The role of phosphorus on planktonic production of the Gironde plume waters in the Bay of Biscay

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Abstract: More and more studies emphasize the status of phosphorus (P) as the principal limiting nutrient of phytoplankton growth, especially in coastal waters under the influence of freshwater discharges. The purpose of the present paper is to investigate the role of P on planktonic production in the waters influenced by the Gironde discharges; the Gironde being one of the two largest rivers on the French Atlantic coast. The survey is based on several cruises made in 1998 and 1999. Two different patterns were observed for waters with salinity below and above 34.5. For waters with salinity < 34.5, P was found to be the first limiting nutrient of winter and spring phytoplankton blooms, based on undetectable phosphate (< 20 nM), high NO3 : PO4 ratios, typically > 100 : 1, short phosphate turnover time (1 to 2 h), high alkaline phosphatase activities (mean of 176 nM h-1 in late May 1999) and ultimately great increases of chlorophyll a (Chl a) and primary production in phosphate-enriched samples relative to controls. This limitation could be partly explained by the Gironde nutrient supplies, which were phosphate deficient compared with the mineral nitrogen(Nmin : PO4 was > 40 within a salinity range 16–33). In summer, corresponding to the period of low influence of Gironde supplies (low runoff and a spreading effect of the plume), phytoplankton growth would be controlled by both P and nitrogen (N), according to low nitrate and the major effect of combined P+N (NH4) enrichment on Chl a and primary production compared with the addition of N or P singly. In early October, after the first autumn gales, the mixed layer was enriched with a sufficient supply of nutrients to support exponential phytoplankton growth for 4 days in enclosures. The pattern was different for waters at the limit of the Gironde plume and Atlantic oceanic waters (within salinity range 34.5–35.4). P would not be the single limiting nutrient of winter blooms and spring phytoplankton growth since low phosphate, and also low nitrate and silicate, availability were recorded and phosphate addition alone had no effect on phytoplankton biomass and production in bioassays. The early P limitation of winter blooms had consequences for the phytoplankton community structure in the Gironde plume waters (salinity < 34.5). Whereas major cells of these blooms were greater than 20 µm in size, phytoplankton growth in spring and autumn was dominated by 3–20 µm (e.g. 53% of Chl a in late April 1999) and < 3 µm cells (e.g. 29% of Chl a). The decreasing size of phytoplankton cells is emphasized by the severe competition between bacteria and algae for phosphate, since bacteria dominated phosphate uptake in spring (e.g. 87% in late April, 77% in late May). Bacteria tended to have greater affinity for phosphate and seemed also to be P limited at certain times in spring, according to results from phosphate enrichment bioassays in late May 1999. The alternative method for phytoplankton to obtain P would be the use of the dissolved organic phosphorus pool by alkaline phosphatase activity. According to the movement of 33P after initial labelling of microbial populations and a subsequent cold chase, the major transfer of P occurred from the bacterial to the dissolved fraction. We hypothesize that algae obtain part of its dissolved organic phosphorus from bacteria-originated organic phosphorus compounds.

INTRODUCTION

Numerous studies over the past two decades have provided contradictory results to the considerations that N is the most often limiting nutrient of phytoplankton growth in oceanic and coastal waters (Ryther and Dunstan, 1971; Oviatt *et al.,* 1995) and P in lake waters (Schindler 1977). If P limitation was actually demonstrated in lakes (Hecky and Kilham, 1988), it was also recently evidenced in coastal (Paasche and Erga, 1988; Harrison *et al.,* 1990; Lignell *et al.,* 1992; Thingstad *et al.,* 1993) and estuarine waters (Fisher *et al.,* 1992; Pennock and Sharp, 1994; Holmboe *et al.,* 1999; Yin *et al.,* 2000). In these areas, characterized by seasonally varying mixtures of fresh- and seawaters (Fisher *et al.,* 1992; Conley, 2000), most studies lead to the general consensus that there are seasonal and spatial variations of the limiting nutrient (D'Elia *et al.*, 1986; Rudek *et al.*, 1991). P limitation was often associated to period of high river runoff with high N:P loading ratio whereas, N or N+P limitation are rather associated to low river runoff, with comparatively greater influence of seawater having balanced N:P ratio (Fisher *et al.,* 1992) and biogeochemical *in situ* processes (Pennock and Sharp, 1994). However, P was also proved to be limiting in the coastal and oligotrophic waters of the western Mediterranean Sea (Dolan *et al.,* 1995; Thingstad *et al.,* 1998; Diaz *et al.,* 2001), in the ultra-oligotrophic waters of the eastern part (Krom *et al.,* 1991; Zohary and Robarts, 1998) and in the North Pacific subtropical gyre (Karl *et al.,* 1995; Björkman *et al.,* 2000). In the Sargasso Sea, phosphate depletion (Wu *et al.,* 2000), N:P ratio far greater than Redfield ratio (Michaels *et al.,* 1994), rapid phosphate turnover times and high alkaline phosphatase activities (Cotner *et al.,* 1997) suggest a P deficiency of the plankton community. Considering the rôle of N_2-fixer community, the recent model of Tyrrell (Tyrrell, 1999) predicts that P should be the "ultimate limiting nutrient" of oceanic primary production, since only external phosphate inputs (by river) would control the long term

primary production in the ocean. If scientists are not unanimous in approving of this concept because it doesn't take into account the regulation of nitrogenase activity by iron (Falkowski, 1997; Cullen, 1999; Toggweiler, 1999), phosphorus is recognized as the most essential of nutrients (Karl, 2000).

We investigated the role of phosphorus on planktonic production of the Gironde plume waters. The Gironde is one of the two largest river (with the Loire) on the French Atlantic coast with annual mean freshwater outflow of about 900 m^3 s^{-1} (Lazure and Jegou, 1998). A large part of the shelf could be influenced by the Gironde plume waters and the knowledge of the nutrients controlling accumulation of algal biomass is of great interest, especially towards a perspective of the food web structure understanding.

Previous studies achieved in May 1995 in the Gironde plume waters pointed out that the spring phytoplankton bloom would be severely P limited, considering the very high values of NO_3:PO_4 ratios and the high C:P and N:P ratios for the particulate organic matter (Herbland et al., 1998). It was hypothesized that winter phytoplankton blooms could occur in the Gironde plume and these blooms may be responsible for the early exhaustion of phosphate within the mixed layer. The existence and the causes of winter blooms are now established as well as their consequence on phosphate depletion (Labry et al., 2001). In the present study, we tried to answer two major questions : (1) Is the phytoplankton growth P limited both in winter and spring and how does this limitation evolve the rest of the year ? (2) What are the consequences of the early P limitation on the structuration of phytoplankton communities in spring ? A particular emphasis was made on the effect of the competition between phytoplankton and bacteria for phosphate on the structuration of phytoplankton communities and its consequences for the microbial phosphorus cycling.

MATERIALS AND METHODS

Sampling strategy

Several cruises were carried out in 1998 from early winter to late spring and in 1999 from late winter to early autumn. BIOMET 2 (8-21 January 1998), BIOMET 3 (25 February-11 March 1998) and PEGASE (7-21 June 1998) were realized aboard *RV Thalassa* whereas, PLAGIA 1 (21-28 February 1999), PLAGIA 2 (24-30 April 1999), PLAGIA 3 (25 May-1 June 1999), PLAGIA 4 (22-25 June 1999), PLAGIA 5 (12-15 July 1999) and PLAGIA 6 (4-7 October 1999) were carried out aboard *RV Thalia* or *RV Gwen Drez*.

BIOMET 2 and BIOMET 3 were composed of a grid of stations (Figure 1) covering the spread of the Gironde plume from the turbid plume (S < 33) to adjacent Atlantic oceanic waters (S > 35.5). Stations were chosen from the predictions of a 3D hydrodynamical model (Lazure and Jegou, 1998), SeaWiFS imagery (Froidefond *et al.,* in press) and ultimately from the continuous subsurface temperature and salinity records by an onboard thermo-salinograph. PLAGIA cruises consisted in the same grid of stations sampled at each period (Figure 1). Two study sites were sampled at PEGASE, one near the Gironde mouth and the other far offshore, on the continental slope (Figure 1).

Samples were collected using Niskin bottles according to salinity and temperature profiles recorded by a CTD probe. The mixed layer was generally sampled twice. At each station, nutrients, chl *a* concentrations and composition (C, N, P) of the particulate organic matter (in 1998 only) were measured. At additional stations, always located on the continental shelf (but sometimes out of the grid in 1999 to reach a salinity greater than 34.5, PLAGIA 2 and 3), supplementary measurements such as chl *a* size fractionation, primary production, bacterial numbers, phosphate uptake and phosphorus release, alkaline phosphatase activity, or phosphate enrichment bioassays were realized.

Chemical and biological variables

Seawater samples were filtered on glass fiber filters (Whatman GF/F) with a syringe filtration system. Filtrates were stored in polyethylene flasks, frozen (-20 °C) and analysed for the determination of nutrient concentrations according to classical methods (Strickland and Parsons, 1972) and as already described in Labry *et al.* (Labry *et al.*, 2001). As an exception, phosphate was analysed on board immediately after sampling on a spectrophotometer (Shimadzu UV 160) with a 10 cm optical path cell.

Particulate organic carbon (POC) and nitrogen (PON) were measured by filtration of seawater on precombusted (12 hours at 400 °C) 25 mm Whatman GF/F filters. The filters were frozen (-20 °C) and analysed within month. At the laboratory, filters were fumed overnight with HCl (36 % final conc.) to remove the inorganic carbon. The amount of organic carbon and nitrogen was measured in a CN analyzer (Carlo Erba 1500). Particulate organic phosphorus (POP) was determined on acid washed (HCl 1 N) precombusted Whatman GF/F in 1998 and on 0.2 µm Nuclepore polycarbonate filters in 1999. After seawater filtration, filters were frozen (-20 °C) and analysed by the persulfate oxidation procedure within month (Valderrama, 1981; Pujo-Pay and Raimbault, 1994).

Total chlorophyll *a* (chl *a*) and pheopigments were determined by filtration of seawater on Whatman GF/F. For size fractionation, 3 µm Nuclepore polycarbonate filters and 20 µm nylon sieve were used. Filters were frozen (-20 °C) and analysed within month by acidification fluorometric procedure in 90 % acetone extracts (Holm-Hansen *et al.,* 1965) at BIOMET 2 and BIOMET 3 and in 100 % methanol extracts (Wright *et al.*, 1997) at PEGASE and all PLAGIA cruises, using a Turner Designs 10-AU fluorometer.

Samples for bacterial counts were preserved with borate buffered formaldehyde (2 % final concentration). Bacteria were enumerated by direct counting after staining with DAPI (Porter and Feig, 1980).

Phytoplankton production and bacterial production

Primary production was measured in 1998 by *in situ* incubation of 300 ml seawater in polycarbonate bottles with 10 µCi of ^{14}C from morning to evening (9 hours to 17 h at BIOMET 2, 9 h to 18 h at BIOMET 3 and 7 h to 21 h at PEGASE). In 1999, potential primary production was measured by incubation of 300 ml seawater with 20 µCi of ^{14}C during 2 hours in an incubator with running surface seawater (*in situ* temperature) and under artificial saturating light of approximately 1660 µEinst m^{-2} s^{-1}. After incubation, filtrations were achieved on different pore size filters according to the size fractionation procedure for chl *a*. Filters were placed in scintillation vials with 200 µl of HCl 1 N, dried overnight at 60 °C then recovered with 4 ml of scintillation cocktail and stocked until counting at the laboratory with a Tri-Carb liquid scintillation analyser model 1500 (Packard). Time zero filtration was also achieved as control to substract non biological adsorption on the filter.

Bacterial production was estimated by the (^{3}H-methyl)-thymidine (TdR) incorporation method (Fuhrman and Azam, 1982). Triplicate samples (20 ml) and a control were incubated with 20 nM final concentration of TdR (50 Ci mmol^{-1}) at *in situ* seawater temperature for 30 min to 1 h. The rate of TdR incorporation into DNA was converted into cell production using 2.18 x 10^{18} cells produced per mole of thymidine incorporated (Fuhrman and Azam, 1982). Values were converted into bacterial carbon production assuming a cell content of 16 fg C, which is close to values found in the literature (Lee and Fuhrman, 1987; Simon and Azam, 1989) and belongs to the range of values (10-18 fg C) previously measured by Artigas (Artigas, 1998) in the Gironde plume waters.

Phosphate uptake and release kinetics

In 1998, extended ^{33}PO$_4^{3-}$ incorporation experiments were realized in parallel with primary production during *in situ* incubation of 300 ml seawater in polycarbonate bottles with 5 µCi

of $^{33}PO_4^{3-}$ from morning to evening in January and March and from morning to midday in June. Controls were prepared by the addition at time T_o of 6 ml formaldehyde (36 %). All filters were rinsed with filtered (< 0.2 µm) seawater.

In 1999, orthophosphate uptake protocol derived from Thingstad *et al.* (Thingstad *et al.,* 1993) was realized by addition of 100 µl of a carrier-free $^{33}PO_4^{3-}$ solution to 10 ml sample ($\approx$ 10^6 cpm ml^{-1} final concentration) in polycarbonate flasks. Incubations were carried out on board in an incubator with running surface seawater (*in situ* temperature) and *in situ* surface light (PLAGIA 1, 6) or 40 % of surface light (PLAGIA 2, 3). At each selected incubation durations (8, 15, 30, 45 minutes and 1, 2, 4, 6, 12, 24 h), $^{33}PO_4^{3-}$ uptake was stopped by a cold chase of 100 µl of 10 mM KH_2PO_4 (0.1 mM final conc.) and by addition of 200 µl of filtered (0.2 µm) formaldehyde (36 %). Controls were prepared by the addition at time T_o of both cold chase and formaldehyde. Initially added radioactivity was measured in triplicates. Samples were filtered on 0.22 µm Millipore filters and 1 µm Nuclepore filters placed on support filters (GF/F) saturated with a KH_2PO_4 solution, using a low suction < 0.15 bar. Filters were dried during approximately 24 hours and then stored in scintillation vials with 4 ml of scintillation cocktail until counting at the laboratory.

According to Thingstad *et al.* (Thingstad *et al.,* 1993) and initially to Sheppard (Sheppard, 1962), the consumed fraction R(t) of added label (substracted control) is related to the turnover time (T_n) of orthophosphate by the theoretical expression : $R(t) = (1-e^{-t/Tn})$ which can be rearranged as - ln [1-R(t)] = t/T_n. - ln [1-R(t)] was plotted against incubation times and the slope of the initial straight line was determined by linear least squares regression. The slope ($1/T_n$) provides directly the turnover rate of orthophosphate. Label in the size fraction 0.2-1 µm was calculated by substracting label in total particulate fraction (> 0.2 µm) to that in the > 1 µm fraction. Contributions of 0.2-1 µm and > 1 µm fractions to total uptake were calculated as the mean of their contribution during the linear phase of uptake.

Kinetic parameters of phosphate uptake were determined during PLAGIA 3 by supplementary addition of 25 to 100 µl of different KH_2PO_4 solutions to 10 ml samples in order to obtain phosphate final concentrations of 25, 50, 75, 100, 200, 400, 800, 1250, 2500 and 5000 nM. Incubation times varied from 1 to 6 hours according to added KH_2PO_4 concentrations. The phosphate uptake rate (V_{po_4}) was calculated by dividing added phosphate final concentration by phosphate turnover time (T_n) which was determined as described above. The determination of kinetic parameters K_t+S_n (K_t : half saturation constant, S_n : natural phosphate concentration), V_m (maximum velocity) was realized by non linear, least squares regression of data fitted to the Michaelis and Menten equation, using Marquardt-Levenberg algorithm (Brooks, 1992).

Measurement of phosphorus release from particulate fractions consisted in cold chase experiments (Dolan *et al.,* 1995; Thingstad *et al.,* 1996a). Natural populations (two 1 l samples in polycarbonate bottles) were firstly labelled with ^{33}P (150 µCi l^{-1}) during 24 hours. Second, a cold chase of 0.1 mM KH_2PO_4 (final conc.) was added to one incubation bottle in order to prevent uptake of $^{33}PO_4^{3-}$ remaining in solution or reassimilation of recycled orthophosphate. Periodically during labelling (1, 3, 6, 12, 24 h) and after the addition of the cold chase (1, 3, 6, 9, 12, 24 h), 10 ml subsamples were removed and immediately filtered on 0.22 µm Millipore filters and 1 µm Nuclepore filters. Filtrates were sampled to estimate labelled dissolved phosphorus. Controls were prepared by the addition of formaldehyde to 10 ml immediately (T_o) subsampled labelled water. Percentage of the added label was plotted against time after the phosphate cold chase and release rates were calculated from linear, least squares regression.

Alkaline phosphatase activity (APA)

The fluorometric method using methyl-umbelliferyl phosphate (MUF-P) as substrate (Hoppe, 1983; Ammerman, 1993) was used to measure alkaline phosphatase activity. All measures were achieved with a saturating substrate concentration of 250 µM. 2ml of whole water samples and prefiltered sample on 0.2 µm Nuclepore polycarbonate filters were incubated in duplicate with 50 µl of substrate solution. The control was carried out like the whole water sample but adding 450 µl of buffered formaldehyde (pH 8.00) at time zero, prior to substrate addition. Both samples and controls were incubated in the dark at *in situ* temperature during approximately ten hours. At the end of incubation, reaction in samples was stopped by the addition of formaldehyde and samples were frozen (- 20 °C). The fluorescence produced by the released methylumbelliferon MUF (excitation at 365 nm and emission at 460 nm) was measured by a fluorescence spectrometer (Kontron SFM 25). Total fluorescence of the control corresponding to the residual fluorescence of water and the substrate (MUF-P) was substracted since it was not negligible. The procedure was calibrated by fluorescence readings of MUF solutions (2 nM to 2 µM). Particulate APA (> 0.2 µm) was calculated by substracting total APA to the dissolved APA (< 0.2 µm fraction).

Phosphate enrichment bioassays

In 1999, enrichment bioassays were conducted on board. Water samples were distributed among two 25 liters polycarbonate bottles. They were prescreened (100 µm sieve) from late May to remove large planktonic organisms. Approximately 0.6 µM KH_2PO_4 final concentration were added to one bottle, such an addition corresponds to phosphate enrichment from winter vertical mixing in the Gironde plume for salinity of 32 (Labry *et al.,* 2001). The other bottle was used as a control. Additional N enrichments were achieved in July 1999 (PLAGIA 5) as NH_4Cl at a final concentration of 9 µM, alone or in combination

with PO$_4$ respecting a N:P ratio of approximately 16. Incubations were achieved in an incubator with running surface seawater (*in situ* temperature) and *in situ* surface light (PLAGIA 1, 6) or 40 % of surface light (PLAGIA 2, 3, 5). Sampling times were chosen according to *in vivo* fluorescence results in such a way to catch the chl *a* maximum. Chl *a*, potential primary production, bacterial numbers and bacterial production were measured. Bacterial predator - free seawater samples were prepared in parallel by filtering seawater through 1 µm polycarbonate filters and by diluting this inoculum with filtered seawater on 0.2 µm polycarbonate filters with a dilution rate of 1/5 and a final volume of 2 liters. Enrichments were the same as those for the whole water. Incubations were achieved at *in situ* temperature in the dark. Periodically subsamples were removed to determine bacterial numbers.

RESULTS

Two different patterns were observed for waters with surface salinity below 34.5 (referred in the following sections to as "Gironde" type) and above 34.5 (referred to as "Oceanic" type). The turbid plume waters were distinguished in January and early March 1998 since they corresponded to very different values for most parameters.

IN SITU MEASUREMENTS

Hydrological characteristics

In January and early March 1998, the Gironde plume spreaded northward and alongshore with strong horizontal salinity gradients (Labry *et al.,* 2001), which explains that salinities as high as 35.6 and 35.7 were recorded (Table I). In June 1998, the Gironde plume spreaded southward and offshore (not shown). In 1999, the southward and offshore extension of the Gironde plume were observed as early as in late February with a surface salinity range 32.0-

35.4 (Table I). In late April as well as in late June and July, the spreading effect of the plume (weak horizontal salinity gradients) was particularly pronounced with narrow surface salinity ranges. In late May, the plume was characterized by low salinities due to particularly high river runoff before the cruise (2668 m^3 s^{-1} on average for the 7 days before the cruise).

Cold temperatures were recorded in January (11.0-13.6 °C) and early March 1998, increasing strongly by June (16.4-18.4 °C) with the establishment of thermal stratification (Table I). In 1999, a gradual increase was measured from late February to July, with a thermocline established from late May. Haline stratification occurred from early March in 1998 (averaged mixed layer depth of 10 m) and from late February in 1999 for the "Gironde" type waters. In early October, thermal stratification remained for "Oceanic" waters (at 36 m, Table I) whereas, thermal and haline stratification were broken down by gales for "Gironde" waters.

Nutrients

In January 1998, nutrient concentrations were relatively high with a negative gradient from the turbid plume to "Oceanic" waters (Table II). They decreased dramatically from January to early March 1998, remaining low or decreasing again in June. Phosphate was particularly low (Table II), frequently below the detection limit of the method (20 nM) whereas nitrate and silicate were especially low within "Oceanic" waters. In late February 1999, phosphate was high near the Gironde mouth and already low (< 0.1 µM) away from the coast (not shown), which explains the high variability of the concentrations for the "Gironde" waters (Table II). It remained low, frequently undetectable thereafter, until it was increased by early October in "Gironde" waters only. Nitrate and silicate decreased strongly between late February and late April then increased by late May within "Gironde" waters only (Table II). Nitrate decreased again until July whereas silicate didn't show significative change.

$NO_3:PO_4$ ratios were high during the whole studied period compared to the Redfield classical value (16), except in "Oceanic" waters in January 1998 (14, Table II) and in "Gironde" waters in early October 1999 (17). Values were particularly high (> 100) from early March 1998 and from late April 1999. $NO_3:Si$ ratios showed balanced values in January 1998 (1.3 and 1.2) and a great decrease by June (0.4 and 0.3). The pattern was similar in 1999 with particularly low values in late June (0.3) and July (0.1) in "Gironde" waters and in early October in "Oceanic" waters (0.1).

Chlorophyll *a* and primary production

Low chl *a* concentrations (0.56 and 0.36 µg l^{-1}, Table II) as well as low integrated chl *a* and primary production (Table III) were recorded in the mixed layer of "Gironde" and "Oceanic" waters in January 1998. They increased dramatically until early March, especially the integrated primary production, implying a strong enhancement of integrated productivity (Assimilation Number AN) as well (Table III). All these parameters increased again by June except chl *a* concentrations in "Oceanic" waters. The corresponding turnover time of the phytoplankton community, assuming a mean C:chl *a* ratio equal to 50, was long in January (20 and 31 days) and very short in early March and in June (2.1 and 0.5 days). Even if the C:chl *a* ratio is expected to have changed seasonally with phytoplankton composition, light availability, etc…, the decreasing trend was strong (factor approximately above 10). In 1999, relatively high chl *a* concentrations were recorded as early as in late February (Table II). They decreased dramatically until late April, then remained low for "Oceanic" waters whereas they increased again by late May in "Gironde" waters. They decreased then gradually until early October.

Generally, size fractionation of chl *a* and primary production gave similar results. In "Gironde" and "Oceanic" waters, cells < 20 µm dominated in January 1998 with high

contribution of 3-20 µm size fraction (56 %) in "Gironde" waters (Figure 2). Whereas, blooms of cells > 20 µm occurred in early March (56 and 53 % of chl *a*). In June, the most important cells are again < 20 µm in "Gironde" waters with 44 % of chl *a* and 53 % of primary production associated with cells < 3 µm. This pattern was more pronounced for "Oceanic" waters. In 1999, contribution of > 20 µm cells was important as early as in late February (Figure 2). It was particularly low in late April, late May and early October to the advantage of 3-20 µm cells in "Gironde" waters and < 3 µm cells in "Oceanic" waters. The contribution of < 3 µm cells to total chl *a* in early october 1999 for "Gironde" waters must be considered with caution since it is underestimated by plugging on 3 µm filters. It is also the case for production of < 3 µm cells in early March 1998 and late April 1999 for "Oceanic" waters.

Particulate organic matter (POM)

In January and early March 1998, the distribution of POM clearly showed a negative gradient from the turbid plume to "Oceanic" waters (Table IV). POC and PON mean concentrations slightly increased in June in "Gironde" waters whereas POP concentrations gradually increased from January to June, except for "Oceanic" waters. A few results recorded in 1999 show similar POC, PON and POP values for "Gironde" waters in late February and late April but a strong increase by late May. Whereas C:N at:at mean ratios were particularly above Redfield ratio in 1998 only, N:P and C:P were greater than Redfield references both in 1998 and 1999.

Alkaline phosphatase activity

Generally, particulate APA (> 0.2 µm) was high within the mixed layer and decreased in the bottom waters (not shown). For "Gironde" waters (Figure 3), low APA was measured in

January 1998 (1.9 nM h^{-1}), increasing until early March (14.4 nM h^{-1}) and reaching the highest values in June (128.4 nM h^{-1}). APA increase was less pronounced for "Oceanic" waters. A similar pattern was recorded in 1999 for the two water type. APA was decreased dramatically by early October.

Phosphate uptake and release kinetics

Phosphate turnover time was particularly long in January 1998 (> 50 days) whereas it decreased dramatically in early March and June, below the upper limit of 13.6 h (Figure 4) calculated from an incubation time of 7 to 10 h, which would correspond to the saturation phase of the $^{33}PO_4^{3-}$ uptake kinetic. Time course realized in 1999 enabled to reach the precise turnover time of phosphate. It was long both in late February (251 h) and early October (217 h) in "Gironde" waters whereas it reached 1.1 and 1.2 h in spring (Figure 4). For "Oceanic" waters, short turnover time remained until early October. A same picture for contribution of microorganisms to phosphate uptake was observed in 1998 and 1999 : during period of long phosphate turnover time, large cells (> 1 µm) dominated whereas the small cells (0.2-1 µm) dominated during periods of short phosphate turnover time.

The phosphate uptake rate (Vpo$_4$) as a function of added phosphate fitted the Michaelis and Menten equation in late May with high variability for the 0.2-1 µm size fraction (Figure 5). The 0.2-1 µm size fraction always displayed (Table V) weaker K_t+S_n (2-38 nM) than the > 1 µm fraction (56-90 nM) and similar or weaker V_m (1.6-4.9 nM h^{-1} against 1.3-44 nM h^{-1}).

The saturation phase of $^{33}PO_4^{3-}$ uptake was attained between 1 to 3 h (Figure 6) for each experiment (except for "Oceanic" waters in late May, between 12 and 24 h). The addition of unlabelled phosphate stopped $^{33}PO_4^{3-}$ uptake and ^{33}P tended to be released at very slow rates

from the particulate fraction compared with controls. The dominant transfer occurred from the 0.2-1 µm fraction to the dissolved fraction (Figure 6, Table V).

BIOASSAYS

Chl *a* and potential primary production showed a maximum increase in the phosphate enriched samples of "Gironde" waters relative to controls 6, 4 and 2 days after time zero in late February, April and May respectively (Figure 7). Even if no replicates were made, chl *a* and primary production differences between control and enriched sample were measured for several incubation time. Whereas, no strong stimulation was observed for "Oceanic" waters. In July, chl *a* was stimulated in the NH_4+PO_4 enriched samples for both "Gironde" type waters G1 (salinity of 33.9) and G2 (salinity of 34.4) whereas, it wasn't the case in single PO_4 or NH_4 enriched samples (Figure 8). Finally, chl *a* and potential primary production of the P enriched sample and the control were not significantly different in early October (decrease of primary production at 3 days in "Gironde" waters is unexplained) (Figure 7).

Bacterial number and bacterial production increased after 1 day incubation in the P enriched sample relative to the control in late May only and in "Gironde" waters only (slightly in "Oceanic" waters; Figure 9). Bacteria numbers also increased in the predator - free, P enriched sample relative to the control.

DISCUSSION

Seasonal limitation of phytoplankton growth

Our results support the distinction of two water types in the Gironde plume with regard to the nutrient limiting phytoplankton growth and its seasonal change.

(1) In the Gironde plume waters of < 34.5 salinity, Labry *et al.* (Labry *et al.*, 2001) already pointed out that winter phytoplankton blooms occurred between January and early March 1998. It is confirmed by increases of integrated chl *a* (factor of 3) and primary production (factor of 16). These blooms had consumed and exhausted phosphate within the mixed layer since low and non-conservative phosphate concentrations were recorded in early March (Labry *et al.*, 2001). The early phosphorus limitation of these winter blooms is suggested by the high $NO_3:PO_4$ ratios, typically > 100:1, the short phosphate turnover times (< 13.6 hours) in March and by a 10 fold increase of particulate APA between January and March, which is classically used as an index of phytoplankton P deficiency (Fitzgerald and Nelson, 1966; Gage and Gorham, 1985; Thingstad *et al.*, 1993). In late February 1999, winter blooms were already initiated, according to relatively high chl *a*, and low phosphate (< 0.1 μM) together with high $NO_3:PO_4$ ratio were already recorded. Phosphate turnover times were still long (~ 10 days), particulate APA was low (on average 1.9 nM h^{-1}) but bioassays showed an increase of phytoplankton growth in the phosphate enriched sample relative to the unenriched control. A transient situation was thus evidenced : winter blooms had not yet depleted phosphate but phosphate was already not sufficient to support phytoplankton growth. In spring 1999 (late April, late May), phosphorus limitation of phytoplankton growth was suggested by $NO_3:PO_4$ above 100:1 as well as $N_{min}:PO_4$ (data not shown) and particulate N:P and C:P largely above Redfield ratios (16 and 106). However external nutrient ratios and particulate matter composition could not be used as simple indicators of nutrient limitation because of the rôle of internal nutrient storage and non-algal particulate matter respectively, the latter being important in the Gironde plume (Labry *et al.*, 2001). Short phosphate turnover times (1.1, 1.2 h), high APA (19 and 176 nM h^{-1}) and ultimately chl *a* and primary production relative increase in phosphate enriched sample confirmed the phosphorus limitation in spring. In contrast, phytoplankton growth in late June and July could to be controlled both by P and N

availability since nitrate decreased dramatically from 6.6 µM in late May to 1.3 µM in late June and 0.5 µM in July, which is close to the half saturation constant for uptake of dinoflagellate (0.5 µM; Kudela and Cochlan, 2000), and low ammonium concentrations persist (data not shown). During the same time, NO_3:Si ratio shifted from 16 ± 30 to 0.3 ± 0.3 and 0.1 ± 0.2. Finally, NH_4+PO_4 additions stimulated chl a in July for both "Gironde" waters whereas NH_4 or PO_4 additions alone had no effect compared to the control. Even if NH_4 addition does not reflect actual nitrogen enrichment by the outflow of the Gironde river, which is mainly a nitrate enrichment, it was indicative of a nitrogen physiological deficiency of phytoplankton. N+P limitation seems to be also actual in June 1998, according to low phosphate, nitrate (0.8 µM), mineral nitrogen (1.1 µM) and NO_3:Si ratio (0.4).

Our results for the Gironde plume waters of < 34.5 salinity support the hypothesis that winter/spring phytoplankton blooms are P limited and that phytoplankton growth would be N+P limited in summer. Silicate mean concentrations as low as 1.9 µM in early March 1998 and late April 1999 were probably also limiting for diatom growth since they were close to their half saturation constant for uptake (2 µM; Del Amo and Brzezinski, 1999). It would be probably responsible for the shift from the winter bloom of diatoms (Labry et $al.,$ 2001) to spring populations dominated by flagellates (data not shown), as commonly observed in other coastal ecosystems (Conley and Malone, 1992; Fisher et $al.,$ 1992; Del Amo et $al.,$ 1997). In early October, the first autumn gales broke down the summer stratification, enriching the mixed layer with nutrients, at ratios close to equilibrated ratios for growth (NO_3:PO_4 = 17 ± 7 and NO_3:Si = 0.7 ± 0.2) and in sufficient supply to support exponential phytoplankton growth during 4 days in enclosure (Figure 7). According to long phosphate turnover time (217 h) and low APA, phytoplankton growth would not be yet P limited.

(2) At the limit of the Gironde plume, as represented by "Oceanic" type waters, low concentrations and short phosphate turnover times were found from late winter, and at least until late spring in 1998 and until early October in 1999. However, it was also accompanied by low nitrate and silicate availability from early March in 1998 (respectively 0.9 and 1.0 µM) and from late April in 1999 (1.8 and 0.8 µM), which may explain that phosphate addition alone had no stimulating effect on chl *a* and primary production in bioassays. Thus, phosphate, nitrate and silicate could have been limiting for winter blooms of large diatoms which also occurred in these waters (Labry *et al.,* 2001), according to their concentrations which were close to their half saturation constant for uptake. In spring, phytoplankton growth could be more probably limited by P+N since nitrate went on decreasing by June 1998 and by late May 1999, whereas silicate remained the same or increased, resulting in low NO_3:Si ratio values compared to the equilibrated N:Si (at:at) ratio of 1 for growth. Lower APA recorded in "Oceanic" waters compared to values in "Gironde" waters in June 1998 and late May 1999 seem to indicate that P limitation was less severe, probably because phosphorus was not the single limiting nutrient. This is confirmed in early October by persisting low nutrients and no stimulation of phytoplankton growth by phosphate addition alone.

P limitation of phytoplankton growth as a contrast to the N limitation dogma has been mostly evidenced in coastal (Paasche and Erga, 1988; Harrison *et al.,* 1990; Thingstad *et al.,* 1993) and estuarine waters (Fisher *et al.,* 1992; Pennock and Sharp, 1994; Holmboe *et al.,* 1999; Yin *et al.,* 2000). In coastal waters under river discharge influence, the status of nutrients with respect to phytoplankton growth was often associated to nutrient loading from the river (D'Elia *et al.,* 1986; Malone *et al.,* 1988; Harrison *et al.,* 1990; Rudek *et al.,* 1991; Conley 2000; Yin *et al.,* 2000). In January 1998, corresponding to the period of low phytoplankton biomass, production and turnover time (20 and 31 days) and relatively high Gironde runoff

(1570 m^3 s^{-1} on average for the 7 days before the cruise), the negative gradient of phosphate, nitrate and silicate from the turbid plume to "Oceanic" type waters reflects the nutrient enrichment by the outflow of the Gironde river. At the outer part of the Gironde estuary, inverse linear relationship between salinity and nutrients were evidenced in January and early March 1998 within salinity range 16-33 (see Labry *et al., 2001*) and the mean NO$_3$:PO$_4$ ratios were respectively 41 ± 10 (n = 17) and 52 ± 3 (n = 15). The Gironde nutrient supplies to the continental shelf are thus phosphate deficient compared with the mineral nitrogen dominated by nitrate, both in early winter (NO$_3$:PO$_4$ = 31 ± 9) and in late winter. The Atlantic oceanic waters (S > 35.5), the second major source of nutrients to the continental shelf, has more balanced NO$_3$:PO$_4$ inputs (14 ± 2, n = 13 in January 1998) but may have little influence during high Gironde runoff. This seems to explain why P was the first limiting nutrient of winter blooms. High river runoff measured in spring, such as in late May 1999 (2668 m^3 s^{-1} on average for the 7 days before the cruise) implying phosphate and nitrate enrichment must contribute to the occurrence of spring phytoplankton blooms as observed in "Gironde" waters (high chl *a*, POC, PON and POP). According to Herbland *et al.* (Herbland *et al., 1998*), estuarine NO$_3$:PO$_4$ ratio would be high in spring (70 ± 9 in May 1995 within salinity range 18-27), which was confirmed by Michel *et al.* (Michel *et al., 2000*) in May 1997. Assumed high NO3:PO4 ratio in the Gironde discharges in late May could explain why P was the first limiting nutrient in spring 1999. In summer 1999 and probably in late spring 1998, phytoplankton growth status shifted from P to N+P limitation. The seasonal shift in the limiting nutrient was often associated to seasonal variations in river flow and nutrient loading (D'Elia *et al., 1986*; Paasche and Erga, 1988; Rudek *et al., 1991*). In Chesapeake Bay, the shift from spring P to summer N limitation was related to the decline in the atomic N:P inputs from spring to summer (Fisher *et al., 1992*). No precise study of N:P seasonal variations was realized in the Gironde estuary. According to the "Réseau National d'Observation" (French

national net monitoring the marine environment quality), nitrate and phosphate would not be conservative within a part of the salinity range 5-35 in Gironde estuarine waters during summers 1974-1984 (RNO, 1989-1990). Thus, nutrients could be partly consumed at the outer part of the Gironde estuary before being supplied to the continental shelf. We actually found low phosphate concentrations at stations close to the Gironde mouth in late June and July (not shown). Moreover, additional N limitation occurs during period of low Gironde supplies influence due to low runoff (608, 533, 252 m^3 s^{-1} respectively in June 98, late June and July 99) and weak horizontal salinity gradients (spreading effect of the plume), which would be favorable to more influence of Atlantic oceanic waters.

Consequences on the phytoplankton community size structure

Phytoplankton size in the Gironde plume waters displayed a pronounced seasonal pattern, which is shown by size fractionation of biomass (chl *a*) and primary production. In "Gironde" type waters, 3-20 µm cells dominated in early winter under light limitation (Labry *et al.,* 2001) and nutrient repleted conditions (56 % of chl *a* and 64 % of production). It was surpassed by winter blooms > 20 µm cells (56 % of chl *a* in 1998 and 49 % in 1999), then being important again or even dominating during spring P limitation (53 % and 71 % of chl *a* in late April and May) and in autumn. Same dominance of chl *a* and primary production < 20 µm were measured in May 1995 during spring bloom when phosphate was exhausted and was associated to a maintenance system with active regeneration processes (Herbland *et al.,* 1998) and a dominant microzooplankton grazing (Sautour *et al.,* 2000). In "Oceanic" type waters, the pattern is yet more pronounced than for "Gironde" waters during periods of small cells dominance (< 20 µm) with a greater contribution of < 3 µm size class (49 % and 64 % of chl *a* in late April and May). This pattern is in good agreement with one of the several statements

of Riegman *et al.* (Riegman *et al.,* 1993), concerning the food web structure, that "small algae are better competitors for light and nutrients than larger algae". In the Gironde plume, light limitation occurs in early winter (Labry *et al.,* 2001) and nutrient limitation (P or N+P) occurs during all spring and until autumn. The competitiveness of small cells could be explained by their relatively high surface/volume (S/V) ratio more favourable for molecular diffusion of nutrients, as demonstrated by Morel *et al.* (Morel *et al.,* 1991) for iron and zinc and by Chisholm (Chisholm, 1992) for nitrogen.

This argument must be also valid for phosphorus limitation. Moreover, in the case of phosphorus, it could also explain the dominance of bacterial phosphate uptake on phytoplankton uptake, as it is largely reported in P limited environment such as lakes (Currie and Kalff, 1984b; Cotner and Wetzel, 1992), coastal waters (Thingstad *et al.,* 1993; 1998) and in P limited cultures (Rothhaupt and Güde, 1992). In the "Gironde" type waters, the 0.2-1 µm size class, which could be assimilated to the bacterial fraction (less than 10 % of chl *a* was < 1 µm in 1999 and only 3 % of bacteria were found in the > 1 µm fraction in late February 1999, unpublished data), dominated phosphate uptake during spring P limitation (87 % in late April, 77 % in late May). When P+N limitation occurred, as in "Oceanic" type waters, the size fraction 0.2-1 µm also dominated phosphate uptake (84 % in late April, 77 % in late May and 69 % in early October). However, in this case, bacterial uptake could be overestimated since chl *a* < 1 µm was found to reach until 40 % of total chl *a* (unpublished data). Taking into account corrections for picophytoplankton, heterotrophic bacterial uptake would reach yet 58 % and 46 % in late May and early October. On the contrary, phytoplankton uptake increased and even dominated during period of phosphate repletion characterized by relatively high phosphate concentrations, long phosphate turnover time, low APA (56 % and 63 % in "Gironde" and "Oceanic" waters in late February) and also long phytoplankton carbon turnover time (as in January 1998, Table III). This is consistent with the results of Currie *et*

al. (Currie *et al.,* 1986) who find that, among 13 studied lakes, phytoplankton contribution is the greatest in the least phosphorus-deficient lakes, leading to the hypothesis that algal-bacterial phosphate uptake partitioning in lakes would be dependent upon the severity of phosphorus limitation. In other coastal waters, phytoplankton contribution was also found to be higher in phosphate repleted conditions (Lebo, 1990; Dolan *et al.,* 1995; Thingstad *et al.,* 1996b) and it is also consistent with numerous experiments showing a shift towards a greater contribution of large cells when phosphate concentrations increased (Lean and White, 1983; Suttle and Harrison, 1988; Suttle *et al.,* 1990; Rothhaupt and Güde, 1992).

Thus, during P depletion, the favourable S/V ratio of bacteria must strengthen P limitation of phytoplankton and the structuration of phytoplankton communities towards small cells. The competition for phosphate is as much strong that first, bacteria tend to have greater affinity for phosphate with a weaker K_t+S_n (2-38 nM) than that of the algal fraction (56-90 nM) in late May, which was already observed in lakes (Chrost and Overbeck, 1987; Cotner and Wetzel, 1992), coastal waters (Thingstad *et al.,* 1993; 1998) and in culture (Currie and Kalff, 1984a) and second, that bacteria could be also P limited at certain time of the year. Indeed, increases of bacterial biomass and production in the whole P enriched sample relative to unenriched controls in late May can be directly attributed to phosphate addition, since bacterial biomass stimulation was also observed in the predator free sample, assumed to be also phytoplankton free. Indirect stimulation via increase of phytoplankton growth and ultimately C compounds excretion (Riemann and Sondergaard, 1986) is not the case in our study.

How can phytoplankton obtain its source of phosphorus when submitted to competition with bacteria? Other unshown results dealing with "Gironde" type waters showed firstly that DOP concentrations were relatively high in spring whereas phosphate was exhausted, secondly that phytoplankton APA per unit biomass increased dramatically from late winter to late spring

and third, that phytoplankton APA was always the major contributor of the total APA (62 %

in 1998 and 61 % in 1999). Therefore, DOP could be an important source of P for

phytoplankton in spring. According to our cold chase experiment, the major transfert of P,

even if it occurs at slow rates, as already found by Thinsgtad *et al.* (Thinsgtad *et al.,* 1993;

1996a) and Dolan *et al.* (Dolan *et al.,* 1995) with the same technique, seems to carry out

directly from the bacterial to the dissolved fraction without net transfer to the > 1 µm

fraction. Based on results of the same authors that label regenerated from bacteria appears

both in dissolved inorganic and organic P pools, we assume that phytoplankton obtain part of

its P-DOP from these bacterial originated organic P compounds. This is in accordance with

Currie and Kalff (Currie and Kalff, 1984b) findings that slow P movement from bacteria to

algae occurs and that the uptake of ^{32}P-labeled lake water filtrate is realized entirely by

phytoplankton. During our cold chase experiment, APA was probably inhibited and possibly

repressed by the addition of phosphate (Chrost, 1990) but not the 5'-nucleotidase activity

(Thingstad *et al.*, 1993). The subsequent coupled uptake of $^{33}PO_4$ by phytoplankton could be

compensated by zooplankton grazing or excretion, or was so slow that no net transfert of P in

the > 1 µm fraction was observed.

Thus bacteria could be central in the phosphorus cycling by dominating phosphate uptake and

liberating organic P compounds via protozoan grazing with low P assimilation efficiency

(Vadstein *et al.,* 1993; Dolan *et al.,* 1995), viral lysis or excretion, these compounds being

used by phytoplankton. However, we cannot exclude other sources of P for phytoplankton.

Since algae are superior competitors at high phosphate concentrations (our results of higher

V_m, Suttle *et al.,* 1990), it is hypothesized that they could take advantage in condition of

nutrient supply inhomogeneities, generated at microbial scale by zooplankton excretion

(Rothhaupt and Güde, 1992) known to be essentially phosphate (Peters and Lean, 1973;

Taylor and Lean, 1981) or at larger scale by upwelling, storm events (Suttle *et al.,* 1988) or

freshwaters supply during floods, the latters being also enriched with organic P compounds. On the other hand, the phagotrophy capacity on bacteria of some mixotrophic species can be another alternative under nutrient limitation condition (Nygaard and Tobiesen, 1993), as it is reported for *Uroglena americana* in condition of short phosphate supply (Urabe *et al.,* 1999).

CONCLUSION

The first hypothesis of this study that phytoplankton growth would be P limited in late winter and spring and then N+P limited in summer is verified for waters the more submitted to Gironde supplies (S < 34.5). In late winter and in spring, the status of phosphorus could be partly explained by the Gironde nutrient supplies which were phosphate deficient compared with the mineral nitrogen. In summer the Gironde influence was weaker (low runoff, spreading effect of the plume) and the influence of Alantic oceanic waters, having more balanced N:P ratios, was greater. For waters at the limit of the plume and Atlantic oceanic waters, P would not be the single limiting nutrient of winter blooms and spring phytoplankton growth since nitrate and silicate were also low and P addition alone had no effect on phytoplankton biomass and production.

The early P limitation of winter blooms favours the structuration of phytoplankton communities towards the dominance of small cells in spring (3-20 µm and < 3 µm). This pattern must be emphasized by the competition of algae and bacteria for phosphate, bacteria dominating phosphate uptake in spring, tending to have greater affinity and being P limited at certain time of spring. Phytoplankton seem to obtain its P from the DOP pool (strong increase of APA in spring) and, according to the major transfert of ^{33}P from the bacterial to the dissolved fraction in cold chase experiments, which could be both organic and inorganic, the bacterial organic P compounds could be partly the source of P-DOP for algae.

A comparative study of nitrate and phosphate winter concentrations by the French national net monitoring the marine environment quality (RNO) leads to the conclusion that $NO_3:PO_4$ ratio would be higher in Loire, the other large river on the French Atlantic coast, than in Gironde (RNO, 1992-1993). Moreover, spring phosphate depletion in the Loire plume and South Brittany waters was very recently observed during a cruise carried out in April-May 2000 (unpublished results). Therefore, we can infer that the phosphorus status could be similarly important for waters under the Loire influence, but new studies are necessary to precise its role on the productivity and the plankton communities.

ACKNOWLEDGEMENTS

LITERATURE CITED

Ammerman,J.W. (1993) Microbial cycling of inorganic and organic phosphorus in the water column. In Kemp,P.F., Sherr,B.F., Sherr,E.B. and Cole,J.J. (eds.), *Handbook of Methods in Aquatic Microbial Ecology*. Lewis Publishers, U.S.A., pp. 649-659.

Artigas,L.F. (1998) Seasonal variability in microplanktonic biomasses in the Gironde dilution plume (Bay of Biscay) : relative importance of bacteria. *Oceanol. Acta*, **21**, 563-580.

Björkman,K., Thomson-Bulldis,A.L. and Karl,D.M. (2000) Phosphorus dynamics in the North Pacific subtropical gyre. *Aquat. Microb. Ecol.*, **22**, 185-198.

Brooks,S.P.J. (1992) A simple computer program with statistical tests for the analysis of enzyme kinetics. *BioTechniques*, **13**, 906-911.

Chisholm,S.W. (1992) Phytoplankton size. In Falkowski,P.G. and Woodhead,A.D. (eds), *Primary productivity and biogeochemical cycles in the sea*. Plenum Press, New York, pp. 213-237.

Chrost,R.J. and Overbeck,J. (1987) Kinetics of alkaline phosphatase activity and phosphorus availability for phytoplankton and bacterioplankton in Lake Pluβsee (North German Eutrophic Lake). *Microb. Ecol.*, **13**, 229-248.

Chrost,R.J. (1990) Microbial ectoenzymes in aquatic environments. In Overbeck,J., Chrost,R.J. (eds), *Aquatic Microbial Ecology: Biochemical and Molecular Approaches*. Springer-Verlag, New York, pp. 47-78.

Conley,D.J. and Malone,T.C. (1992) Annual cycle of dissolved silicate in Chesapeake Bay: implications for the production and fate of phytoplankton biomass. *Mar. Ecol. Prog. Ser.*, **81**, 121-128.

Conley,D.J. (2000) Biogeochemical nutrient cycles and nutrient management strategies. *Hydrobiologia*, **410**, 87-96.

Cotner,J.B., Ammerman,J.W., Peele,E.R. and Bentzen,E. (1997) Phosphorus-limited bacterioplankton growth in the Sargasso Sea. *Aquat. Microb. Ecol.,* **13**, 141-149.

Cotner,J.B. and Wetzel,R.G. (1992) Uptake of dissolved inorganic and organic phosphorus compounds by phytoplankton and bacterioplankton. *Limnol. Oceanogr.*, **37**, 232-243.

Cullen,J.J. (1999) Iron, nitrogen and phosphorus in the ocean. *Nature*, **402**, 372.

Currie,D.J., Bentzen,E. and Kalff,J. (1986) Does algal-bacterial phosphorus partitioning vary among lakes? A comparative study of orthophosphate uptake and alkaline phosphatase activity in freshwater. *Can. J. Fish. Aquat. Sci.,* **43**, 311-318.

Currie,D.J. and Kalff,J. (1984a) A comparison of the abilities of freshwater algae and bacteria to acquire and retain phosphorus. *Limnol. Oceanogr.,* **29**, 298-310.

Currie,D.J. and Kalff,J. (1984b) The relative importance of bacterioplankton and phytoplankton in phosphorus uptake in freshwater. *Limnol. Oceanogr.*, **29**, 311-321.

D'Elia,C.F., Sanders,J.G. and Boynton,W.R. (1986) Nutrient enrichment studies in a coastal plain estuary: phytoplankton growth in large-scale, continuous cultures. *Can. J. Fish. Aquat. Sci.*, **43**, 397-406.

Del Amo,Y. and Brzezinski,M.A. (1999) The chemical form of dissolved Si taken up by marine diatoms. *J. Phycol.*, **35**, 1162-1170.

Del Amo,Y., Le Pape,O., Treguer,P., Queguiner,B., Menesguen,A. and Aminot,A. (1997) Impacts of high-nitrate freshwater inputs on macrotidal ecosystems. I. Seasonal evolution of nutrient limitation for the diatom-dominated phytoplankton of the Bay of Brest (France). *Mar. Ecol. Prog. Ser.*, **161**, 213-224.

Diaz,F., Raimbault,P., Boudjellal,B., Garcia,N. and Moutin,T. (2001) Early spring phosphorus limitation of primary productivity in a NW Mediterranean coastal zone (Gulf of Lions). *Mar. Ecol. Prog. Ser.*, **211**, 51-62.

Dolan,J.R., Thingstad,T.F. and Rassoulzadegan,F. (1995) Phosphate transfer between microbial size-fractions in Villefranche Bay (N.W.Mediterranean sea), France in autumn 1992. *Ophelia*, **41**, 71-85.

Falkowski,P.G. (1997) Evolution of the nitrogen cycle and its influence on the biological CO_2 pump in the ocean. *Nature*, **387**, 272-275.

Fisher,T.R., Peele,E.R., Ammerman,J.W. and Harding,L.W. (1992) Nutrient limitation of phytoplankton in Chesapeake Bay. *Mar. Ecol. Prog. Ser.*, **82**, 51-63.

Fitzgerald,G.P. and Nelson,T.C. (1966) Extractive and enzymatic analyses for limiting or surplus phosphorus in algae. *J. Phycol.*, **2**, 32-37.

Froidefond,J.M., Lavender,S., Laborde,P., Herbland,A. and Lafon,V. (in press) SeaWiFS data interpretation in a coastal area in the Bay of Biscay. *Int. J. of Remote Sensing*

Fuhrman,J.A. and Azam,F. (1982) Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. *Mar. Biol.*, **66**, 109-120.

Gage,M.A. and Gorham,E. (1985) Alkaline phosphatase activity and cellular phosphorus as an index of the phosphorus status of phytoplankton in Minnesota lakes. *Fresh. Biol.*, **15**, 227-233.

Harrison,P.J., Hu,M.H., Yang,Y.P. and Lu X. (1990) Phosphate limitation in estuarine and coastal waters of China. *J. Exp. Mar. Biol. Ecol.*, **140**, 79-87.

Herbland,A., Delmas,D., Laborde,P., Sautour,B. and Artigas,F. (1998) Phytoplankton spring bloom of the Gironde plume waters in the Bay of Biscay: early phosphorus limitation and food-web consequences. *Oceanol. Acta*, **21**, 279-291.

Holm-Hansen,O., Lorenzen,C.J., Holmes,R.W. and Strickland,J.D.H. (1965) Fluorometric determination of chlorophyll. *J. Cons. Perm. Int. Exp. Mer*, **30**, 3-15.

Holmboe,N., Jensen,H.S. and Andersen,F.O. (1999) Nutrient addition bioassays as indicators of nutrient limitation of phytoplankton in an eutrophic estuary. *Mar. Ecol. Prog. Ser.*, **186**, 95-104.

Hoppe,H.G. (1983) Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. *Mar. Ecol. Prog. Ser.*, **11**, 299-308.

Wright,S.W., Jeffrey,S.W. and Mantoura,R.F.C. (1997) Evaluation of methods and solvents for pigment extraction. In Jeffrey,S.W., Mantoura,R.F.C. and Wright,S.W. (eds), *Phytoplankton pigments in oceanography: guidelines to modern methods*. UNESCO Publishing, Paris, pp. 261-282.

Karl,D.M., Letelier,R., Hebel,D., Tupas,L., Dore,J., Christian,J. and Winn,C. (1995) Ecosystem changes in the North Pacific subtropical gyre attributed to the 1991-92 El Nino. *Nature*, **373**, 230-234.

Karl,D.M. (2000) Phosphorus, the staff of life. *Nature*, **406**, 31-33.

Krom,M.D., Kress,N. and Brenner,S. (1991) Phosphorus limitation of primary productivity in the eastern Mediterranean Sea. *Limnol. Oceanogr.*, **36**, 424-432.

Kudela,R.M. and Cochlan,W.P. (2000) Nitrogen and carbon uptake kinetics and the influence of irradiance for a red tide bloom off southern California. *Aquat. Microb. Ecol.,* **21**, 31-47.

Labry,C., Herbland,A., Delmas,D., Laborde,P., Lazure,P., Froidefond,J.M., Jegou,A.M. and Sautour,B. (2001) Initiation of winter phytoplankton blooms within the Gironde plume waters in the Bay of Biscay. *Mar. Ecol. Prog. Ser.*, **212**, 117-130.

Lazure,P. and Jegou,A.M. (1998) 3D modelling of seasonal evolution of Loire and Gironde plumes on Biscay Bay continental shelf. *Oceanol. Acta*, **21**, 165-177.

Lean,D.R.S. and White,E. (1983) Chemical and radiotracer measurements of phosphorus uptake by lake plankton. *Can. J. Fish. Aquat. Sci.*, **40**, 147-155.

Lebo,M.E. (1990) Phosphate uptake along a coastal plain estuary. *Limnol. Oceanogr.*, **35**, 1279-1289.

Lee,S. and Fuhrman,J.A. (1987) Relationships between biovolume and biomass of naturally derived marine bacterioplankton. *Appl. Environ. Microbiol.*, **53**, 1298-1303.

Lignell,R., Kaitala,S. and Kuosa,H. (1992) Factors controlling phyto- and bacterioplankton in late spring on a salinity gradient in the northern Baltic. *Mar. Ecol. Prog. Ser.*, **84**, 121-131.

Malone,T.C., Crocker,L.H., Pike,S.E. and Wendler,B.W. (1988) Influences of river flow on the dynamics of phytoplankton production in a partially stratified estuary. *Mar. Ecol. Prog. Ser.*, **48**, 235-249.

Michaels,A.F. and others (1994) Seasonal patterns of ocean biogeochemistry at the U.S. JGOFS Bermuda Atlantic Time series Study site. *Deep. Sea Res. I*, **41**, 1013-1038.

Michel,P., Boutier,B. and Chiffoleau,J.F. (2000) Net fluxes of dissolved Arsenic, Cadmium, Copper, Zinc, Nitrogen and Phosphorus from the Gironde estuary (France): seasonal variations and trends. *Est. Coast. Shelf Sci.*, **51**, 451-462.

Morel,F.M.M., Hudson,R.J. and Price,N.M. (1991) Limitation of production by trace metal in the sea. *Limnol. Oceanogr.*, **36**, 1742-1755.

Nygaard,K. and Tobiesen,A. (1993) Bacterivory in algae: a survival strategy during nutrient limitation. *Limnol. Oceanogr.*, **38**, 273-279.

Oviatt,C., Doering,P., Nowicki,B., Reed,L., Cole,J. and Frithsen,J. (1995) An ecosystem level experiment on nutrient limitation in temperate coastal marine environments. *Mar. Ecol. Prog. Ser.*, **116**, 171-179.

Paasche,E. and Erga,S.R. (1988) Phosphorus and nitrogen limitation of phytoplankton in the inner Oslofjord (Norway). *Sarsia*, **73**, 229-243.

Pennock,J.R. and Sharp,J.H. (1994) Temporal alternation between light- and nutrient-limitation of phytoplankton production in a coastal plain estuary. *Mar. Ecol. Prog. Ser.*, **111**, 275-288.

Peters,R. and Lean,D. (1973) The characterization of soluble phosphorus released by limnetic zooplankton. *Limnol. Oceanogr.*, **18**, 270-279.

Porter,K.G. and Feig,Y.S. (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.*, **25**, 943-948.

Pujo-Pay,M. and Raimbault,P. (1994) Improvement of the wet-oxidation procedure for simultaneous determination of particulate organic nitrogen and phosphorus collected on filters. *Mar. Ecol. Prog. Ser.*, **105**, 203-207.

Riegman,R., Kuipers,B.R, Noordeloos,A.A.M. and Witte,H.J. (1993) Size-differential control of phytoplankton and the structure of plankton communities. *Neth. J. Sea Res*., **31**, 255-265.

Riemann,B. and Sondergaard,M. (1986) Regulation of bacterial secondary production in two eutrophic lakes and in experimental enclosures. *J. Plankton Res.*, **8**, 519-536.

RNO (Réseau National d'Observation de la qualité du milieu marin) (1989-1990) Surveillance du milieu marin. Travaux du RNO. Ministère de l'Environnement and IFREMER (eds). INSTAPRINT, Tours, pp. 7-13.

RNO (Réseau National d'Observation de la qualité du milieu marin) (1992-1993) Surveillance du milieu marin. Travaux du RNO. Ministère de l'Environnement and IFREMER (eds). INSTAPRINT, Tours, pp. 9-13.

Rothhaupt,K.O. and Gude H. (1992) The influence of spatial and temporal concentration gradients on phosphate partitioning between different size fractions of plankton: further evidence and possible causes. *Limnol. Oceanogr.*, **37**, 739-749.

Rudek,J., Paerl,H.W., Mallin,M.A. and Bates,P.W. (1991) Seasonal and hydrological control of phytoplankton nutrient limitation in the lower Neuse River Estuary, North Carolina. *Mar. Ecol. Prog. Ser.*, **75**, 133-142.

Ryther,J.H. and Dunstan,W.M. (1971) Nitrogen, phosphorus and eutrophication in the coastal marine environment. *Science*, **171**, 1008-1013.

Sautour,B., Artigas,L.F., Delmas,D., Herbland,A. and Laborde,P. (2000) Grazing impact of micro- and mesozooplankton during a spring situation in coastal waters off the Gironde estuary. *J. Plankton Res.*, **22**, 531-552.

Schindler,D.W. (1977) Evolution of phosphorus limitation in lakes. *Science*, **195**, 260-262.

Sheppard,C.W. (1962) Basic principles of the tracer method. Wiley, New York, pp. 1-29.

Simon,M. and Azam,F. (1989) Protein content and protein synthesis rates of planktonic marine bacteria. *Mar. Ecol. Prog. Ser.*, **51**, 201-213.

Strickland,J.D.H. and Parsons,T.R. (1972) A practical handbook of seawater analysis, 2nd edn *Bull. Fish. Res. Board Can.*, **167**

Suttle,C.A., Fuhrman,J.A. and Capone,D.G. (1990) Rapid ammonium cycling and concentration-dependent partitioning of ammonium and phosphate: implications for carbon transfer in planktonic communities. *Limnol. Oceanogr.*, **35**, 424-433.

Suttle,C.A. and Harrison,P.J. (1988) Ammonium and phosphate uptake kinetics of size-fractionated plankton from an oligotrophic freshwater lake. *J. Plankton Res.*, **10**, 133-149.

Suttle,C.A., Stockner,J.G., Shortreed,K.S. and Harrison,P.J. (1988) Time courses of size-fractionated phosphate uptake: are larger cells better competitors for pulses of phosphate than smaller cells? *Oecologia*, **74**, 571-576.

Taylor,W.D. and Lean,D.R.S. (1981) Radiotracer experiments on phosphorus uptake and release by limnetic microzooplankton. *Can. J. Fish. Aquat. Sci.*, **38**, 1316-1321.

Thingstad,T.F., Dolan,J.R. and Fuhrman,J.A. (1996a) Loss rate of an oligotrophic bacterial assemblage as measured by ^{3}H-thymidine and ^{32}PO$_4$: good agreement and near-balance with production. *Aquat. Microb. Ecol.*, **10**,29-36.

Thingstad,T.F., Riemann,B., Havskum,H. and Garde,K. (1996b) Incorporation rates and biomass content of C and P in phytoplankton and bacteria in the Bay of Aarhus (Denmark) June 1992. *J. Plankton Res.*, **18**, 97-121.

Thingstad,T.F., Skjoldal,E.F. and Bohme,R.A. (1993) Phosphorus cycling and algal-bacterial competition in Sandsfjord, western Norway. *Mar. Ecol. Prog. Ser.*, **99**, 239-259.

Thingstad,T.F., Zweifel,U.L. and Rassoulzadegan,F. (1998) P limitation of heterotrophic bacteria and phytoplankton in the northwest Mediterranean. *Limnol. Oceanogr.*, **43**, 88-94.

Toggweiler,J.R. (1999) An ultimate limiting nutrient. *Nature*, **400**, 511-512.

Tyrrell,T. (1999) The relative influences of nitrogen and phosphorus on oceanic primary production. *Nature*, **400**, 525-531.

Urabe,J., Gurung,T.B. and Yoshida,T. (1999) Effects of phosphorus supply on phagotrophy by the mixotrophic alga *Uroglena americana* (Chrysophyceae). *Aquat. Microb. Ecol.*, **18**, 77-83.

Vadstein,O., Olsen,Y. and Reinertsen,H. (1993) The role of planktonic bacteria in phosphorus cycling in lakes-sink and link. *Limnol. Oceanogr.*, **38**, 1539-1544.

Valderrama,J.C. (1981) The simultaneous analysis of total nitrogen and total phosphorus in natural waters. *Mar. Chem.*, **10**, 109-122.

Wu,J.F., Sunda,W., Boyle,E.A. and Karl,D.M. (2000) Phosphate depletion in the western North Atlantic Ocean. *Science*, **289**, 759-762.

Yin,K., Qian,P.Y., Chen,J.C., Hsieh,D.P.H. and Harrison,P.J. (2000) Dynamics of nutrients and phytoplankton biomass in the Pearl River estuary and adjacent waters of Hong Kong during summer: preliminary evidence for phosphorus and silicon limitation. *Mar. Ecol. Prog. Ser.*, **194**, 295-305.

Zohary,T. and Robarts,R.D. (1998) Experimental study of microbial P limitation in the eastern Mediterranean. *Limnol. Oceanogr.*, **43**, 387-395.

Fig. 1. Grid of stations at the different cruises BIOMET 2, BIOMET 3, PEGASE and PLAGIA.

Fig. 2. Mean contribution of chl *a* and primary production size fractions > 20 µm, 3-20 µm and < 3 µm in the mixed layer of "Gironde" and "Oceanic" waters in 1998 and 1999

Fig. 3. Mean particulate APA (> 0.2 µm) in the mixed layer of "Gironde" and "Oceanic" type waters in 1998 and 1999

Fig. 4. Mean phosphate turnover time (h) and mean contribution (%) of size fractions 0.2-1 µm and > 1 µm to particulate phosphate uptake for "Gironde" and "Oceanic" type waters. In March and June 1998, upper limits of T_n and ^{33}P partitioning among size fractions at the saturation phasis of the $^{33}PO_4^{3-}$ uptake kinetic are represented.

Fig. 5. Phosphate uptake rate (V_{po_4}) as a function of added phosphate concentration for two "Gironde" and one "Oceanic" type waters in late May 1999. Lines correspond to the fitted Michaelis and Menten equation for particulate (> 0.2 µm), > 1 µm and 0.2-1 µm size fractions.

Fig. 6. Time course distribution of ^{33}P (as % of added label) in particulate, > 1µm, 0.2-1 µm and dissolved fractions in bottles without (filled symbols) and with (open symbols) a phosphate cold chase added 24 h after the addition of $^{33}PO_4^{3-}$ (arrows). Experiments were realized for representative "Gironde" and "Oceanic" waters in late April and late May 1999.

Fig. 7. Evolution of chl *a* and potential primary production in control (open symbol) and PO_4 enriched sample (filled symbol) during bioassays conducted from late February to early October 1999 on "Gironde" waters (circle) and "Oceanic" waters (triangle).

Fig. 8. Evolution of chl *a* in control (open circle), PO_4 (p), NH_4 (n) and NH_4+PO_4 (n + p) enriched samples during bioassays conducted in July 1999 on two "Gironde" type waters, G1 (salinity of 33.9) and G2 (salinity of 34.4).

Fig. 9. Evolution of bacteria numbers (x 10^6 cells ml^{-1}) and bacterial production ($\mu gC\ l^{-1}\ d^{-1}$) in control (open symbol), PO_4 enriched sample (filled symbol) of whole water and predator free water during bioassays conducted in late February and in late May 1999 on "Gironde" waters (circle) and "Oceanic" waters (triangle).

Table I. Range of surface salinity, temperature and depth of the mixed layer during cruises in 1998 and in 1999. Mean ($\pm$ sd) of the depth of the mixed layer was calculated when the range of values was not too large. Values in parentheses are sample numbers.

Table II. Mean value ($\pm$ sd) in the mixed layer of nutrient concentrations (μM), $NO_3:PO_4$ and $NO_3:Si$ ratios, and chl *a* ($\mu g\ l^{-1}$) during cruises in 1998 and in 1999. *n* represents sample numbers. Mean phosphate concentrations were calculated, assimilating undetectable values (< 20 nM) to zero. When phosphate was undetectable, mean $NO_3:PO_4$ ratio were calculated, assuming a mean phosphate concentrations of 0.01 μM. They were indeterminate when both nitrate and phosphate concentrations were low.

Table III. Mean of integrated chl *a*, primary production (ΔC), assimilation number (AN) and turnover time (Tnphyto) of the phytoplankton community assuming a mean C:chl *a* of 50 in 1998. *n* represents sample numbers.

Table IV. Mean concentration (μM; $\pm$ sd) in the mixed layer and composition (atomic ratios) of the particulate organic matter in terms of carbon (POC), nitrogen (PON) and phosphorus (POP) during cruises in 1998 and in 1999. *n* represents sample numbers.

Table V. Mean ($\pm$ sd) release rates (% h^{-1}) of ^{33}P during cold chase experiments achieved in late April and late May 1999 in particulate, $> 1\mu$m, 0.2-1 μm and dissolved fractions and mean kinetic parameters for phosphate uptake determined in late May 1999 for representative "Gironde" and "Oceanic" waters. Negative value indicates a decrease of ^{33}P in a fraction whereas positive value represents an increase of label.

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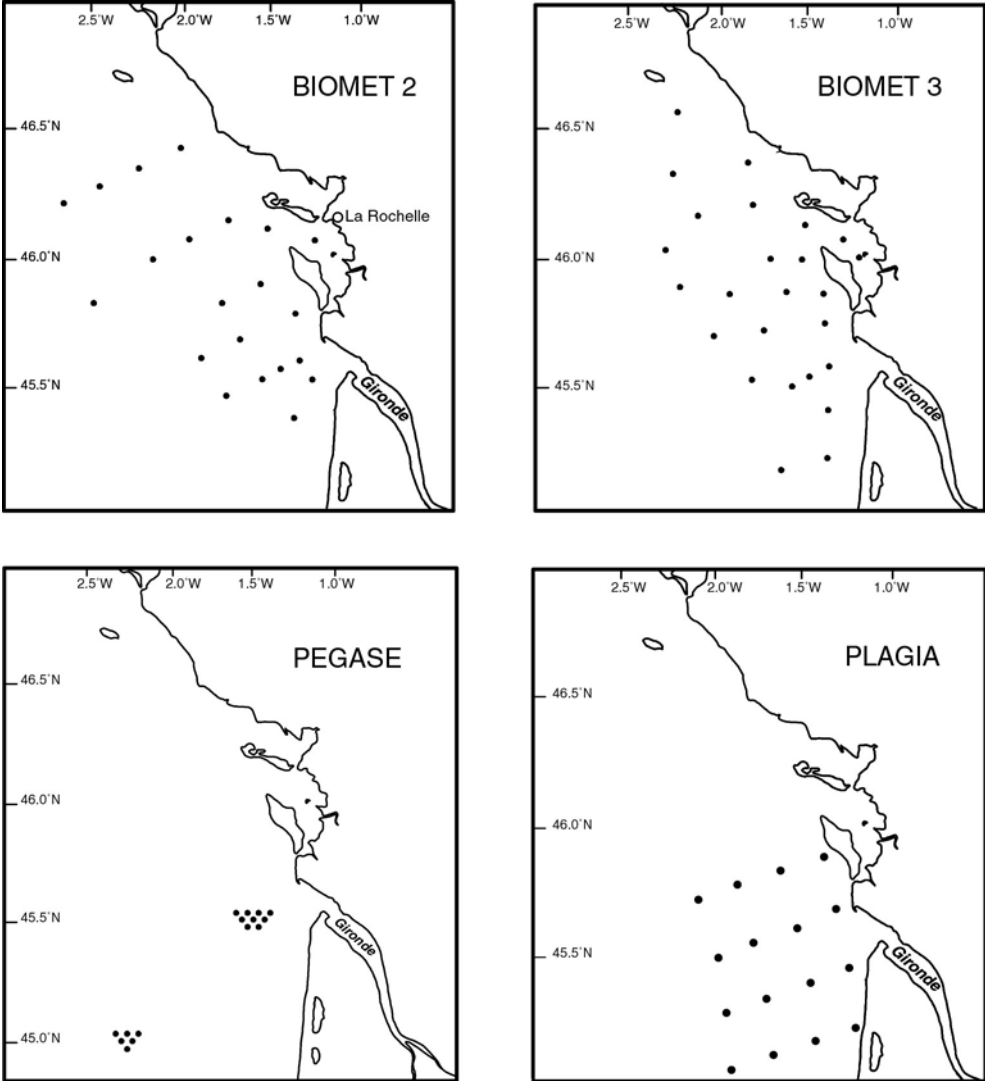
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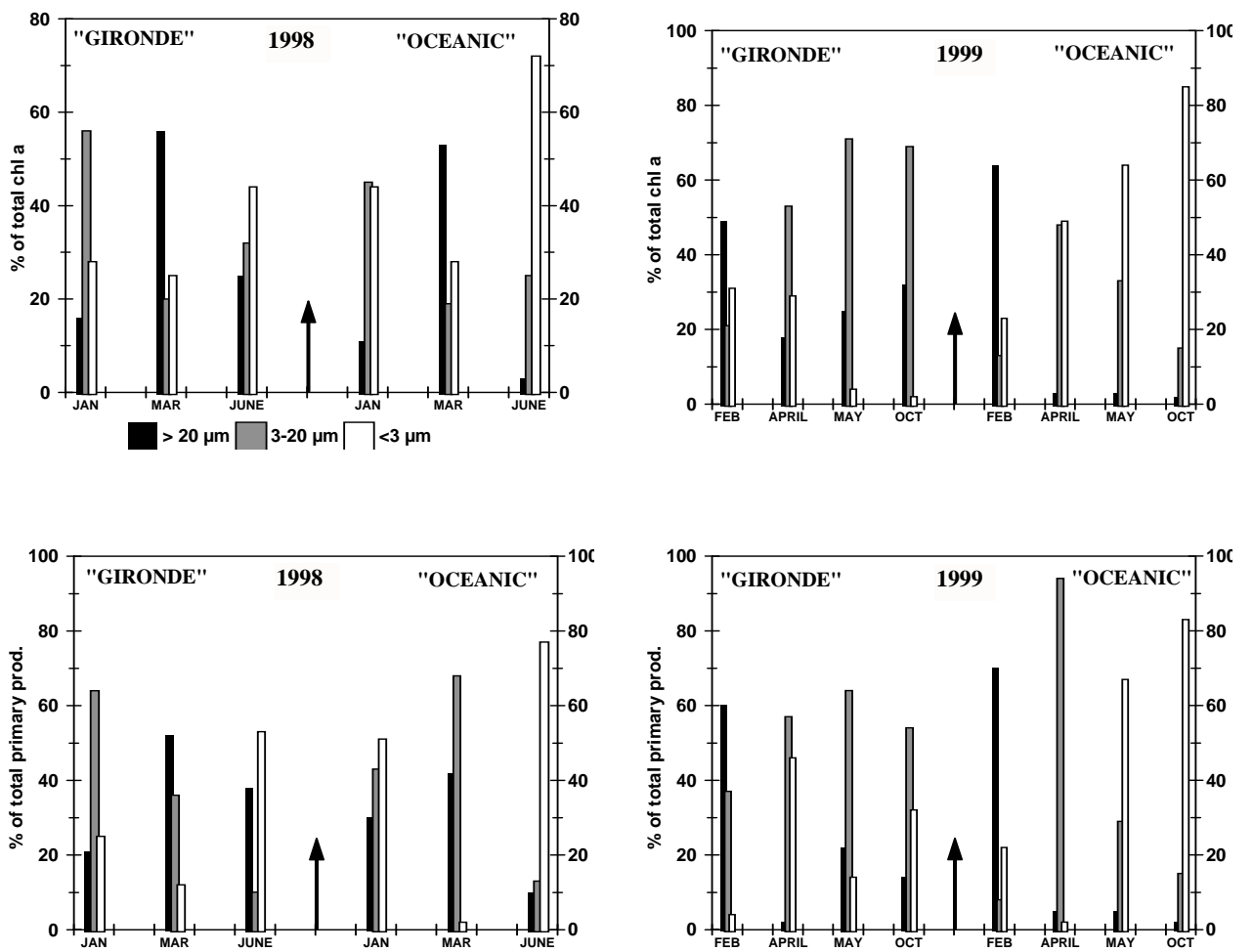
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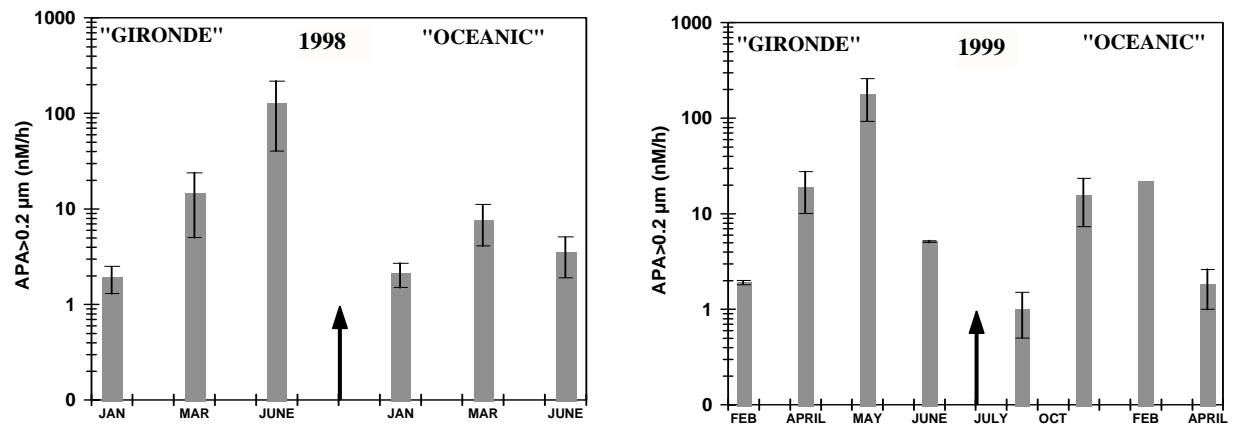
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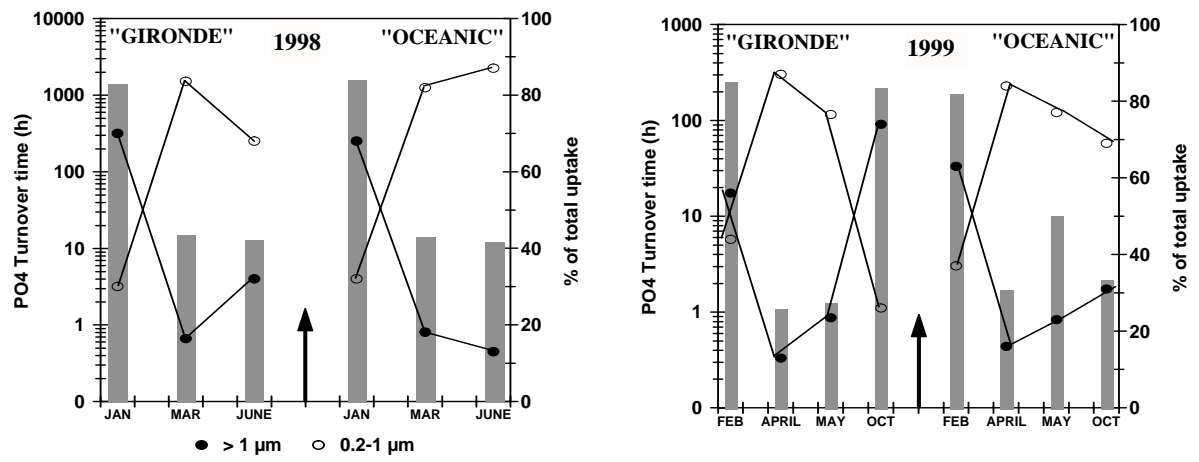
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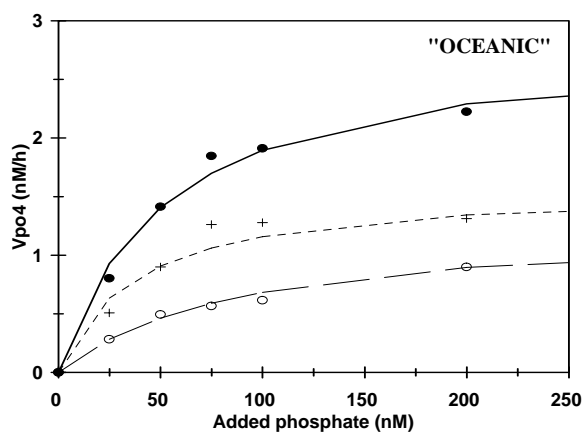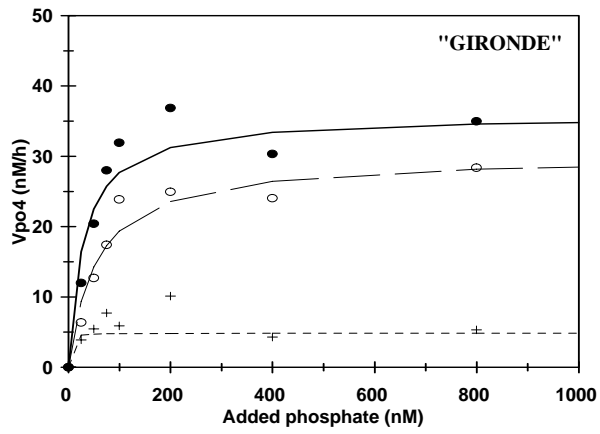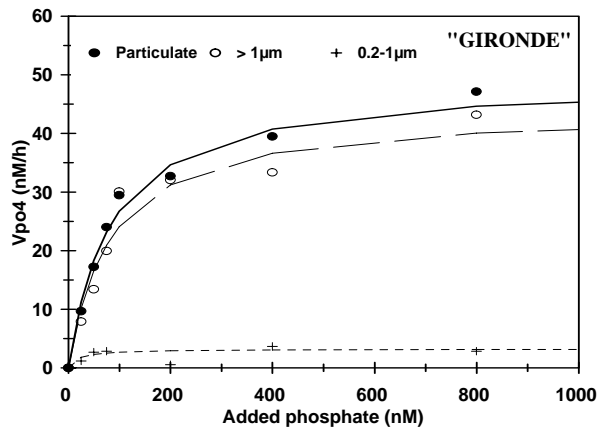
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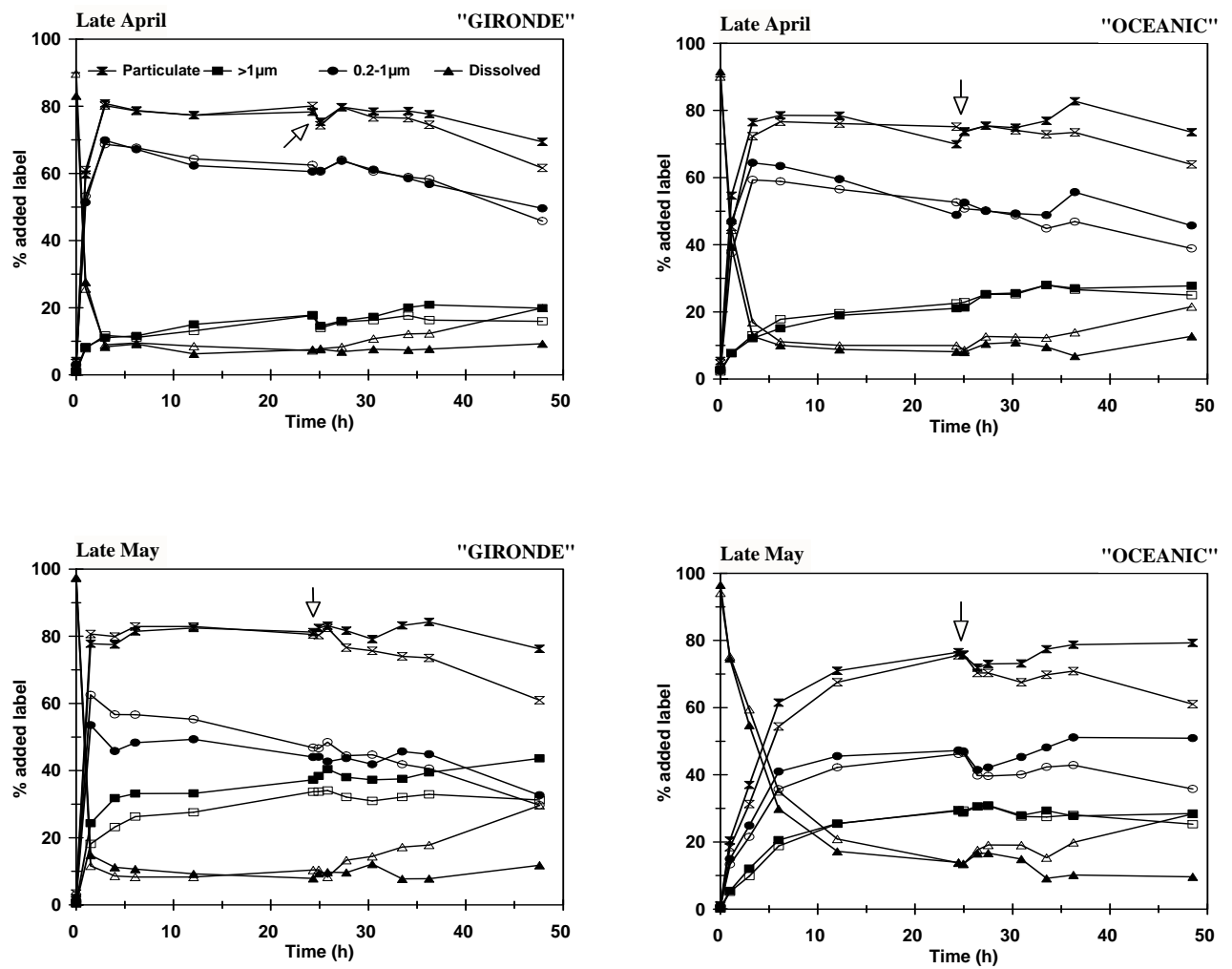
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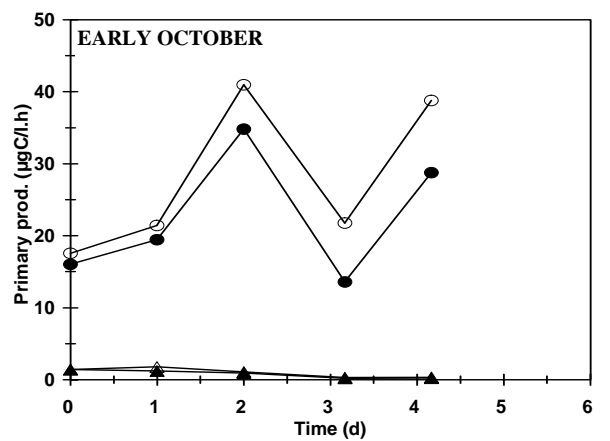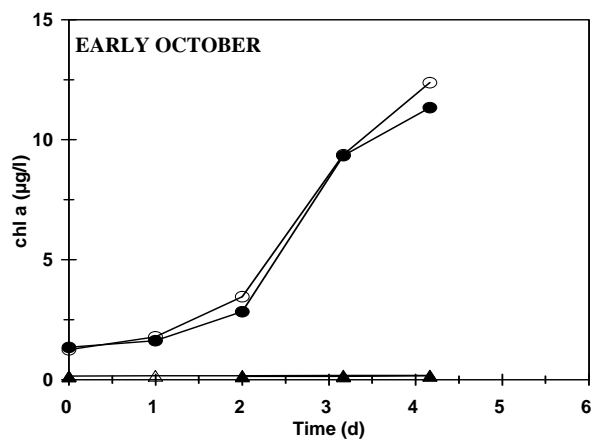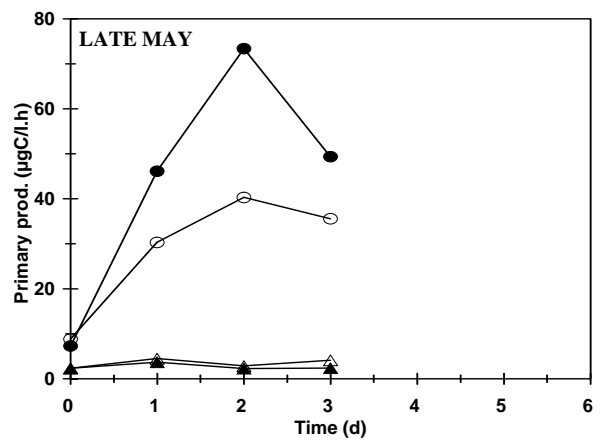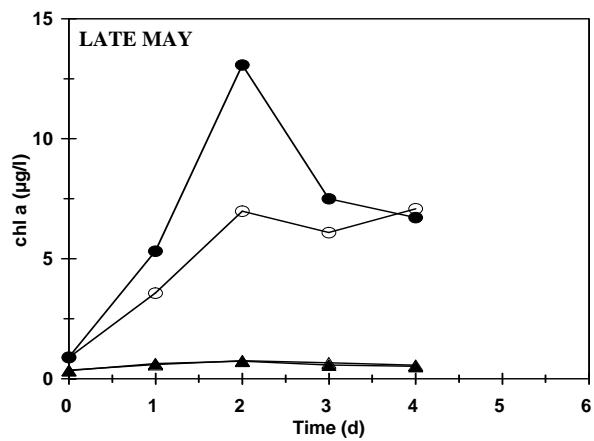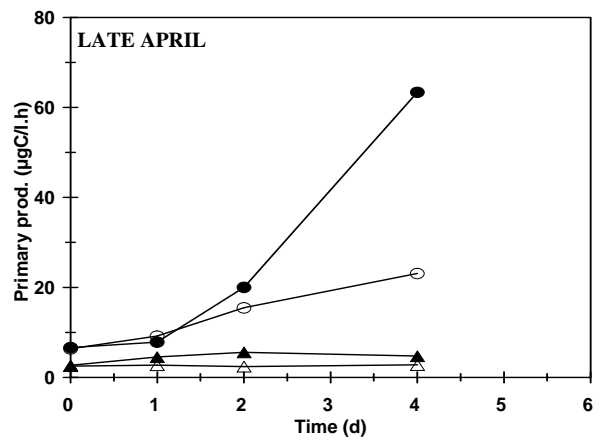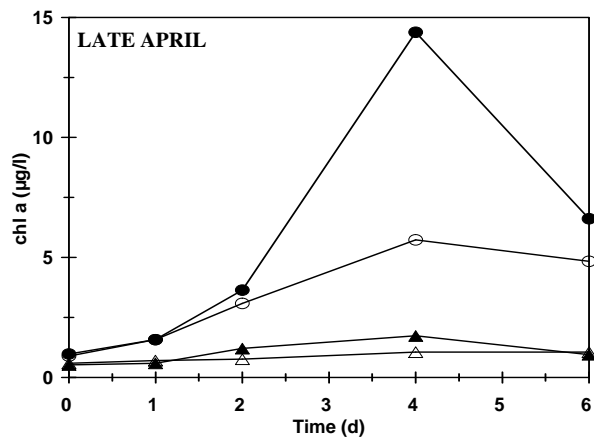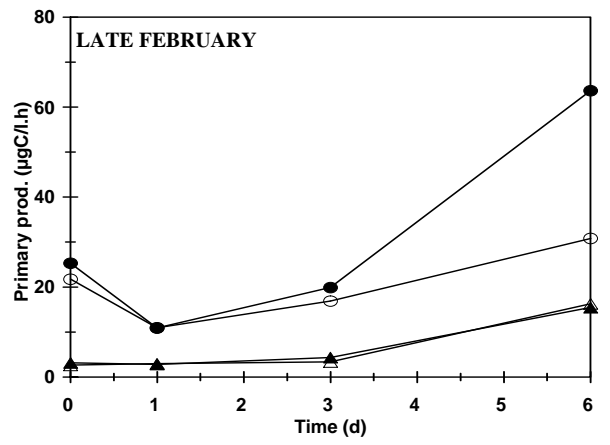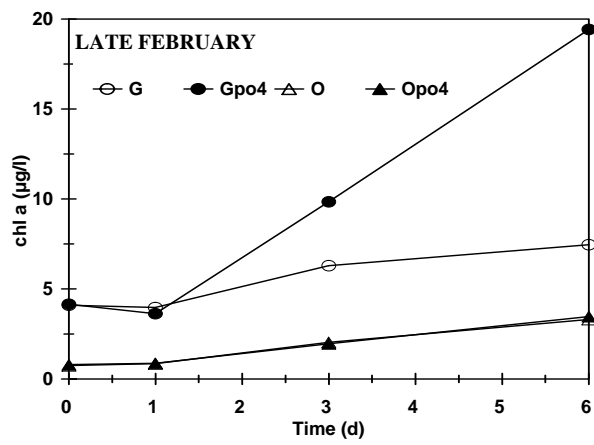
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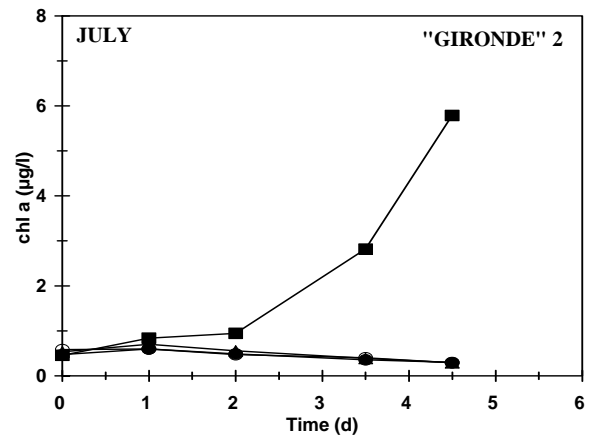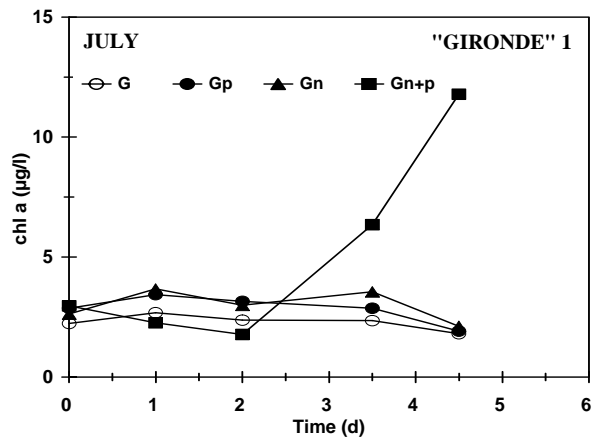
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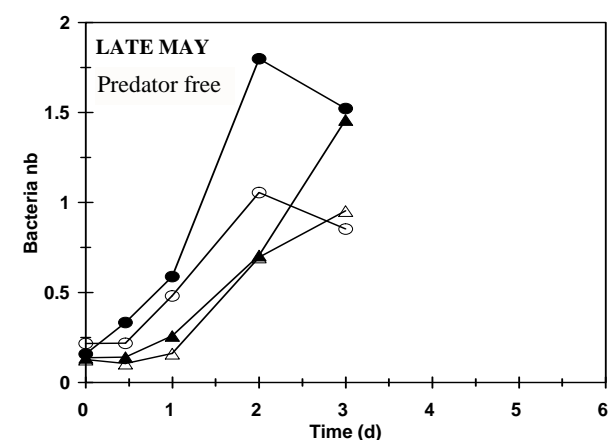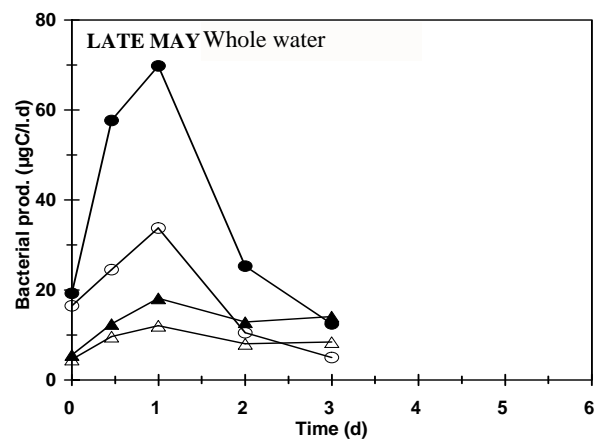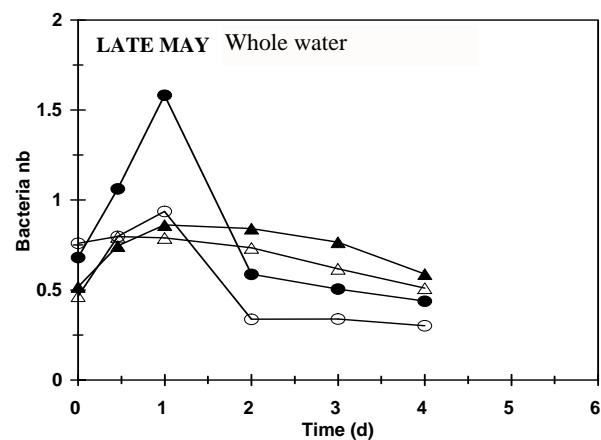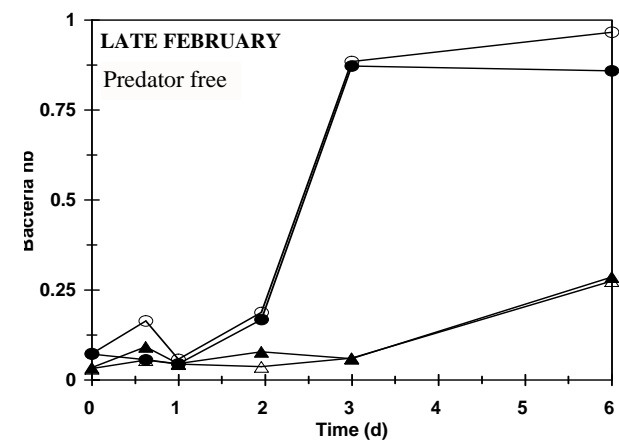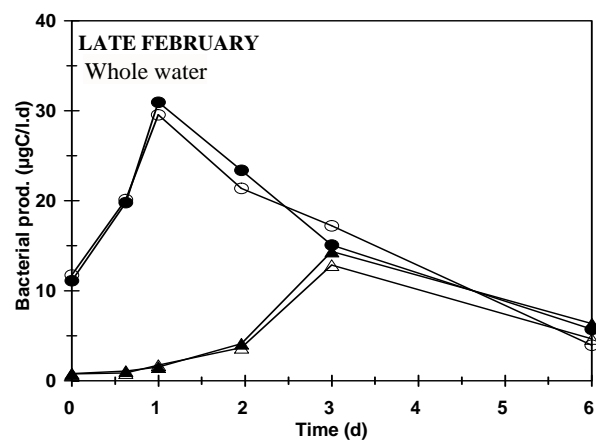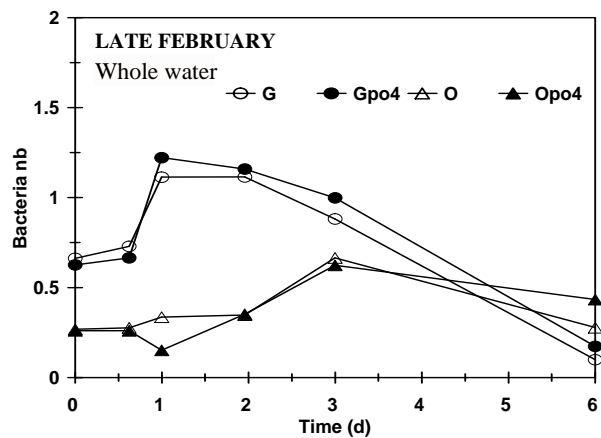
			Salinity	**Temperature (°C)**	**Mixed layer depth (m)**
1998	**January**	**Turbid plume**	29.9-32.4	11.0-12.0	2-20
	(BIOMET 2)	**"Gironde"**	33.1-34.3	11.3-12.4	10-30
		"Oceanic"	34.7-35.6	12.2-13.6	16-100
	Early March	**Turbid plume**	31.2-32.9	10.3-10.9	10 ± 7 (3)
	(BIOMET 3)	**"Gironde"**	33.2-34.4	10.2-11.5	10 ± 5 (13)
		"Oceanic"	34.5-35.7	11.0-12.9	3-74
	June	**"Gironde"**	32.8-34.1	16.4-18.4	13 ± 3 (9)
	(PEGASE)	**"Oceanic"**	35.3-35.5	16.8-17.5	23 ± 3 (6)
1999	**Late February**	**"Gironde"**	32.0-34.5	9.4-10.5	1-28
	(PLAGIA 1)	**"Oceanic"**	34.5-35.4	10.2-11.6	24-97
	Late April	**"Gironde"**	33.2-34.4	12.0-12.8	2-31
	(PLAGIA 2)	**"Oceanic"**	34.8-35.0	12.1-12.9	38 ± 8 (2)
	Late May	**"Gironde"**	28.6-33.8	15.0-17.8	7 ± 4 (18)
	(PLAGIA 3)	**"Oceanic"**	34.7	15.5-16.5	11 ± 6 (2)
	Late June	**"Gironde"**	32.8-34.4	14.5-19.6	11 ± 4 (14)
	(PLAGIA 4)				
	July	**"Gironde"**	33.3-34.4	17.6-22.0	10 ± 4 (18)
	(PLAGIA 5)				
	Early October	**"Gironde"**	33.3-34.3	17.5-18.8	10 ± 2 (5)
	(PLAGIA 6)	**"Oceanic"**	34.5-35.0	18.4-19.4	36 ± 7 (13)

Table I.

		PO$_4$	NO$_3$	Si(OH)$_4$	NO$_3$:PO$_4$	NO$_3$:Si	chl *a*	*n*
1998								
Turbid plume	**January**	**0.586** ± 0.086	**26.2** ± 3.7	**17.9** ± 3.3	**47** ± 6	**1.4** ± 0.1	**0.38** ± 0.05	*4 - 5*
	Early March	**0.215** ± 0.064	**13.1** ± 1.5	**8.1** ± 2.2	**67** ± 25	**1.7** ± 0.6	**2.17** ± 0.54	*4*
"Gironde"	**January**	**0.320** ± 0.087	**9.9** ± 3.6	**7.5** ± 2.0	**31** ± 9	**1.3** ± 0.2	**0.56** ± 0.14	*17 - 19*
	Early March	**0.011** ± 0.017	**4.9** ± 2.6	**1.9** ± 1.0	**> 100**	**2.4** ± 1.1	**1.76** ± 0.99	*20 - 22*
	June	**0.007** ± 0.011	**0.8** ± 0.5	**2.3** ± 0.6	**-**	**0.4** ± 0.4	**2.17** ± 0.46	*21 - 23*
"Oceanic"	**January**	**0.213** ± 0.042	**2.8** ± 0.5	**2.7** ± 1.0	**14** ± 3	**1.2** ± 0.3	**0.36** ± 0.11	*27 - 46*
	Early March	**0.034** ± 0.027	**0.9** ± 1.2	**1.0** ± 0.7	**> 100**	**0.8** ± 0.8	**0.89** ± 0.24	*40*
	June	**0.014** ± 0.014	**0.3** ± 0.3	**1.6** ± 0.5	**-**	**0.3** ± 0.3	**0.17** ± 0.05	*18*
1999								
"Gironde"	**Late Feb**	**0.289** ± 0.236	**9.8** ± 4.1	**6.2** ± 2.2	**51** ± 33	**1.6** ± 0.4	**1.18** ± 0.86	*19 - 21*
	Late April	**0.009** ± 0.022	**4.3** ± 2.1	**1.9** ± 1.2	**> 100**	**3.5** ± 3.1	**0.62** ± 0.29	*45*
	Late May	**0.018** ± 0.029	**6.6** ± 5.9	**3.2** ± 4.6	**> 100**	**16** ± 30	**1.96** ± 1.17	*18 - 22*
	Late June	**0.003** ± 0.005	**1.3** ± 1.6	**3.3** ± 1.7	**-**	**0.3** ± 0.3	**1.18** ± 0.51	*10 - 26*
	July	**0.006** ± 0.015	**0.5** ± 0.9	**3.6** ± 2.1	**-**	**0.1** ± 0.2	**0.93** ± 0.58	*32*
	Early Oct	**0.284** ± 0.062	**4.9** ± 2.5	**6.6** ± 1.7	**17** ± 7	**0.7** ± 0.2	**0.74** ± 0.26	*12*
"Oceanic"	**Late Feb**	**0.127** ± 0.041	**3.4** ± 1.0	**2.6** ± 0.9	**30** ± 16	**1.5** ± 0.9	**1.13** ± 0.79	*25 - 27*
	Late April	**0.000** ± 0.000	**1.8** ± 0.6	**0.8** ± 0.3	**> 100**	**2.7** ± 1.4	**0.34** ± 0.17	*4*
	Late May	**0.000** ± 0.000	**1.0** ± 0.3	**2.0** ± 1.0	**> 100**	**0.6** ± 0.4	**0.42** ± 0.08	*2*
	Early Oct	**0.022** ± 0.031	**0.2** ± 0.2	**1.6** ± 0.7	**-**	**0.1** ± 0.1	**0.36** ± 0.25	*43*

Table II.

		chl*a*	ΔC	AN	Tnphyto	*n*
		mg m^{-2}	mgC m^{-2} d^{-1}	mgC mgChl*a*$^{-1}$ d^{-1}	d	
1998						
January	**"Gironde"**	**9.1** ± 2.9	**28** ± 18	**2.9** ± 1.4	**20** ± 8	*3*
	"Oceanic"	**9.1** ± 0.2	**15** ± 3	**1.6** ± 0.4	**31** ± 8	*2*
Early March	**"Gironde"**	**29.5** ± 14.0	**447** ± 87	**16.9** ± 5.7	**3.2** ± 1.0	*4*
	"Oceanic"	**37.7** ± 10.5	**716** ± 664	**17.2** ± 12.8	**4.0** ± 3.0	*2*
June	**"Gironde"**	**53.9** ± 15.0	**1329** ± 446	**24.4** ± 1.6	**2.1** ± 0.1	*3*
	"Oceanic"	**10.8** ± 0.6	**990** ± 77	**92.1** ± 11.9	**0.5** ± 0.1	*2*

Table III.

		POC	PON	POP	C:N	N:P	C:P	*n*
1998								
Turbid plume	**January**	**26.6** ± 9.9	**3.0** ± 1.0	**0.097** ± 0.047	**8.9** ± 0.7	**26.7** ± 14.7	**227** ± 111	*4 - 5*
	Early March	**31.4** ± 7.1	**2.9** ± 0.3	**0.177** ± 0.066	**10.9** ± 1.9	**17.6** ± 6.4	**184** ± 89	*3 - 4*
"Gironde"	**January**	**17.3** ± 4.8	**1.8** ± 0.6	**0.086** ± 0.056	**9.4** ± 0.7	**26.7** ± 14.1	**250** ± 130	*18 - 20*
	Early March	**18.5** ± 6.9	**2.0** ± 0.7	**0.115** ± 0.071	**9.1** ± 1.1	**23.0** ± 12.2	**209** ± 108	*21 - 22*
	June	**24.7** ± 4.6	**3.4** ± 0.6	**0.184** ± 0.053	**7.2** ± 0.6	**19.6** ± 4.6	**142** ± 34	*10*
"Oceanic"	**January**	**12.9** ± 2.8	**1.4** ± 0.4	**0.068** ± 0.043	**9.2** ± 0.6	**27.6** ± 15.1	**251** ± 135	*46 - 48*
	Early March	**13.4** ± 3.8	**1.2** ± 0.3	**0.075** ± 0.027	**11.1** ± 1.3	**17.8** ± 6.9	**196** ± 65	*39 - 40*
	June	**14.9** ± 2.1	**1.4** ± 0.4	**0.036** ± 0.005	**9.1** ± 1.4	**39.5** ± 12.3	**422** ± 77	*6*
1999								
"Gironde"	**Late Feb**	**19.9** ± 9.4	**3.1** ± 1.7	**0.122** ± 0.021	**6.7** ± 0.8	**24.9** ± 11.9	**162** ± 66	*3*
	Late April	**24.4** ± 4.9	**3.3** ± 0.8	**0.107** ± 0.039	**7.6** ± 0.8	**31.9** ± 6.3	**241** ± 55	*4*
	Late May	**44.5** ± 18.0	**6.0** ± 2.4	**0.169** ± 0.051	**7.3** ± 0.5	**34.8** ± 3.9	**256** ± 39	*3*
	Early Oct	**21.1** ± 2.5	**3.2** ± 0.1	**0.130** ± 0.003	**6.6** ± 0.9	**24.4** ± 1.1	**162** ± 15	*2*
"Oceanic"	**Late Feb**	**7.7** ± 2.6	**1.0** ± 0.4	**0.044** ± 0.031	**7.7** ± 0.7	**27.1** ± 9.5	**210** ± 75	*5*
	Late April	**17.7** ± 2.8	**2.7** ± 0.4	**0.066** ± 0.014	**6.7** ± 0.5	**41.5** ± 10.6	**277** ± 84	*4*
	Late May	**24.4**	**2.7**	**0.055**	**9.2**	**46.3**	**425**	*1*
	Early Oct	**11.0** ± 3.2	**1.3** ± 0.4	**0.027** ± 0.015	**8.6** ± 0.9	**53.6** ± 17.5	**473** ± 206	*4*

Table IV.

			Particulate	0.2-1μm	> 1μm	Dissolved
Cold Chase exp	**Late April**					
	"Gironde"		**- 0.66** ± 0.16	**- 0.68** ± 0.12	**+ 0.01** ± 0.07	**+ 0.53** ± 0.04
	"Oceanic"		**- 0.43** ± 0.09	**- 0.53** ± 0.06	**+ 0.10** ±0.09	**+ 0.46** ± 0.08
	Late May					
	"Gironde"		**- 0.84** ± 0.09	**- 0.74** ± 0.07	**- 0.10** ± 0.05	**+ 0.88** ± 0.06
	"Oceanic"		**- 0.51** ± 0.13	**- 0.31** ± 0.13	**- 0.20** ± 0.06	**+ 0.51** ± 0.12
Kinetics	**Late May**					
	"Gironde"	K_t+S_n	**85** ± 9	**20** ± 13	**83** ± 17	**-**
		V_m	**49** ± 1	**3.2** ± 0.4	**44** ± 2	**-**
	"Gironde"	K_t+S_n	**30** ± 8	**2** ± 5	**56** ± 10	**-**
		V_m	**36** ± 2	**4.9** ± 0.3	**30** ± 1	**-**
	"Oceanic"	K_t+S_n	**53**	**38**	**90**	**-**
		V_m	**2.9**	**1.6**	**1.3**	**-**

Table V.