-1}) and mean temperature in the incubation chamber during primary productivity experiments.

| Day	Outdoor incubation temperature			PAR (μE m^{-2}s^{-1})			PP incubation temperature		
	Winter	Summer	c-Winter	Winter	Summer	c-Winter	Winter	Summer	c-Winter
0	18.0	26.0	21.0	591.2	725.0	508.4	20.5	32.0	20.0
1	19.3	26.0	18.5	908.5	1095.9	516.3	19.5	26.0	20.0
2	19.6	28.0	19.5	808.8	825.1	503.0	20.5	23.5	20.5
3	20.0	28.0	21.0	824.5	941.8	526.7	23.5	23.5	22.0
4	18.0	27.0	19.5	405.6	654.3	388.2	21.5	19.0	21.0
5	17.5	27.0	20.0	1043.1	620.7	492.4	20.8	22.0	20.0
6	17.5	27.0	20.0	667.5	1038.8	477.2	19.5	25.0	20.0
7	16.8	27.0	21.0	842.9	1002.8	495.5	20.0	24.0	20.0
8	18.0	27.0	19.5	931.5	1167.0	560.4	21.5	24.0	20.0
9		27.5	20.5		950.3	579.2		25.0	22.0
10		28.0	21.0		950.3	544.1		21.0	20.0
11		27.0	21.0		1029.7	435.8		26.5	21.0
12		27.0	21.0		912.3	596.0		26.0	20.0
13		27.0	20.5		1033.7	196.7		25.0	21.0
14		27.0	20.5		901.3	131.8		25.0	20.5
15		27.0			245.5			24.5	
Mean	18.3	27.1	20.3	761.5	923.2	487.1	20.8	24.5	20.5
St. Dev.	1.1	0.6	0.8	199.4	157.6	99.6	1.2	2.8	0.7
Maximum	20.0	28.0	21.0	1043.1	1167.0	596.0	23.5	32.0	22.0
Minimum	16.8	26.0	18.5	405.6	245.5	131.8	19.5	19.0	20.0
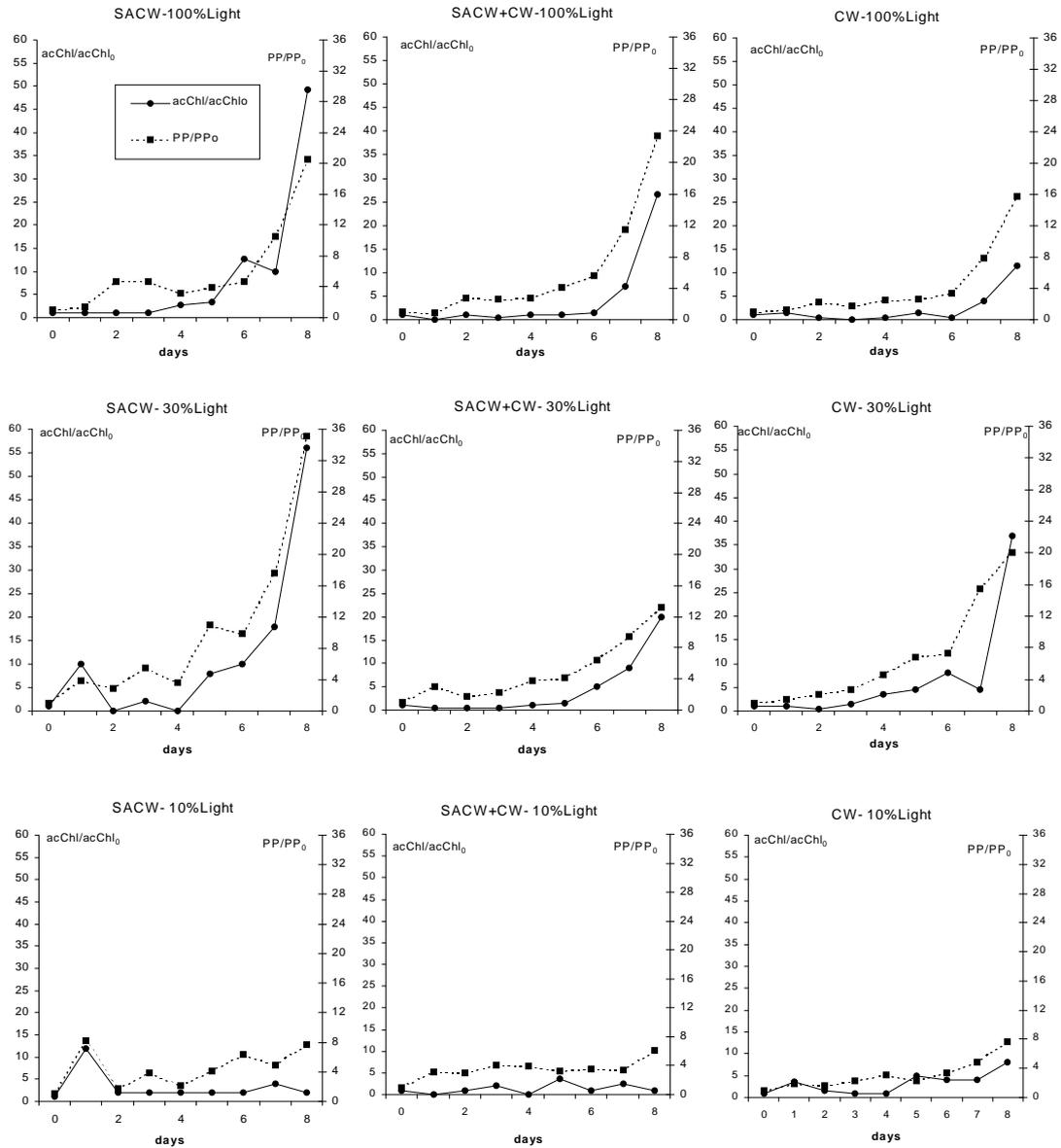
Fig. 1. Temporal variation of active chlorophyll-a (acChl) and primary production rates (PP) normalized by the time zero value for each treatment (acChl/acChl$_o$ and PP/PP$_o$), observed during the winter microcosms incubations.
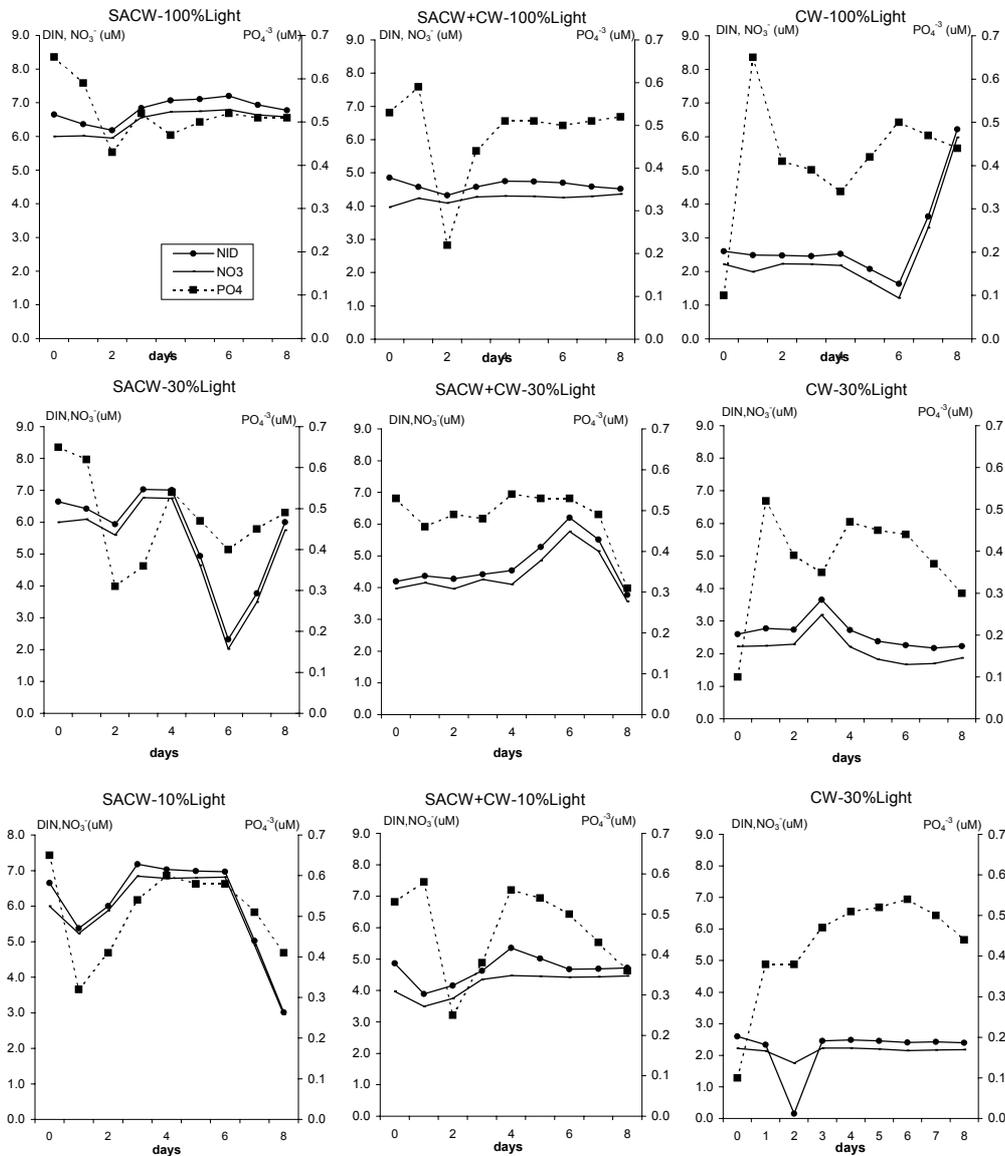
Fig. 2. Temporal variation of dissolved inorganic nitrogen (DIN), nitrate (NO_3^-) and phosphate (PO_4^{-3}) in the nine microcosm treatments performed during the winter microcosms (units in μM).

Summer

At the time of the seawater sampling for summer microcosms, the water column was stratified, presenting a thermal gradient of almost 10°C in 40 m of depth (Table 2). In CW, Chl and nutrients were low, indicating the oligotrophic condition usually detected in the mixing layer. On the other hand,

According to the preceding experience in winter, the incubation period was established to last 15 days to ensure that the complete phytoplankton growth would be observed. The mean temperature in the outdoor incubation tank was 27.1 ± 0.6 °C (Table 3), almost 4°C and 13°C higher than *in situ* temperatures of CW and SACW, respectively. Mean value of PAR was 923.2 μE m^{-2} s^{-1}, typical for tropical latitudes.

Unfortunately, on the 4th day of incubation the bottle of microcosm CW10 was broken and it was impossible to recover the water content. So, only the data up to day 3 are available for this microcosm.

Many differences were found in summer comparing to the winter. The more conspicuous was the reduced lag-phase observed in all microcosms: maximum of 2 days (against a minimum of 4 days in winter), high biomass yields were obtained (Fig. 3) and nutrients were actively consumed (Fig. 4).
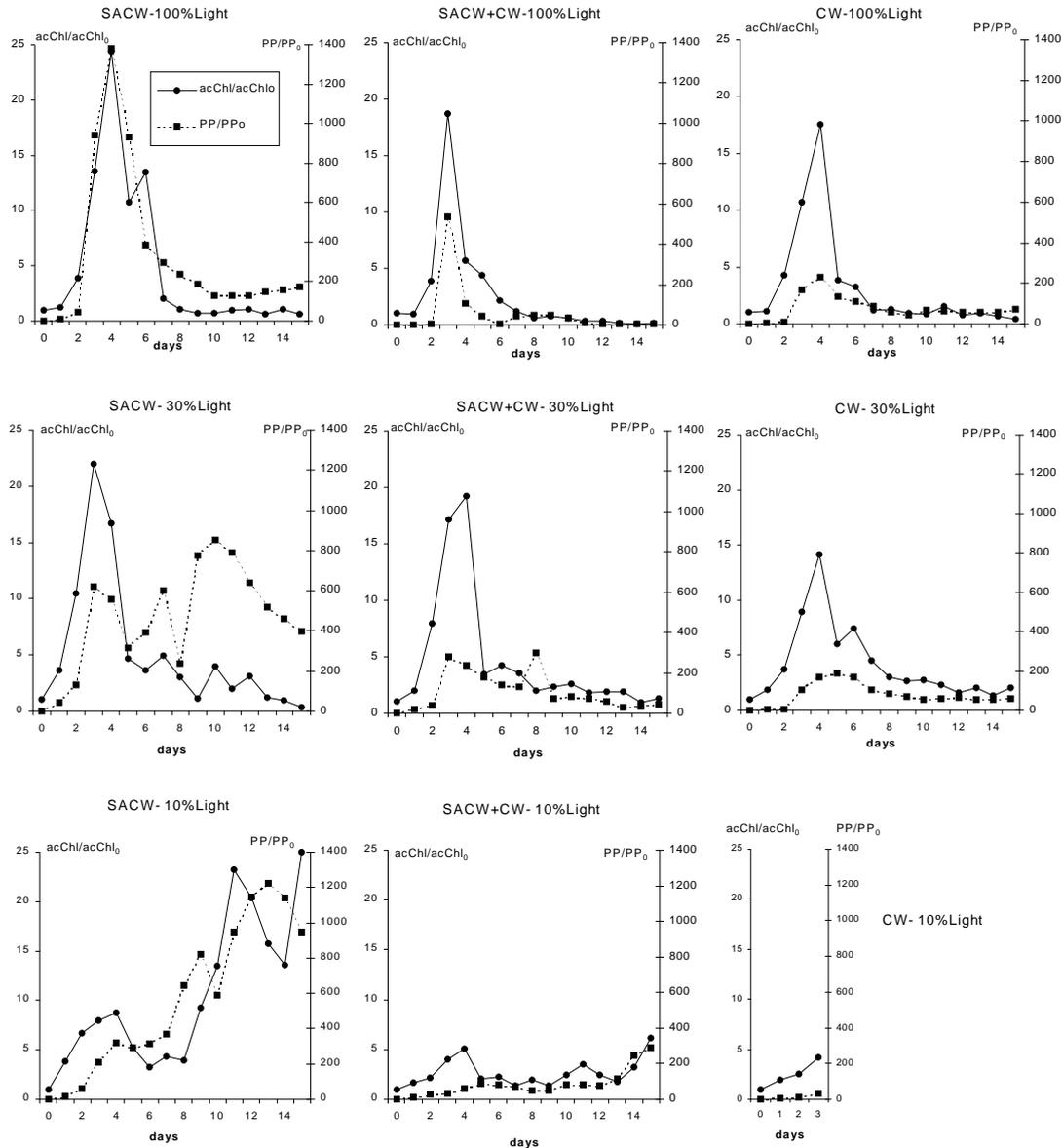

Fig. 3. Temporal variation of active chlorophyll-a (acChl) and primary production rates (PP) normalized by the time zero value of each treatment (respectively acChl/acChl$_0$ and PP/PP$_0$), observed during summer incubations.
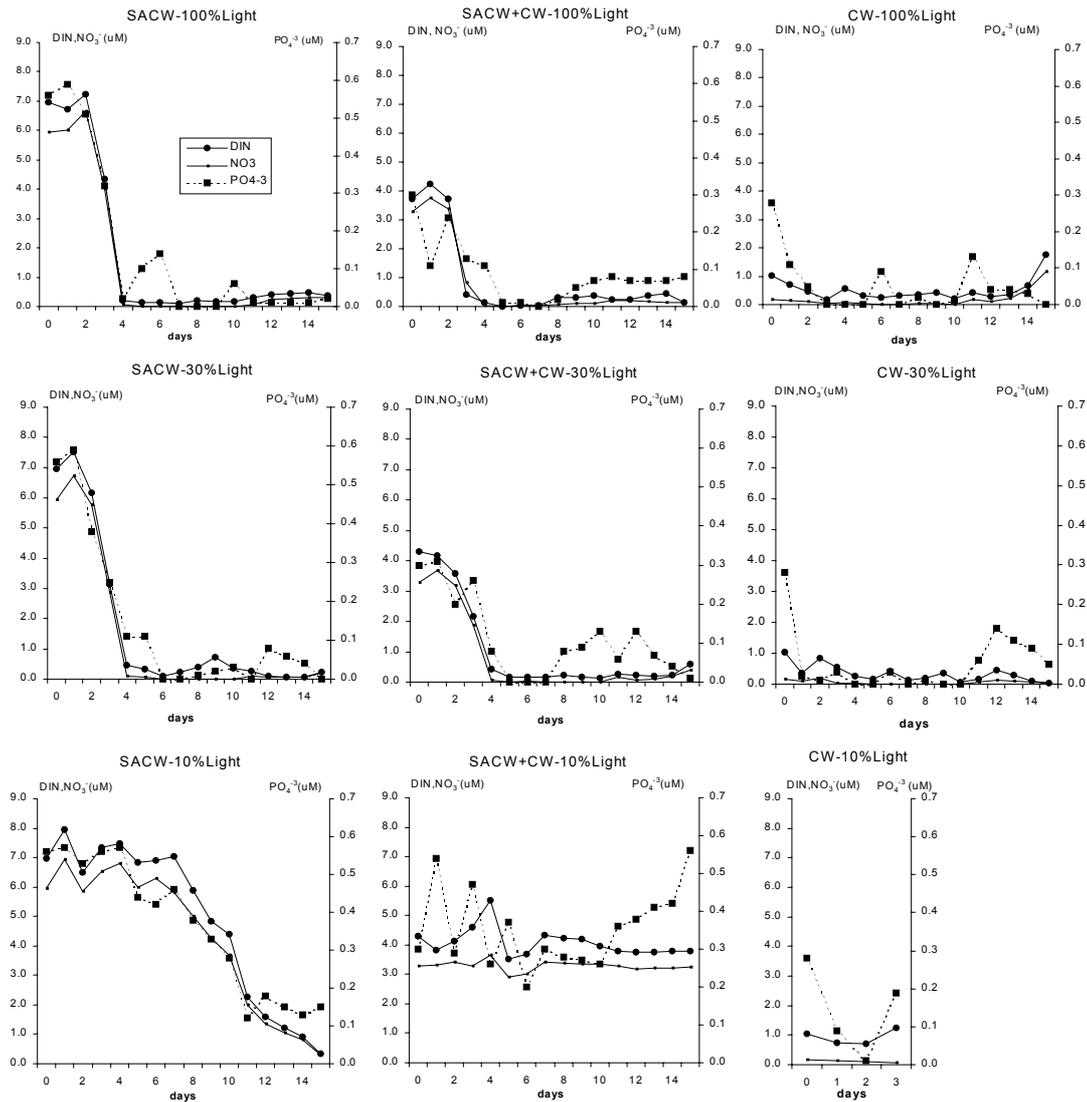
Fig. 4. Temporal variation of dissolved inorganic nitrogen (DIN), nitrate (NO_3^-) and phosphate (PO_4^{-3}) in the nine microcosm treatments performed during the summer (units in μM).

Light level influenced both the biomass yield and the nutrient assimilation pattern. At 100 and 30% PAR, PP/PP_0 and $acChl/acChl_0$ maxima were observed on the third or fourth day of incubation (Fig. 3). Preceding these maxima a significant decrease in DIN and phosphate was observed (Fig. 4), especially in SACW and SACW+CW. Diatoms were the more abundant group in the biomass peak at 100% PAR, while flagellates dominated at 30% PAR (Fig. 5). Under 10% PAR, microcosm responses were distinct. SACW10 presented the greatest yields in terms of $[Chl/Chl_0]$, $[acChl/acChl_0]$

and $[PP/PP_0]$ among all microcosms (Table 4) and nutrient concentrations declined sharply after day 7, preceding the second and higher biomass peak (whose absolute value was 16.5 mgChl m^{-3}) dominated by diatoms, in opposition to the initial biomass peak which was dominated by flagellates without a prior relevant nutrient consumption (Figs 3 to 5). A high degree of correlation between DIN consumption and $\mu_{mean}acChl$ (91.2%, P=0.0016) and $v_{max}DIN$ and $[PP/PP0]$ (81.8%, P=0.013) suggests that the biomass production is primarily linked to N uptake.
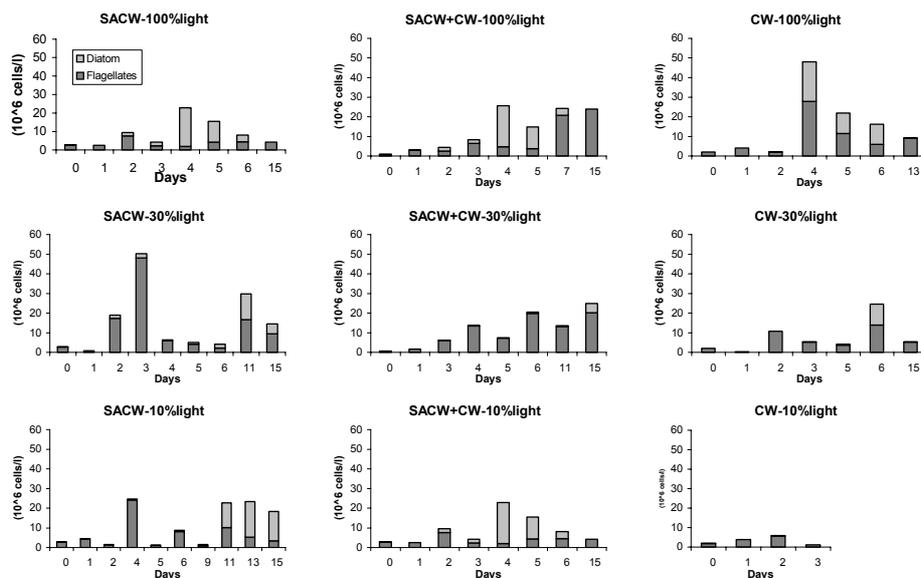
Fig. 5. Temporal variation of total phytoplankton cell density (10^6 cells l^{-1}) in the summer microcosms, emphasizing the dominance of the groups flagellates (phytoflagellates + dinoflagellates + silicoflagellates + cocolitophorids) and diatoms.

Table 4. Parameters derived from the 8 summer microcosms growth curves: maximum value obtained for Chl/Chl$_0$, acChl/acChl$_0$, PP/PP$_0$, PP/acChl, cell/cell$_0$ (variables normalized by the time zero value); time integrated values (represented by the brackets) of [Chl/Chl$_0$], [acChl/acChl$_0$] and [PP/PP$_0$]; maximum growth rate (μ_{max}) in terms of Chl/Chl$_0$, acChl/acChl$_0$ and cell/cell$_0$; mean growth rates in the exponential phase (μ_{mean}) obtained for Chl/Chl$_0$ and acChl/acChl$_0$ and maximum assimilation rate of DIN and PO$_4^{-3}$ per active Chl units- v_{max}DIN and v_{max}PO$_4^{-3}$ (in μmol μgChl^{-1} d^{-1}). (M=SACW+CW)

Summer Microcosm	max Chl/Chl$_0$	max acChl/acChl$_0$	$\left\langle \frac{Chl}{Chl_0} \right\rangle$ (d)	$\left\langle \frac{acChl}{acChl_0} \right\rangle$ (d)	max PP/PP$_0$	$\left\langle \frac{PP}{PP_0} \right\rangle$ (d)	max PP/acChl (mgC/mg Chl h)	max cell/cell$_0$	μ_{max} Chl (d^{-1})	μ_{max} acChl (d^{-1})	μ_{mean} Chl (d^{-1})	μ_{mean} acChl (d^{-1})	μ_{max} cell (d^{-1})	vmax DIN (μmol / μgChl d)	vmax PO$_4^{-3}$ (μmol / μgChl d)
SACW100	26.54	24.35	69.38	72.01	1382.3	5197	38.90	8.14	2.49	1.26	7.21	5.90	1.69	10.56	1.11
M100	42.81	18.70	50.77	40.49	534.9	899	39.17	38.71	1.86	1.57	8.79	5.60	1.90	12.63	0.54
CW100	5.85	17.54	21.91	49.50	228.3	1173	72.67	24.51	1.01	1.35	1.37	4.26	1.55	3.70	0.71
SACW30	10.34	21.94	52.55	81.95	851.4	7132	146.89	17.86	1.16	1.28	3.06	6.97	3.08	16.03	1.32
M30	10.01	19.26	42.89	72.01	299.0	1693	40.64	19.09	1.60	1.39	2.49	5.17	1.19	6.09	0.51
CW30	6.02	14.11	29.02	63.75	189.4	1210	17.89	12.51	0.84	0.88	0.63	1.00	3.26	4.63	1.55
SACW10	14.83	27.23	111.14	153.67	1222.1	8560	22.17	8.82	0.82	1.34	1.42	1.05	2.05	16.06	1.43
M10	5.35	6.17	33.20	38.79	287.3	1211	20.01	35.56	1.47	0.65	0.14	0.49	1.33	4.88	0.45

The greatest values of v$_{max}$DIN and v$_{max}$PO$_4^{-3}$ were registered in SACW microcosms followed by the intermediate values in the SACW+CW ones (Table 4), although v$_{max}$PO$_4^{-3}$ in CW had been higher than in the SACW+CW microcosms, as a result of an intense PO$_4^{-3}$ uptake in the first days in CW microcosms.

Cluster analysis applied to the data on Table 4 corroborates the visual interpretation of Figure 3, separating 3 groups: one composed by SACW at the three light levels, characterized by greater [Chl/Chl$_0$], [PP/PP$_0$], v$_{max}$DIN, v$_{max}$PO$_4^{-3}$ but with lower cell/cell$_0$ than the other microcosms at the same light level. Another group comprising SACW+CW100, CW100 and SACW+CW30 presented the maximum yield in terms of cell density and values of [acChl/acChl$_0$] and [PP/PP$_0$] a bit smaller than the first group ones. The last group, composed by CW30 and SACW+CW10, presented the lower assimilation ratios (PP/acChl) and lower acChl yields. Discriminant analysis pointed out [PP/PP$_0$] and v$_{max}$DIN as the main parameters for group separation.

C-winter

This microcosm series was conducted to highlight the phytoplankton dynamics in a very common condition in wintertime: a physically homogeneous water column occupies the continental shelf, as SACW generally remains around the shelf-break. The question was: are there differences between surface and bottom phytoplankton communities into the same water mass?

At the sampling time, phytoplankton biomass and inorganic nutrients in both depths were similar (Table 2), except to the higher ammonium concentrations at surface, that allowed supposing that a better growth could be expected for surface populations. However, time series of $acChl/acChl_0$ and PP/PP_0 (Fig. 6), clearly demonstrate the differences between surface and bottom water microcosm responses: in surface, $acChl/acChl_0$ values were most of the time below 1 (indicating a biomass decrease related to initial concentration) whereas in the bottom ones, a significant phytoplankton biomass growth was verified at the three light levels (Table 5). The phytoplankton community present in bottom water was able to develop; meanwhile the surface one seemed to be depleted. These results indicate the existence of a vertical physiological stratification in the phytoplankton community at the sampling time.

Ammonium was the dominant nitrogen form in all microcosms and the variability in DIN concentrations was generally related to it. In microcosms S100 and S30 there was a slight tendency to phosphate and DIN consumption with time, which was not evident in S10 (Fig. 7). In B100 and B30, DIN and phosphate concentrations decreased rapidly after day 6, preceding the biomass peak. In B10, DIN tended to decrease since day 1 and phosphate consumption was more intense after day 8, also preceding the Chl peak. Bottom water microcosms results showed the importance of light for biomass development: B100 attained the greater growth rates, using almost every available nutrient, while B30 and B10 were not significantly different in terms of biomass production and the nutrient consumption was lower. Maximum correlation was found between $[PP/PP_0]$ and $[acChl/acChl_0]$ (92%, P=0.001), $v_{max}PO_4^{-3}$ and $\mu_{max}Chl$ (82%, P=0.022) and also $v_{max}DIN$ and max PP/acChl (73.5%, P=0.037).

Cluster analysis applied to the data presented in Table 5 separated the microcosms in 3 groups: one represented by B100, which displayed the maximum Chl and PP yields, another one represented by S100 that did not present growth, and the last one composed by the other microcosms which displayed intermediate Chl and PP yields. Discriminant analysis pointed out that the main distinctive parameters were C assimilation ratio (PP/acChl), maximum cell/cell$_0$ and $[PP/PP_0]$.
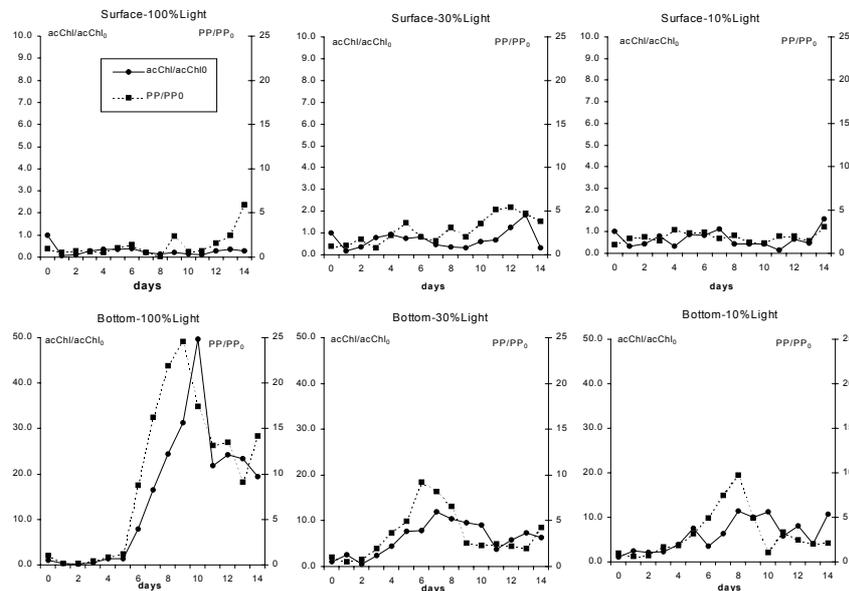

Fig. 6. Temporal variation of active chlorophyll-a (acChl) and primary production rates (PP) normalized by the time zero value of each treatment (respectively $acChl/acChl_0$ and PP/PP_0), observed during c-winter microcosm incubations, from surface and bottom layers.

Table 5. Parameters derived from the 6 c-winter microcosm growth curves: maximum value obtained for Chl/Chl_0, $acChl/acChl_0$, PP/PP_0, $PP/acChl$, $cell/cell_0$ (variables normalized by the time zero value); time integrated values (represented by the brackets) of $[Chl/Chl_0]$, $[acChl/acChl_0]$ and $[PP/PP_0]$; maximum growth rate (μ_{max}) in terms of Chl/Chl_0, $acChl/acChl_0$ and $cell/cell_0$; mean growth rates in the exponential phase (μ_{mean}) obtained for Chl/Chl_0 and $acChl/acChl_0$ and maximum assimilation rate of DIN and PO_4^{-3} per active Chl units- $v_{max}DIN$ and $v_{max}PO_4^{-3}$ (in μmol $\mu gChl^{-1} d^{-1}$).

| c-Winter Microcosm	max Chl/Chl_0	max $acChl/acChl_0$	$\left\langle \frac{Chl}{Chl_0} \right\rangle$ (d)	$\left\langle \frac{acChl}{acChl_0} \right\rangle$ (d)	max PP/PP_0	$\left\langle \frac{PP}{PP_0} \right\rangle$ (d)	max $PP/acChl$ (mgC / mg Chl h)	max $cell/cell_0$	μ_{max} Chl (d^{-1})	μ_{max} $acChl$ (d^{-1})	μ_{mean} Chl (d^{-1})	μ_{mean} $acChl$ (d^{-1})	μ_{max} $cell$ (d^{-1})	$vmax$ DIN (μmol / $\mu gChl$ d)	$vmax$ PO_4^{-3} (μ mol / $\mu gChl$ d)
Bottom100	46.71	49.63	209.03	213.16	24.54	135.1	7.77	27.29	1.18	1.71	5.63	5.85	0.72	19.02	3.34
Bottom30	14.29	11.88	97.85	86.94	9.20	50.1	6.65	15.2	1	1.47	2.41	2.17	0.57	17.68	2.43
Bottom10	13.82	11.38	101.2	84.41	9.73	45.4	6.65	12.62	0.9	0.98	1.39	1.07	0.60	3.61	0.99
Surface100	1	1	3.93	3.66	5.96	16.65	15.59	1.44	1.15	1.1	0.06	0.07	0.30	9.28	3.74
Surface30	1.83	1.83	10.71	10.01	5.51	39.92	5.67	4.41	0.74	0.79	0.29	0.29	0.32	2.49	1.35
Surface10	1.67	1.6	9.88	8.58	3.03	26.12	4.24	8.85	0.92	1.51	0.10	0.11	0.70	13.86	2.07
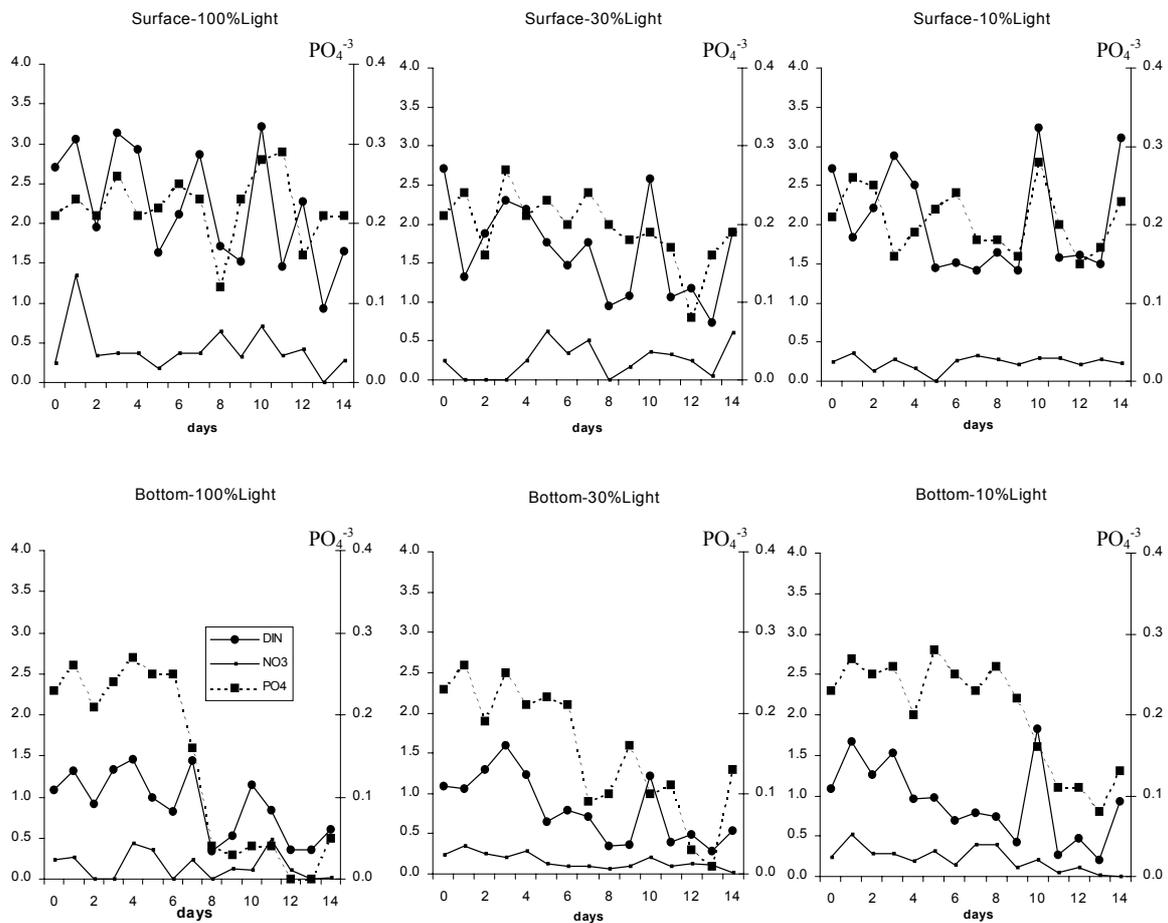
Fig. 7. Temporal variation of dissolved inorganic nitrogen (DIN), nitrate (NO_3^-) and phosphate (PO_4^{-3}) in the c-winter microcosms (units in μM).

DISCUSSION

Several studies pointed out the importance of the SACW presence over the continental shelf in increasing phytoplankton productivity. However, the present study constitutes the first tentative of quantifying the nutritional as well as thend also biological potential of this water mass based on phytoplankton growth rates.

Carpenter (1996), discussing about the microcosms application in ecological studies, states "*microcosms have become an important tool for some ecologists. However, they also have serious limitations… The size and duration of microcosm experiments exclude or distort important features of communities and ecosystems*". He also emphasizes that "*The rich context of ecology, our fundamental understanding of phenomena at multiple scales... depend on appropriately scaled field studies. While microcosm experiments have many advantages, their primary role is supportive and heuristic*". Daehler & Strong (1996) assert that the value of microcosms certainly depends on the questions being asked and the ecosystem or community being studied.

Even considering the limitations and biases inherent to microcosm approaches in representing natural conditions and given that all microcosms were submitted to the same "restrictions" allowing inter-comparisons, the present study was able to demonstrate the importance of the mixture of the nutrient rich SACW with the oligotrophic waters of the mixed layer (dominated by CW) in the Southeast Brazilian shelf, for the input of new nutrients and hence new production. Also, it was evident that the autochthonous phytoplankton community of SACW acts as a biological seeder when it reaches the euphotic layer developing high biomass.

Maximum Chl yields here obtained can be slightly overestimated by at least two reasons: as microcosms enclose amounts of water, they prevent cell losses by physical factors (Estrada *et al.,* 1987) and the small-scale turbulence generated by the bubbling air inside the bottles also could have a positive effect on biomass increase as verified by Alcaraz *et al*. (2002).

Eppley (1972) demonstrated that the ratio of cell division increases exponentially in the 0-40°C interval, so the differences between lag-phase for phytoplankton growth observed in the experiments of summer and winter may be related to the differences in water temperature at the outdoor tank in both periods.

Albeit there are great differences between SACW *in situ* temperature and the temperature in the incubation tanks, especially in the summer experiments ($\Delta T=14°C$), the results obtained represent the potential of biomass production of SACW inside the euphotic zone, considering the intensive nutrient consumption in all SACW microcosms. If the incubation temperature had been lower, probably the biomass yields would have been smaller, and so the potential yield would have been underestimated. Field observations in different SACW upwelling events at Cabo Frio (RJ) and neighboring area reported by several authors (Valentin *et al.,* 1986*,* Lins da Silva *et al.*, 1988; Gonzalez-Rodrigues *et al.*, 1992, Moser & Gianesella-Galvão, 1997, among others) showed that the phytoplankton development after an upwelling depends on the water heating. Indeed, Gonzalez-Rodriguez (*op. cit*.) characterized the upwelling phenomenon in three sequential phases where the second one, named as "productive phase", is typified by the water heating accompanied by the phytoplankton biomass increase.

Delgadillo-Hinojosa *et al.* (1997) observed that the increase in daily-integrated primary production was positively correlated to nitrate concentrations. Marañón *et al.* (2003) found that the rate of nutrient supply to the euphotic layer, estimated from variations in the nitracline depth, appeared as the most relevant environmental factor in explaining the observed variability in the primary productivity. The same feature was observed in the summer microcosms where the main nitrogen source was nitrate. The analyzed parameters $v_{max}DIN$ and $v_{max}PO_4^{-3}$ reflect the degree of phytoplankton adaptation to the environmental trophic conditions: the lowest $v_{max}DIN$ values were registered in CW microcosms while SACW+CW presented intermediate values and the highest occurred in SACW, independently of the light level (Table 4). This feature indicates that the more oligotrophic adapted the community is, the lower is the nutrient demand per Chl unit.

The mean values and the ranges observed for PAR were characteristic for tropical regions and agree with observations reported for Ubatuba (Teixeira, 1973; Aidar *et al.,* 1993). Certainly light was not a limiting factor for growth in microcosms submitted to 100% PAR in both winter and summer, although results indicate that it is a determinant factor in growing characteristics and also in the nutrient assimilation pattern. However, in c-winter, PAR levels were significantly smaller than in the other periods, and they could constitute a limiting factor in this specific case, leading to the low biomass yields observed. Light had a determinant role in the community composition: under higher PAR levels, an ephemeral diatom peak developed first (associated to fast nutrient depletion) while under lower PAR levels, the first peak was composed by flagellates which were latter substituted by diatoms, with a slower nutrient consumption. Such observations are important in the sense that in applying them to ocean conditions, different responses to a SACW upwelling event can be

expected depending on its characteristics: if the SACW stays at deeper portions of the euphotic zone, the nutrient consumption and autochthonous phytoplankton development will be different from a shallower upwelling condition. According to the present data, in the first case a slow nutrient consumption and a slow biomass increase but with higher yields would be expected, whereas if the SACW reaches the upper layers of the euphotic zone, an ephemeral and high biomass diatom peak following an intense nutrient consumption will be observed. In terms of nutrient profit, each condition results in different degrees of system enrichment: opportunist diatom peaks with higher growth rates could be uncoupled to zooplankton population able to graze them efficiently. In this case, most of that phytoplankton biomass will settle down, constituting a food source for benthos in shallow areas and a substrate to bacterial nutrient remineralization, allowing the nutrient turnover into the mixing layer. In the case where the nutrients were consumed slowly, the biomass peak is longer and grazing can be an important route to export nutrients to pelagic fish. This means that the upwelling characteristics will determine the main enrichment route into the trophic web. Similar conclusions were presented by Valentin *et al.* (1991) that states that the best biological results are obtained when the SACW penetration occurs slowly and continuously, allowing the establishment of a well-structured phytoplankton community. The phytoplankton community composition also has an important role in the energy flux in terms of food quality for the herbivores. Planktonic diatoms are the usual food for zooplankton and filter feeding fishes and contribute in a direct way to large fishable populations in coastal zones. In contrast, flagellates are frequently poor foods for most grazers and can lead to undesirable eutrophication effects (Officer & Ryther, 1980).

Odebrecht & Djurfelt (1996) observed that nutrient injection into the euphotic zone through bottom driven turbulence both during upwelling and downweling conditions, fuels the subsurface growth of large diatoms, leading to a surface chlorophyll maximum in a layer of relative low mixing rates, which may provide optimal food, for most pelagic filter feeders. In this case, the authors affirm that a simple and short trophic system, with low losses, would be expected, as a result of the growth of large centric diatoms.

Valentin *et al.* (1986) following an upwelling event at Cabo Frio (RJ), observed a diatom species succession related to the water warming: under lower temperatures and high nutrients the genera *Thalassionema, Diploneis* and *Pleurosigma* dominate, while in warmer and lower nutrient upwelled water

the dominating genera were *Chaetoceros, Nitzschia, Rhizosolenia* and *Leptocylindrus,* indicating specific adaptive mechanisms. In summer microcosms, under high incubation temperature, the diatom bloom was mainly composed by the genera *Nitzschia, Thalassiosira* and unidentified nanoplanktonic centrics.

Saldanha-Corrêa (1999) verified dynamic stable conditions during a strong SACW upwelling in São Sebastião coast, when this water reached the 25m depth and returned to deeper layers after 36h. In this occasion, SACW nutrients were not introduced into the mixed layer, due to the lack of turbulent mixing. Conversely, in this same period, a biomass increase was detected in the SACW domain (temperature was higher than 17°C), representing the autochthonous phytoplankton development, as the water entered into the euphotic layer. This observation agrees with the fact that phytoplankton responses occur on a scale of hours to days (Ferris & Christian, 1991 & Prézelin *et al.*, 1991) and corroborates the existence of a viable autotrophic community living outside the euphotic zone, which can growth significantly when light becomes available (Murphy & Cowles, 1997).

In the c-winter, the water column was homogeneous in terms of temperature and salinity and nutrient concentrations were similar between surface and bottom waters. However, the differences in biomass yields between surface and bottom microcosms indicate that bottom waters possessed some kind of bio-stimulant substance, not evaluated in the nutrient analysis performed. The sediment suspension due to the bottom friction promoted by water movements should be responsible for the introduction of these substances into the deeper layers of the water column, where they became available for phytoplankton. Indeed, Gianesella-Galvão & Saldanha-Corrêa (1995) based on fixed station data where physical and chemical conditions of the water column and phytoplankton biomass were monitored, verified an event of SACW intrusion in the outer shelf during the winter with a simultaneous phytoplankton productivity decrease. These authors discuss that, as the intrusion was very slight and occurred under dynamic stable conditions, the mixture between the water masses was negligible and in addition SACW acted as a physical barrier, isolating the remaining water column from the bottom, preventing the upward flow of settled remineralized substances from the sediment-water interface to the oligotrophic water column.

Such experimental results contribute to the understanding of the dynamics of SACW nutrient utilization by phytoplankton related to different hydrographic structure and position into the euphotic zone in the natural environment.

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