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Phytoplankton seasonal dynamics in a Mediterranean coastal lagoon: emphasis on the picoeukaryote community

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Abstract: The dynamics of the phytoplankton community were investigated in a marine coastal lagoon (Thau, NW Mediterranean) from February 1999 to January 2000. Dilution experiments, chlorophyll a (Chl a) size-fractionation and primary production measurements were conducted monthly. Maximum growth and microzooplankton grazing rates were estimated from Chl a biomass fractions to separate pico- from nano- and microphytoplankton and by flow cytometry to distinguish between picoeukaryotes and picocyanobacteria. In spring, the phytoplankton community was dominated by Chaetoceros sp. and Skeletonema costatum, which represented most of biomass (B) and primary production (P). Nano- and microphytoplankton growth was controlled by nutrient availability and exceeded losses due to microzooplankton grazing (g). Picoeukaryote and cyanobacteria growth was positively correlated with water temperature and/or irradiance, reaching maximum values in the summer (2.38 and 1.44 day–1 for picoeukaryotes and cyanobacteria, respectively). Picophytoplankton accounted for 57% of the biomass-specific primary productivity (P/B). Picophytoplankton was strongly controlled by protist grazers (g = 0.09–1.66 day–1 for picoeukaryotes, g = 0.25–1.17 day–1 for cyanobacteria), and microzooplankton consumption removed 71% of the daily picoplanktonic growth. Picoeukaryotes, which numerically dominate the picoplankton community, are an important source of organic carbon for the protistan community and contribute to the carbon flow to higher trophic levels.

Keywords: phytoplankton, Picoeukaryote, NW Mediterranean, coastal lagoon

INTRODUCTION

Picophytoplankton (class size: 0.2 to 2 µm) comprises photosynthetic prokaryotes, represented primarily by the genera *Synechococcus* and *Prochlorococcus* which are major contributors of biomass and primary productivity in oligotrophic oceanic ecosystems (Partensky et al., 1999), and picoeukaryotic algae which are generally less abundant except in coastal waters where their relative importance increases (Johnson and Sieburth, 1982; Shapiro and Guillard, 1986). Among the photosynthetic picoeukaryote community, prasinophytes are probably the most widespread and abundant organisms (Shapiro and Guillard, 1986; Biegala et al., 2003; Not et al., 2004). Although outnumbered by photosynthetic prokaryotes, picoeukaryotes have been recognized as contributing significantly to biomass and primary production in the oceans (Li, 1994). Because of their larger size than *Prochlorococcus* and *Synechococcus*, picoeukaryotes may dominate in terms of carbon biomass, and may be the most important picoautotrophic carbon source for microzooplankton in coastal marine systems (Reckerman and Veldhuis, 1997; Brown et al., 1999). Furthermore, picophytoplankton appear to be an essential component of microbial food webs and carbon flow, particularly in the summer in coastal environments (Kuosa, 1991; Malone et al., 1991; Worden et al., 2004). The influence of irradiance and water temperature on the seasonal distribution of the abundance and biomass of picocyanobacteria (Kuosa, 1991; Agawin et al., 1998) and picoeukaryotes (Vaquer et al., 1996) has been established. Nevertheless, the dominance of autotrophic picoplankton may also result from size-differential responses of phytoplankton to diffusion limitation of nutrient transport and nitrogen source supply (Chisholm 1992, and references therein). Due to its small cell size, picophytoplankton has a competitive advantage to acquire nutrients in resource-limited environments (Raven, 1998), such as oligotrophic systems in which primary production is generally supported by regenerated nitrogen (ammonium recycled from bacteria and zooplankton). In contrast, under high nutrient levels, primary production is supported by new inputs of nitrogen (nitrate from river runoff, upwelling) and is dominated by large phytoplankton (mainly diatoms) whose growth is released from diffusion limitation (Chisholm, 1992). However, picophytoplankton are tightly controlled by protistan grazers (Kuosa, 1991; Reckermann and Veldhuis, 1997), which can achieve high growth rates close to those of their picoprey, while potential predators of larger phytoplankton (i.e. mesozooplankton grazers) exhibit slow reproduction rates (Riegman et al., 1993). Hence, in order to estimate the role of autotrophic picoeukaryotes in carbon cycling, it is necessary to investigate their growth capability as well as their losses to microzooplankton grazing in their natural environment.

The smallest photosynthetic picoeukaryote described to date, the prasinophyte *Ostreococcus tauri* (Chrétiennot-Dinet et al., 1995), was discovered in the marine Mediterranean Thau lagoon (Courties

et al., 1994) where it numerically dominates the phytoplankton community and represents the main component of picophytoplankton throughout the year (Vaquer et al., 1996). Picocyanobacteria were indeed occasionally observed, but their abundances were always lower than those of picoeukaryotes (Chrétiennot-Dinet et al., 1995; Dupuy et al., 2000). Maximum values for biomass contribution and abundance of *Ostreococcus tauri* were observed during the warm season (Chrétiennot-Dinet et al., 1995; Vaquer et al., 1996), when ammonium benthic fluxes increase (Mazouni et al., 1996). The Thau lagoon phytoplankton community is composed of diatoms, cryptophyceae, dinophyceae and small phytoflagellates (Vaquer et al., 1996) supporting some of the highest growth rates of farmed oysters in France (Gangnery et al., 2003). In contrast, picophytoplankton escapes grazing by filter-feeding bivalves because of their small size (Dupuy et al., 2000). This latter study, which was carried out in August 1998, also brought to light the role of the heterotrophic protistan community in top-down control of picophytoplankton in the Thau lagoon.

In the present paper, we investigate the dynamics of the phytoplankton community throughout an annual cycle in the Thau lagoon. The aim of this study was to identify factors implicated in the seasonal control of picophytoplankton dynamics, as compared to larger size phytoplankton, focusing on bottom-up as well as top-down processes. Accordingly, an annual sampling program, coupled with *in situ* carbon assimilation and dilution experiments, was carried out: (1) to describe seasonal variations in the abundances of picoeukaryotes and cyanobacteria, (2) to determine the relative contribution of picophytoplankton to chlorophyll *a* biomass and primary production by means of size-fractionation (2 µm), and (3) to estimate the ambient and maximum growth rates of phytoplankton and its mortality rates due to microzooplankton grazing.

METHOD

Study site and sampling procedures

The Thau lagoon is a shallow marine lagoon located on the French Mediterranean coast (43°24'N-3°36'E) covering 75 km^2 (Fig. 1). It has a mean depth of 4 m with a maximum depth of 10 m. The lagoon is connected to the sea by 3 narrow channels. Three shellfish farming zones are located along the northwestern shore. The sampling station ZA (8.5 m depth) was located within a wide corridor of the northeast oyster farming area (see Souchu et al., 2001, for precise location). The presence of shellfish results in a decrease in zooplankton biomass (Lam-Höai et al., 1997) and phytoplankton (Souchu et al., 2001), with deficits of about 30% and 40%, respectively. At the sampling station, the proportion of zooplankton and phytoplankton biomass is weakly affected in comparison to the rest of the lagoon. In addition, oysters being excluded from dilution experiments, we assumed that the estimates of phytoplankton growth and microzooplankton grazing rates were

representative of the majority of the lagoon (4/5 of the total surface). Dilution experiments were carried out monthly from February 1999 to January 2000. During the seasonal cycle, August was not sampled because of technical problems. Water samples were collected at 8:30 am inside oyster farming zones (Fig. 1) by immersing two 20 L polycarbonate (PC) jars to a depth of 0.1 m and were immediately brought to the shore laboratory.

Experimental procedures

At the shore laboratory, collected water was passed through a 1000 µm mesh to eliminate larger debris without removing large diatoms (Dupuy et al., 2000), and was then transferred to a 60 L polyethylene carboy that was tested for non-toxicity (Collos et al., in press). Serial dilution experiments were carried out according to Landry and Hassett (1982). Part of the water was gravity filtered through in-line 0.2 µm Suporcap cartridges (Pall-Gelman) that had been previously rinsed with 1 L of deionized water and 1 L of seawater. Different fractions of whole water were mixed with filtered water to obtain five serial dilutions (0.15, 0.25, 0.45, 0.75 and 1.0 unfiltered water) in duplicate 1L PC bottles. Each bottle received a nutrient enrichment based on K medium (Keller et al., 1987) resulting in a final nitrate concentration of 10 µM, which had been previously verified to be sufficient to satisfy phytoplankton nitrogen demands over 24 h (Fouilland et al., 2002). All other nutrients contained in K medium were added in stoichiometric proportions to the original K composition. Two control bottles were filled with whole seawater without added nutrients. Within 2 hours of the sample collection, all bottles were incubated *in situ* for 24 h at the sampling site near the oyster pen. Initial samples (t_0) were taken in triplicate prior to dilution for chlorophyll *a* and cell abundance determination, whereas initial concentrations in the diluted bottles were calculated.

Physical and chemical variables

Surface photosynthetic available radiation (PAR) was continuously measured over 24 h with a LI-COR (Model LI-190SA) quantum sensor and was recorded by a LI-1000 data logger. Around noon, underwater irradiance was measured every 50 cm with a LI-COR (Model 193SA) spherical quantum sensor. The visible light extinction coefficient was calculated from linear regression of the logarithm of irradiance versus depth. Water temperature and salinity were recorded using a WTW LF 196 conductimeter.

To determine ammonium (NH_4^+) concentrations, samples (50 mL) were immediately fixed with reagents and measured in the laboratory using the method of Koroleff (1976). For the other nutrients, samples (75 mL) were frozen in Pyrex flasks after filtration through precombusted (450°C for 6 h) Whatman glass-fiber filters (GF/F), except for reactive silicate samples, which were stored in polyethylene bottles at 4°C (Souchu et al., 1998). Nitrate (NO_3^-), nitrite (NO_2^-), soluble

reactive phosphorus (SRP), and reactive silicate (Si) were measured with a segmented flow analyser (Tréguer and Le Corre, 1975). Detection limits were 0.05, 0.005, 0.03 and 0.1 µM for NO_3^-, NO_2^-, SRP and Si, respectively.

Pigment determination

For chlorophyll *a* measurements, seawater aliquots were filtered (40 mL) under a vacuum <10 cm Hg through Whatman GF/F filters (25 mm diameter), which were then stored in Corning glass tubes at –20°C. Filters were ground in 90% acetone and extracted for 24 h in the dark at 4°C. Chl *a* concentration was also determined after size-fractionation (100 mL) through Nuclepore membranes (2 µm pore size) to determine the contribution of picophytoplankton to total phytoplankton biomass. The pigment content (µg Chl *a* L^{-1}) was measured spectrofluorimetrically (Perkin-Elmer LS50 b) and calculated according to Neveux and Lantoine (1993).

C assimilation

Phytoplankton primary production was estimated using the standard ^{14}C technique (Steemann-Nielsen, 1952). For each sample, carbon fixation (µg C L^{-1} h^{-1}) was measured in 125 mL PC light bottles. The added bicarbonate activity was 148 to 370 kBq/bottle (Amersham specific activity 1.95 GBq/mmole). *In situ* incubations lasted 2 hours on average, and were stopped by adding formalin to the bottles (1% final concentration) according to Riemann and Jensen (1991). Subsamples of 50 mL raw water and prefiltered water (on 2 µm Nuclepore membranes) from each bottle were gently filtered through Whatman GF/F glass fiber (25 mm). Nuclepore membranes and glass filters were air dried and acidified with 100 µL 1N HCl, placed in 4 mL of scintillation cocktail (Packard Ultima Gold MV), and assayed with a Packard Tricarb 2100TR liquid scintillation counter. The biomass-specific primary productivity (P/B, µg C (µg Chl *a*)$^{-1}$ h^{-1}) was calculated as the carbon fixation (P) per unit of chlorophyll *a* biomass (B).

Flow cytometry analyses

Phytoplankton abundances were estimated by flow cytometry (FACSCalibur, Becton Dickinson) fitted with a 488 nm laser. Samples (1000 µL) were fixed with 2% (final concentration) formaldehyde (Troussellier et al., 1995) and stored in liquid nitrogen. Eukaryotic phytoplankton cells (PEUK and NANO, for picoeukaryotes and nanophytoplankton, respectively) were distinguished on the basis of light diffraction (FSC Forward Scatter, related to cell size) and red fluorescence emissions (chlorophyll *a*, wavelength > 650 nm). Populations of coccoid cyanobacteria (CYAN) were identified by their orange fluorescence emissions (phycoerythrin - PE, 542-585 nm). A mixture of fluorescent beads of 0.96 and 1.8 µm diameter ("Fluoresbrite" YG

beads, Polysciences, Inc., Warrington, PA) was added to all sample analyses in order to normalize all parameters and to distinguish between pico- and nanophytoplankton. Duplicate subsamples were run for 6 min at a medium rate (25-30 µL min^{-1}). Data were logged using CellQuest software, and analyzed with "Attractors" software (Becton Dickinson, Inc., USA).

Data analyses

Initial ($P_{x,o}$) and final ($P_{x,t}$) phytoplankton concentrations (Chl *a* or cell density) were used to compute the apparent growth rate k(x) for each dilution (x = 1 for undiluted sample, and x = 0 at infinite dilution):

$$k\,(x,t) = 1/t \ln (P_{x,t} / P_{x,o}) \qquad (1)$$

Linear regression between apparent growth rate (k) and dilution factor (x) (Landry and Hassett, 1982) allows to determine simultaneously the instantaneous growth rate (μ_{max} = Y-axis intercept) and the phytoplankton mortality rate due to microzooplankton grazing (g = slope of linear regression) for nutrient-enriched treatments:

$$k\,(x) = \mu_{max} - g\,x \qquad (2)$$

The phytoplankton growth rate without added nutrients (μ_0) was subsequently calculated according to Landry et al. (1998) by adding the mortality rate (g) estimated for nutrient-enriched treatments to the apparent growth rate (k_0) estimated for undiluted unenriched treatments ($\mu_0 = k_0 + g$). The ratio μ_0/μ_{max} is used to assess the impact of inorganic nutrient enrichment on algal growth and estimate the nutrient sufficiency for phytoplankton growth (Landry et al., 1998).

Non-parametric correlation (Spearman's rank correlation, r_s) or regression analysis between parameters were calculated to determine the relationship between the different measured variables.

RESULTS

Variations of physical and chemical parameters

All physical and chemical data for the different sampling times are reported in Table I. Water temperature varied seasonally, with a minimum of 6.5°C in January and a maximum of 23.3°C in July and September. For surface irradiance, the highest values were also observed in July and September (852 and 915 µmol photons m^{-2} s^{-1}, respectively) while the lowest value was measured in March (199 µmol m^{-2} s^{-1}). Salinity ranged from 31.3 in November to 38.8 in April, with a first decrease in May and June, followed by a more significant decrease in the winter. Dissolved inorganic nitrogen concentrations (DIN = NO_3^- + NO_2^- + NH_4^+) were generally low, except during the autumn when ammonium, which was the main source of inorganic nitrogen throughout the sampling period, strongly increased (8.70 µM). SRP concentrations were low (< 1 µM), but the DIN:SRP ratio ranged from 2 to 9 and was lower than the Redfield ratio (N:P 16), suggesting

potential nitrogen limitation. Silicate concentrations were generally higher than the concentrations of other nutrients, and exhibited significant fluctuations throughout the year. In May, a strong decrease in nutrient concentrations (NO_3^-, NO_2^-, Si) was observed, with values near or below detection limits.

Phytoplankton pigments and density

Chlorophyll *a* concentrations (Table I) ranged from 0.39 µg Chl *a* L^{-1} in November to 5.38 µg Chl *a* L^{-1} in May. The seasonal cycle of phytoplankton Chl *a* biomass was characterized by a spring peak corresponding to diatom blooms that were numerically dominated by *Chaetoceros* sp. (94.5 and 99.2% in April and May, respectively), and by generally low concentrations during the autumn and winter (<1 µg Chl *a* L^{-1}). The spring bloom of phytoplankton coincided with a 6°C increase in water temperature and a decline in nutrient concentrations that occurred between April and May. Picophytoplankton contributed from 7.4 to 50.8% (annual mean = 29%) of the phytoplankton Chl *a* biomass (Table I). The picoplanktonic contribution to Chl *a* biomass was close to that of the larger-sized fraction (>2 µm) between February and April, but was much lower the following month, during the *Chaetoceros* sp. bloom, reaching its lowest value of the year. In the summer, the picophytoplankton represented up to one-third of the phytoplankton biomass (34.2% in July). Autotrophic picoeukaryotes numerically dominated the phytoplankton community, and represented between 55 and 99.7% of the picoplanktonic cell density. The seasonal cycle of picoeukaryote abundances was characterized by two peaks, in April and July, and by a strong decline in May during the diatom bloom. Abundances ranged more than 10-fold (Fig. 2A), with a minimum in September (5.2×10^6 cells L^{-1}) and a maximum in April (90.8×10^6 cells L^{-1}). In the lagoon, PE-rich cyanobacteria were observed at very low levels in the autumn and winter (background < 0.25×10^6 cells L^{-1}), but abundances increased in May and reached a maximum in July (8.20×10^6 cells L^{-1}). The highest cyanobacteria contribution to picoplanktonic density was observed in October. Nanophytoplanktonic abundances (size class: 2 to 20 µm) ranged from 45.6×10^3 cells L^{-1} in September to 514×10^3 cells L^{-1} in April (Fig. 2B). In February, the nanophytoplankton density was too low to permit an accurate estimation by flow cytometry (more than 20 min of cytometry acquisition).

Maximum growth and mortality rates based on chlorophyll a

Chlorophyll-based estimates of maximum phytoplankton growth and microzooplankton grazing rates are indicated in Table II. For the entire phytoplankton community, growth rates for enriched treatments (μ_{max}) and mortality rates exhibited similar patterns, with the highest values occurring between May and September (Fig. 3A&B). Maximum growth rates ranged from - 0.23 d^{-1} in

February to 2.63 d^{-1} in June. The phytoplankton growth rate, as gauged from changes in Chl *a*, was low and constant (mean μ_{max} = 0.41 d^{-1}) in the early spring and in the autumn-winter period, and increased strongly in the summer (mean μ_{max} = 2.21 d^{-1}, from May to September). Microzooplankton grazing rates ranged from - 0.28 d^{-1} ($p < 0.05$) in February to 1.13 d^{-1} in June. For all dilution experiments, growth rates for the enriched treatments always exceeded microzooplankton grazing losses.

For both Chl *a* fractions (Fig. 3C&E), seasonal growth rate variations were similar to the variation observed in the overall phytoplankton community. Growth rates (μ_{max}) significantly increased between May and September. However, the maximum values for the two fractions occurred at different times. For the >2 µm fraction (Fig. 3C), the growth rate increased strongly in May (2.30 d^{-1} or 3.3 divisions per day), during the *Chaetoceros* sp. bloom, to reach a maximum value (2.93 d^{-1} or 4.22 divisions per day) in June. In contrast, for the <2 µm fraction (Fig. 3E), the maximum value (2.67 d^{-1} or 3.85 divisions per day) was observed in July. These two maximum values coincided with the two highest growth rates observed for the total Chl *a* biomass (Fig. 3A). Microzooplankton grazing rates were generally higher for the <2 µm fraction than they were for the >2 µm fraction (Fig. 3D&F), ranging from – 0.09 (n.s., $p > 0.05$) to 1.89 d^{-1} in July for the <2 µm fraction, and from – 0.34 (n.s., $p > 0.05$) to 1.46 d^{-1} in June for the >2 µm fraction. Five of the estimates of grazing rate were negative, but these values were not significantly different from zero (Table II). Maximum grazing rates for both fractions coincided with the two highest grazing rates observed for the total Chl *a* biomass.

Maximum growth and microzooplankton grazing rates based on flow cytometry

For the picophytoplankton community, abundance-based estimates of maximum growth rates (μ_{max}) and microzooplankton grazing rates (Fig 4A&B) exhibited seasonal variations similar to the variation observed in the Chl *a* <2 µm fraction (Fig. 3E&F). Rates based on cell density and on the Chl *a* <2 µm fraction were significantly correlated (Spearman correlation coefficient r = 0.84, n = 20, $p < 0.0001$). For picoeukaryotes, maximum growth rates (Fig. 4A) varied between 0.31 d^{-1} (February) and 2.44 d^{-1} (July). For picocyanobacteria, growth and grazing rates were only calculated from May to October because the abundances were too low during the autumn-winter period. In the summer, seasonal growth rate patterns for cyanobacteria and picoeukaryotes were similar. Cyanobacteria growth rates (Fig. 4A) ranged from 0.42 d^{-1} (October) to 1.64 d^{-1} (July), and were always lower than picoeukaryote growth. Mortality grazing rates (Fig. 4B) ranged from 0.25 d^{-1} (September) to 1.17 d^{-1} (June) for cyanobacteria, and from – 0.09 d^{-1} (n.s. $p < 0.05$, February) to 1.66 d^{-1} (June) for picoeukaryotes. From May to July, the grazing pressure was higher on picoeukaryotes than it was on cyanobacteria.

Nanophytoplankton growth rate variations (Fig. 5) were different from the variation observed in the >2 µm fraction (Fig. 3C). Growth rates also increased from May and peaked in July (2.81 d^{-1} corresponding to 4 divisions per day), and a lower value was observed in November (0.14 d^{-1}). The maximum grazing rate was observed in July (0.41 d^{-1}) and, as with the Chl *a* >2 µm fraction (Fig. 3D), most g values were not significantly different from zero.

Nutrient enrichment impact

Chlorophyll-based estimates of growth rates without added nutrients (μ_0) varied between –0.48 d^{-1} in February and 1.72 d^{-1} in June for the >2 µm fraction, and between 0.37 d^{-1} in March and 2.6 d^{-1} in July for the <2 µm fraction (Table II). There was a strong nutrient enrichment effect on the growth rate of the >2 µm fraction (Fig. 6) between April and July (μ_0/μ_{max} range = -0.02 - 0.58), corresponding to the period with the highest values for maximum growth rate (Fig. 3C). In contrast to the nutrient impact on the larger fraction, enrichment only had an effect on the growth rate of the <2 µm fraction in May and June (μ_0/μ_{max} = 0.47). The highest values for maximum growth rates of the smallest fraction occurred in July and September (Fig. 3E) while the ratio μ_0/μ_{max} was near or above 1 (though not significantly different from 1).

Phytoplankton carbon assimilation

Annual variations of size-fractionated primary production and P/B quotients (carbon fixation per unit of biomass based upon Chl *a*) are shown in Figure 7. The contribution of the two size classes to the total primary production was close to their contribution to the total biomass (data not shown). Throughout the year, primary production was higher for the larger fraction than it was for the smaller fraction, with an annual mean (± SD) of 5.68 ± 7.27 µg C L^{-1} h^{-1} and 1.48 ± 1.67 µg C L^{-1} h^{-1}, respectively. For the >2 µm fraction, primary production increased strongly during diatom blooms, with a maximum of 20.3 µg C L^{-1} h^{-1} in May which was six times higher than picoplanktonic production and which corresponded to 86% of the total primary production (Fig. 7A). Picoplanktonic primary production ranged between 0.15 µg C L^{-1} h^{-1} in January and 5.11 µg C L^{-1} h^{-1} in July. The picophytoplankton contribution to primary production amounted to 25% of the total primary production and ranged from a minimum of 11% in January 1999 to a maximum of 42% in February 1998.

In contrast, the <2 µm fraction made a greater contribution to P/B quotients. Picoplanktonic P/B quotients ranged from 0.92 µg C (µg Chl *a*)$^{-1}$ h^{-1} in January to 9.18 µg C (µgchl *a*)$^{-1}$ h^{-1} in May, while larger cell P/B varied between 0.59 µg C (µg Chl *a*)$^{-1}$ h^{-1} in January and 6.48 µg C (µg Chl *a*)$^{-1}$ h^{-1} in June (Fig. 7B). Annual P/B values of the two size fractions were almost similar and were 3.99 ± 3.20 µg C (µg Chl *a*)$^{-1}$ h^{-1} and 3.89 ± 2.02 µg C (µg Chl *a*)$^{-1}$ h^{-1} for <2 µm and >2 µm

fraction, respectively. Contrary to primary production, the picoplanktonic P/B quotient tended to be higher than the quotient for the largest fraction in the late spring and in the summer, in particular in May when the P/B for the <2 µm fraction was twice that measured for the >2 µm fraction. Thus, the mean percent contribution of picophytoplankton to total P/B reached 57% between May and September, while its percent contribution to total primary production was 21% during the same period.

DISCUSSION

Phytoplankton succession: composition, abundance and biomass

During the year-long sampling period described in the present study, the abundance of autotrophic picoplankton (mean annual density = 35 x 10^6 cells L^{-1}) was within the range of values previously reported for the lagoon (Vaquer et al., 1996; Dupuy et al., 2000). Also, the seasonal pattern, which was characterized by a maximum occurring during the warm season and lower values in the autumn, was similar to those observed during a sampling period from 1991 to 1994 (Vaquer et al., 1996). However, the maximum value (90 x 10^6 cells L^{-1}) observed in the current study was two times lower than the maximal abundance measured in the summer in a previous study (Vaquer et al., 1996). Our results confirm that photosynthetic picoeukaryotes numerically dominated the picoalgal community throughout the year. In the Thau lagoon, the picoeukaryote community comprises different species belonging to prasinophytes whose the most abundant component is *Ostreococcus tauri* (Courties et al., 1994, Chrétiennot-Dinet et al., 1995, Chrétiennot-Dinet and Courties, 1997). Recent work using genetic tools has revealed that the picoplanktonic genus *Ostreococcus* is widely distributed in various oceanic and coastal environments (Guillou et al., 2004). Recently, this picoalga was observed at a Pacific Ocean coastal site at a density of 59 x 10^6 cells L^{-1} (Worden et al., 2004), and in Long Island bay during a transient bloom reaching 500 x 10^6 cells L^{-1} (O'Kelly et al., 2003). This latter value has never been observed in the Thau lagoon in any of various seasonal monitoring studies (Courties et al., 1994; Chrétiennot-Dinet et al., 1995; Vaquer et al., 1996). The widespread occurrence of this tiny prasinophyte suggests that it could play an important ecological role in marine systems. Unlike in the Mediterranean Sea (Agawin et al., 1998; Jacquet et al., 1998), PE-rich cyanobacteria abundances were always low in the Thau lagoon, even though their density increased during the summer. In temperate waters, a seasonal peak of PE-rich cyanobacteria usually occurs during the warm summer months (Iriarte and Purdie, 1994; Agawin et al., 1998).

In terms of biomass, the annual mean contribution of picophytoplankton (29%) fits well within the range reported in previous studies (Courties et al., 1994; Chrétiennot-Dinet et al., 1995; Vaquer et al., 1996), with minimum and maximum contributions occurring in the spring and winter,

respectively. In the spring, several rainy days before the sampling period (marked by a decrease of salinity in May and June) probably caused allochthonous nutrient inputs, particularly DIN as nitrate coming from the watershed (Souchu et al., 2001). An increase in water temperature, coupled with new nutrient inputs, can support the bloom of chain-forming diatoms of the genera *Chaetoceros* sp. and *Skeletonema costatum*. Such diatom blooms are responsible for new primary production (Chisholm, 1992), and resulted in the lowest contribution of picophytoplankton to Chl *a* biomass observed during that year-long survey. The decline of the diatom bloom could be due to nutrient limitation resulting from the exhaustion of inorganic nutrients in the water column (NO_3^-, NO_2^- and Si concentrations were below or near the detection limit). Such nutrient-limited conditions for the larger phytoplankton, associated with maximum values for temperature and irradiance, support a greater picoplanktonic contribution to the Chl *a* biomass (Kuosa, 1991; Iriarte and Purdie, 1994). By virtue of its small size and high surface to volume ratio, picophytoplankton is able to use scarce resources more efficiently than larger cells (Raven, 1998). This confers on small phytoplankton a competitive advantage relative to large phytoplankton under nutrient-limited conditions (Agawin et al., 2000). Furthermore, DIN fluxes, largely dominated by ammonium in summer in the Thau lagoon (Souchu et al., 2001), are more likely to sustain growth of pico- and nanophytoplankton, which depend on regenerated primary production (Collos et al., 2003), than of microphytoplankton (i.e. diatoms), which might depend on new rather than recycled nutrients (Ferrier and Rassoulzadegan, 1991; Selmer et al., 1993).

Factors controlling seasonal variations in phytoplankton growth

In the Thau lagoon, maximum growth rates (μ_{max}) based on chlorophyll *a* were in the high range of values that have been reported using the dilution technique (Murrell et al., 2002), and the highest value (2.93 d^{-1}) is ascribed to the larger fraction of phytoplankton (Table II). In terms of growth, the response of the phytoplankton community to nutrient inputs differs in time between the >2 µm and <2 µm fractions. In the spring, under nutrient-depleted conditions, nutrient enrichment during incubation stimulates the >2 µm fraction growth more than it does picoplanktonic growth. Subsequent to new nitrogen inputs, microphytoplankton growth is released from nutrient diffusion limitation, and can outcompete the picoplankton community (Agawin et al., 2000). The highest values for instantaneous growth rates for the >2 µm fraction, which were observed in May and June, can be attributed to fast-growing diatoms (*Chaetoceros* sp. and *Skeletonema costatum*, respectively). These genera are able to grow rapidly in response to nutrient pulses because both exhibit a short time lag and rapidly transform nutrients into new biomass (Collos, 1986). On the other hand, the microzooplankton grazing pressure on the two Chl *a* fractions was different. For the larger phytoplankton, most grazing rates based on the Chl *a* >2 µm fraction and on cell density were

not significantly different from zero, indicating that grazing pressure on larger algae was negligible in bottle incubations. In the spring, phytoplankton production for the larger fraction partly escaped microzooplankton consumption under ambient nutrient conditions (g/μ_0 = 0.75 in May and 0.85 in June), whereas loss rates of picophytoplankton to microzooplankton grazing exceeded growth rates (g/μ_0 = 1.5 in May and 1.57 in June). Thus, the dominance of large diatoms in the spring was related not only to their growth characteristics but also to their escape from size-selective microzooplankton grazing, in contrast to picophytoplankton (Riegman et al., 1993; Strom et al., 2001). However, the transient imbalance between growth and grazing rates observed for larger phytoplankton may also be due to a suppression of losses, since nano- and micro-phytoplankton (in particular diatoms) in the Thau lagoon are mainly controlled top-down by bivalve suspension feeders (Dupuy et al., 2000), which were excluded from incubations.

Concerning picoplanktonic growth, the <2 µm fraction growth rates were higher than those of the larger Chl *a* fraction in July and September. In contrast to the latter one, the picoplanktonic community grew at close to its maximal growth rate under ambient nutrient conditions (μ_0/μ_{max} near 1). Indeed, growth rates (μ_0) based on cell abundance were 2.38 and 1.44 d^{-1} for picoeukaryotes and cyanobacteria, respectively. Cyanobacteria growth rates were of the same order of magnitude as those recorded for *Synechococcus* in a coastal Mediterranean system, where the maximum growth rate was also achieved in summer under low nutrient levels (0.2 to 1.5 d^{-1}, Agawin et al., 1998). For picoeukaryotes, growth rates were in the range of the maximum rates reported in the Thau lagoon in August 1998 (Dupuy et al., 2000). The strong increase in the growth rate may be related to an increased ammonium concentration in the water column (Table II), favoring regenerated primary production (Collos et al., 2003). Moreover, during this period, although grazing pressure on picophytoplankton increased, picoplanktonic production exceeded losses due to microzooplankton grazing (g/μ_0 range : 0.40 - 0.73). In the summer, temperature and irradiance could be key factors controlling picophytoplankton growth. For both picoeukaryotes and cyanobacteria, the highest values for maximum growth rates were measured during the warmest and brightest months (July and September), and the annual variations in growth rates displayed a strong seasonality which could be related to seawater temperature and/or irradiance. Indeed, in contrast to the >2 µm fraction phytoplankton, maximum growth rates for picoeukaryotes and cyanobacteria were positively correlated with temperature (Table III). These results agree with previous observations which found a strong correlation between the *Synechococcus* sp. growth rate and seawater temperature in coastal NW Mediterranean Sea (Agawin et al., 1998). A correlation between picoeukaryotic growth rate and temperature was also observed in the Pacific Ocean (Worden et al., 2004). On the other hand, in the Thau lagoon, the maximum values for growth rates were observed for picoeukaryotes, whose growth appears to be positively correlated with irradiance (Table III), in contrast to the growth of

picocyanobacteria. This might be due to differences in photoadaptive responses to high irradiance between prokaryote and eukaryote picoplankton. Indeed, in surface waters of the Mediterranean Sea, high irradiances seem to have a less deleterious effect on picoeukaryotes than on *Synechococcus* (Jacquet et al., 1998). Moreover, the dominant picoeukaryote in the Thau lagoon, *Ostreococcus tauri*, contains high cellular concentration of violaxanthin (Chrétiennot-Dinet et al., 1995). This carotenoid pigment is a major light-harvesting pigment and has also the capacity to dissipate excessive energy under high light conditions via the xanthophyll cycle, avoiding photooxidative damage (Lawlor, 1993). In contrast to higher plants and algae, cyanobacteria do not have the xanthophyll cycle which constitutes a major photoprotective system (Hirschberg and Chamovitz, 1995).

In contrast to large phytoplankton, the picophytoplankton biomass was limited by zooplankton predation. In fact, microzooplankton grazing exerted a high pressure on picoeukaryotes, which constitute the most abundant food resource for the heterotrophic protist (tintinnids, oligotrich ciliates and flagellates) community in the Thau lagoon (Lam-Höai et al., 1997; Dupuy et al., 2000). These results corroborate recent observations showing that heterotrophic nanoflagellates can play a significant role in the regulation of *Ostreococcus* populations in the coastal waters of Long Island (O'Kelly et al., 2003). Moreover, the grazing mortality of picoeukaryotes (-0.09 to 1.66 d^{-1}) was higher than that of cyanobacteria (0.25 to 1.17 d^{-1}), and was in the high range of previously reported values (0.26 to 0.59 d^{-1}, Samuelsson and Andersson, 2003; 0.27 to 1.09 d^{-1}, Worden et al., 2004). Several explanations could account for the differential mortality due to grazing among the picoplankton community. Protist grazing on picoplankton could be prey density dependent (Kuipers et al., 2003). However, in marine coastal sites, selective grazing activity on picoeukaryotes has been reported, although picocyanobacteria outnumbered eukaryote picoalgae (Samuelsson and Andersson, 2003; Worden et al., 2004), suggesting that picoeukaryotes could be an important carbon source for the microzooplankton in these systems (Reckerman and Veldhuis, 1997; Brown et al., 1999). The absence of a cell wall in the *Ostreococcus* cell structure could explain the preferential grazing (Worden et al., 2004). Microzooplankton grazers therefore removed a substantial part of picophytoplankton production throughout the year in the Thau lagoon (71% on average). These results support the role of microzooplankton grazing as an important loss process for phytoplankton in coastal waters (Strom et al., 2001), and the tight correlation between picoplanktonic growth and microzooplankton grazing (Table III) suggests a high transfer efficiency of picophytoplankton production to higher trophic levels. Moreover, within the mesozooplankton, the abundance of *Oikopleura dioica* Fol (T. Lam-Höai, Montpellier, personal communication) was enhanced from May to September, attaining its highest values (20-100 individuals L^{-1}). This

appendicularian may play an important role as a predator of picoplankton in eutrophic coastal areas (Nakamura et al., 1997), and thus in the transfer of picoplanktonic production from the microbial food web to higher trophic levels in the summer.

Size-fractionated primary production

The total primary production was similar to values previously reported in the Thau lagoon (Souchu et al., 2001). Larger phytoplankton (>2 µm) was the most productive fraction throughout the year, particularly in the spring, when diatoms are responsible for most primary production. The relative contribution of picophytoplankton to the total primary production (25%) was lower than previous estimates for the Thau lagoon (38.3%, Vaquer et al., 1996), but was close to values reported for eutrophic coastal Mediterranean sites (Magazzù and Decembrini, 1995; Modigh et al., 1996). Furthermore, the picoplanktonic contribution to primary production was lower during the warmer months (May to September) than it was during the rest of the year (21% and 27%, respectively). In warm and productive waters, the decreased contribution of picophytoplankton has been hypothesized to result from increased loss rates, such as strong grazing pressure (Agawin et al., 2000). Therefore in the Thau lagoon, the decreasing contribution of autotrophic picoplankton to primary production in the summer could be due to preferential grazing by microzooplankton. On the other hand, picophytoplankton had the greatest contribution to biomass-specific primary productivity (P/B), especially in the summer with a mean picoplanktonic contribution of 57%. Picoplanktonic P/B quotient values as well as seasonal variations were similar to those reported for other coastal systems (Malone et al., 1991). In contrast to primary production, picoplanktonic P/B was linearly correlated with water temperature (Table III). These results indicate a rapid turnover of carbon per unit chlorophyll for algal picoplankton in the summer, and they are consistent with the high microzooplankton grazing pressure on picophytoplankton that was observed in the current study. Hence, in the Thau lagoon the high picoplanktonic production in the summer appears to be an important source of organic carbon for the community of protists grazers. This is in agreement with previous observations which found that picophytoplankton is an important component of the coastal Mediterranean food web (Agawin et al., 1998). In the Thau lagoon, picoeukaryotes, which dominate the photosynthetic picoplankton community, could play a significant role in the trophic transfer of carbon to farmed oysters via the protist community, as previously suggested for picocyanobacteria (Le Gall et al., 1997, Loret et al., 2000).

In summary, the different groups within the phytoplankton community seem to have a competitive advantage according to the season. Nano- and microphytoplankton growth were mainly limited by nutrient availability, but rapidly responded to new nutrient inputs in the spring. The dominance of picoeukaryotes in the summer is attributable to their great capacity to acquire regenerated nutrients

present at low concentrations. Furthermore, picoeukaryote growth was positively correlated with temperature and irradiance. In contrast, picoeukaryotes are strongly controlled by microzooplankton grazing. Although representing a weak carbon source in terms of biomass for farmed oysters in the Thau lagoon (Dupuy et al 2000), picoeukaryotes equaled or exceeded nano - and microphytoplankton in terms of productivity (especially in the summer). Picoeukaryotes constitute an essential component of the microbial food web, and contribute significantly to the carbon flow to higher trophic levels.

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LEGENDS FOR TABLES AND FIGURES

Table I. Temp: temperature, n.d. non determined, b.d. below detection.

Table II. μ_{max}: maximum growth rate (d^{-1}), g: microzooplankton grazing rate (d^{-1}), μ_0: growth rate without added nutrients (d^{-1}), r^2: coefficient of determination of linear regression analysis, n: number of observation. The significance level of the regression (i.e. slope, g, was significantly different from zero, $p < 0.05$) is indicated by p: ns: not significant, * $p<0.01$, ** $p<0.001$.

Table III. μ_{max}: maximum growth rate (d^{-1}), g: microzooplankton grazing rate (d^{-1}), P/B: biomass-specific primary productivity (µg C µg $Chla^{-1}$ h^{-1}), T: temperature (°C), Irr: irradiance (µE m^{-2} s^{-1}), Peuk: cell density of picoeukaryotes, Cyan: cell density of cyanobacteria, Chl *a* >2 µm and Chl *a* <2 µm: fraction of phytoplankton chlorophyll *a* biomass. n: number of observation, r^2 : coefficient of determination, p : probability for significant slope. ns: not significant (for $p > 0.05$), * $p<0.01$, ** $p<0.001$, *** $p<0.0001$.

Fig. 1. Location of the sampling site (ZA) in Thau lagoon inside the northeast shellfish farming zone.

Fig. 2. Temporal variations in picophytoplankton (A) and nanophytoplankton (B) cell abundances from February 1999 to January 2000 in Thau lagoon. (A): Picoeukaryotes (filled bars), cyanobacteria (hatched bars). Error bars represent standard deviation for each month. Note difference in scale between pico- and nano-phytoplankton abundances.

Fig. 3. Temporal variations in maximum growth (μ_{max}, d^{-1}) and microzooplankton grazing (g, d^{-1}) rates from dilution experiments based on total Chl *a* (A&B), >2 µm Chl *a* fraction (C&D) and <2 µm Chl *a* fraction (E&F) from February 1999 to January 2000. Error bars represent standard error.

Fig. 4. Temporal variations in maximum picophytoplankton growth (A) and grazing (B) rates based on cell density from February 1999 to January 2000. Picoeukaryotes (filled bars), cyanobacteria (hatched bars). Cyanobacteria growth and grazing rates could be estimated only from May to October (see text for details). Error bars represent standard error.

Fig. 5. Temporal variations in maximum nanophytoplankton growth (filled bars) and grazing (open bars) rates based on cell density from February 1999 to January 2000. Error bars represent standard error.

Fig. 6. Temporal variations in relative growth (μ_0 / μ_{max}), ratio between no nutrient and nutrient-enriched phytoplankton growth based on Chl *a* from February 1999 to January 2000. Solid and open bars represent >2 µm and <2 µm fraction respectively. Stars above histograms indicate a significant difference between μ_{max} and μ_0 ($p > 0.05$).

Fig. 7. Comparison of temporal variations in primary production (A) and biomass-specific primary productivity (B) from February 1999 to January 2000. Solid and open bars represent >2 µm and <2 µm fraction respectively.

Table I. Summary of environmental conditions, chlorophyll *a* concentrations and contribution of the Chl *a* fractions to total biomass for *in situ* incubations experiments in Thau lagoon.

Date	Salinity	Temp (°C)	Mean PAR (µmol m^{-2} s^{-1})	SRP	Si	NO_3^-	NO_2^-	NH_4^+	Chl *a* ($\mu g\ L^{-1}$)	>2 µm (%)	<2 µm (%)
				µM							
Feb-99	38.4	12.2	616	0.31	5.80	0.12	0.24	0.60	0.48	58.4	41.6
Mar-99	38.5	12.1	199	0.23	2.20	0.38	0.06	0.56	0.80	49.2	50.8
Apr-99	38.8	13.9	470	0.21	6.20	0.06	0.31	0.57	1.29	53.8	46.2
May-99	36.4	19.7	405	0.17	0.40	b.d.	b.d.	0.31	5.38	92.6	7.4
Jun-99	36.6	21.7	752	0.07	8.60	b.d.	b.d.	0.61	3.59	85.6	14.4
Jul-99	38.2	23.3	852	0.47	4.70	0.16	0.05	2.26	2.04	65.8	34.2
Sep-99	37.4	23.3	915	0.95	13.00	1.03	0.42	4.89	0.62	85	15
Oct-99	36.4	17.5	444	n.d.	n.d.	n.d.	n.d.	8.70	1.19	70.2	29.8
Nov 99	31.3	10.5	244	n.d.	n.d.	n.d.	n.d.	4.80	0.39	76.9	23.1
Dec 99	32.9	7.4	247	n.d.	n.d.	n.d.	n.d.	0.60	n.d.	n.d.	n.d.
Jan 00	33.4	6.5	363	n.d.	n.d.	n.d.	n.d.	0.06	2.22	92.5	7.5

Table II. Maximum growth rates and microzooplankton grazing rates estimated by linear regression from dilution experiments conducted in Thau lagoon from February 1999 to January 2000, and based on Chl *a*.

Date	Chl *a*	μ_{max} ± SE	g ± SE	μ_0	r^2	p value	n
Feb-99	total	-0.23 ± 0.06	-0.28 ± 0.10	-0.17	0.50	< 0.05	10
	>2 µm	-0.20 ± 0.11	-0.12 ± 0.17	-0.48	0.14	n. s.	5
	<2 µm	0.34 ± 0.21	-0.09 ± 0.34	0.15	0.02	n. s.	5
Mar-99	total	0.60 ± 0.04	0.17 ± 0.06	0.59	0.51	< 0.05	10
	>2 µm	0.47 ± 0.01	0.32 ± 0.02	0.62	0.99	**	5
	<2 µm	0.84 ± 0.03	0.27 ± 0.05	0.81	0.92	**	5
Apr-99	total	0.57 ± 0.02	0.11 ± 0.03	0.48	0.64	*	10
	>2 µm	0.60 ± 0.16	-0.35 ± 0.27	-0.01	0.36	n. s.	5
	<2 µm	0.39 ± 0.09	0.79 ± 0.15	0.61	0.90	< 0.05	5
May-99	total	2.13 ± 0.06	0.36 ± 0.11	0.38	0.62	< 0.05	9
	>2 µm	2.28 ± 0.10	0.46 ± 0.15	0.61	0.75	n. s.	5
	<2 µm	1.41 ± 0.18	1.00 ± 0.29	0.67	0.80	< 0.05	5
Jun-99	total	2.63 ± 0.05	1.13 ± 0.08	1.23	0.96	***	10
	>2 µm	2.93 ± 0.05	1.46 ± 0.08	1.72	0.99	**	5
	<2 µm	1.83 ± 0.08	1.35 ± 0.13	0.86	0.97	**	5
Jul-99	total	2.42 ± 0.05	0.78 ± 0.07	1.61	0.94	***	10
	>2 µm	1.76 ± 0.23	-0.08 ± 0.38	0.70	0.02	n. s.	5
	<2 µm	2.67 ± 0.25	1.89 ± 0.41	2.60	0.87	< 0.05	5
Sep-99	total	1.65 ± 0.09	0.10 ± 0.15	1.71	0.06	n. s.	10
	>2 µm	0.76 ± 0.52	-0.34 ± 0.19	0.90	0.05	n. s.	5
	<2 µm	2.14 ± 0.48	0.95 ± 0.80	2.35	0.32	n. s.	5
Oct-99	total	0.39 ± 0.07	0.26 ± 0.11	0.37	0.41	< 0.05	10
	>2 µm	0.57 ± 0.08	0.65 ± 0.18	1.05	0.86	n. s.	5
	< 2 µm	0.40 ± 0.13	0.26 ± 0.23	0.28	0.39	n. s.	5
Nov-99	total	0.66 ± 0.04	0.28 ± 0.06	0.61	0.74	*	9
	>2 µm	0.77 ± 0.16	0.66 ± 0.27	0.89	0.67	n. s.	5
	<2 µm	0.60 ± 0.19	0.47 ± 0.34	0.65	0.42	n. s.	5
Jan-00	total	0.49 ± 0.02	0.11 ± 0.04	0.44	0.49	< 0.05	10
	>2 µm	0.47 ± 0.03	0.37 ± 0.05	0.05	0.97	< 0.05	5
	<2 µm	0.64 ± 0.28	0.20 ± 0.42	0.90	0.10	n. s.	5

Table III. Significance of linear relationships between temperature, irradiance and biological rates measured during the annual cycle in Thau lagoon.

Variable	Equation	n	r^2	p
Maximum growth rate				
Chl *a* >2 µm Growth vs. Temperature	-	10	0.39	n.s.
Chl *a* <2 µm Growth vs. Temperature	μ_{max} = 0.12 (± 0.03) T - 0.90 (± 0.51)	10	0.68	*
Peuk Growth vs. Temperature	μ_{max} = 0.14 (± 0.02) T - 1.11 (± 0.32)	10	0.88	***
Cyan Growth vs. Temperature	μ_{max} = 0.18 (± 0.03) T - 2.63 (± 0.57)	5	0.93	*
Chl *a* >2 µm Growth vs. Irradiance	-	10	0.08	n.s.
Chl *a* <2 µm Growth vs. Irradiance	μ_{max} = 0.003 (± 0.001) Irr - 0.40 (± 0.43)	10	0.63	*
Peuk Growth vs. Irradiance	μ_{max} = 0.003 (± 0.001) Irr - 0.23 (± 0.49)	10	0.55	<0.05
Cyan Growth vs. Irradiance	-	5	0.68	n.s.
Microzooplankton grazing rate				
Chl *a* >2 µm Grazing vs. Temperature	-	10	0.21	n.s.
Chl *a* <2 µm Grazing vs. Temperature	g = 0.08 (± 0.02) T - 0.65 (± 0.39)	10	0.62	*
Peuk Grazing vs. Temperature	g = 0.08 (± 0.02) T - 0.71 (± 0.44)	10	0.54	<0.05
Cyan Grazing vs. Temperature	-	5	0.00	n.s.
Chl *a* >2 µm Grazing vs. Chl *a* >2 µm Growth	g = 0.45 (± 0.17) μ_{max} - 0.41 (± 0.24)	10	0.48	<0.05
Chl *a* <2 µm Grazing vs Chl *a* <2 µm Growth	g = 0.64 (± 0.11) μ_{max} + 0.01 (± 0.15)	10	0.80	**
Peuk Grazing vs Peuk Growth	g = 0.56 (± 0.16) μ_{max} - 0.09 (± 0.23)	10	0.61	*
Cyan Grazing vs Cyan Growth	-	5	0.04	n.s.
C Assimilation				
(P/B) >2 µm vs. Temperature	P/B = 0.29 (± 0.07) T - 0.70 (± 1.15)	10	0.69	*
(P/B) <2 µm vs. Temperature	P/B = 0.46 (± 0.10) T - 3.49 (± 1.70)	10	0.73	*
(P/B) >2 µm vs. Irradiance	-	10	0.36	n.s.
(P/B) <2 µm vs. Irradiance	-	10	0.40	n.s.

Fig. 1

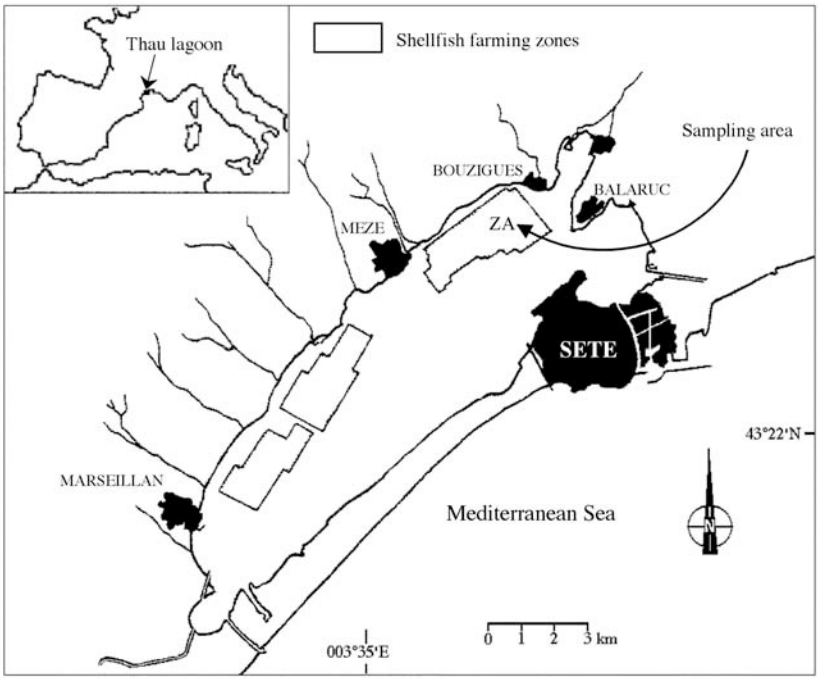

Thau lagoon
Shellfish farming zones
Sampling area
BOUZIGUES
BALARUC
MEZE
ZA
SETE
43°22'N
MARSEILLAN
Mediterranean Sea
003°35'E
0 1 2 3 km

Fig. 2

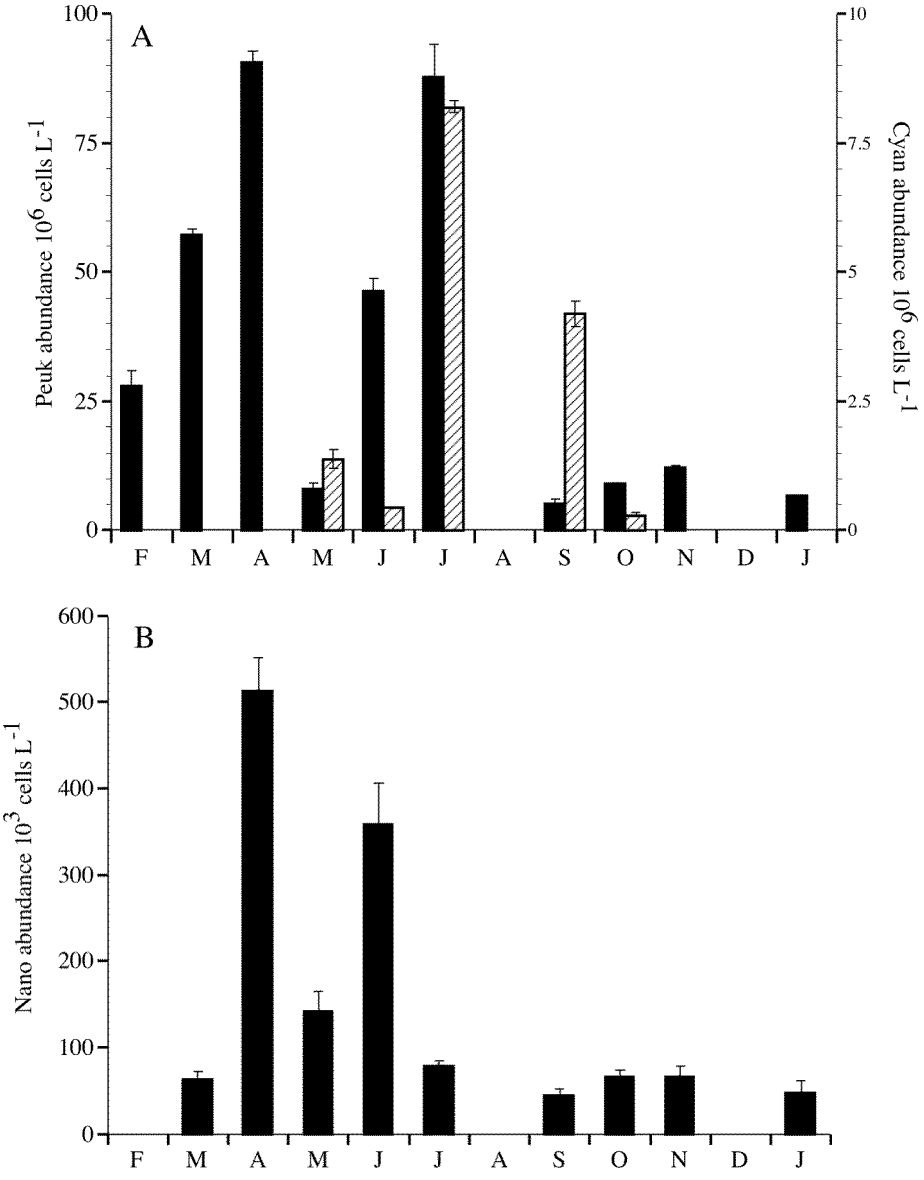

A
Peuk abundance 10^6 cells L^{-1}
Cyan abundance 10^6 cells L^{-1}
100
75
50
25
0
10
7.5
5
2.5
0
F M A M J J A S O N D J
B
Nano abundance 10^3 cells L^{-1}
600
500
400
300
200
100
0
F M A M J J A S O N D J

Fig. 3

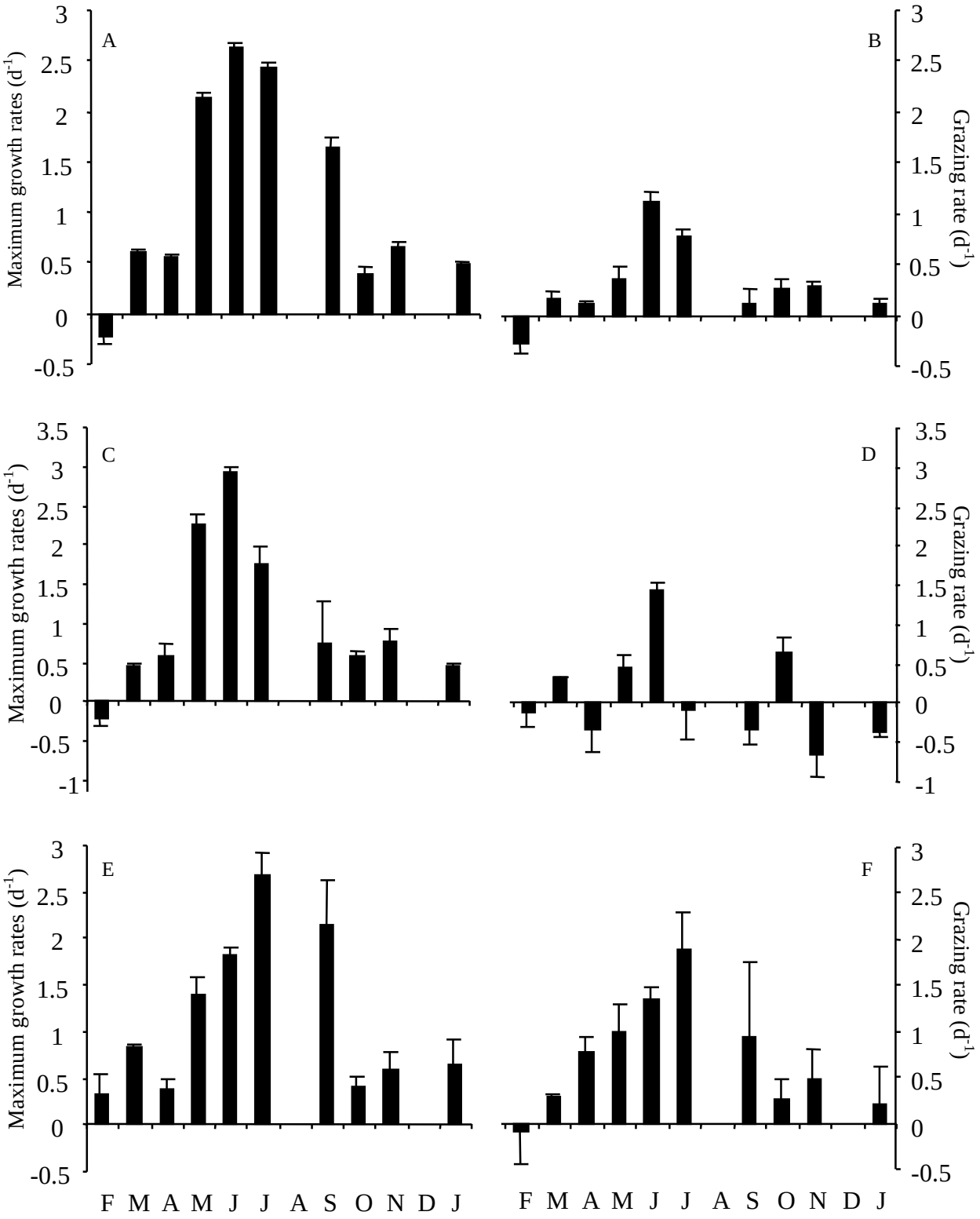

A
B
C
D
E
F
Maximum growth rates (d-1)
Grazing rate (d-1)
3
2.5
2
1.5
1
0.5
0
-0.5
3.5
-1
F M A M J J A S O N D J

Fig. 4

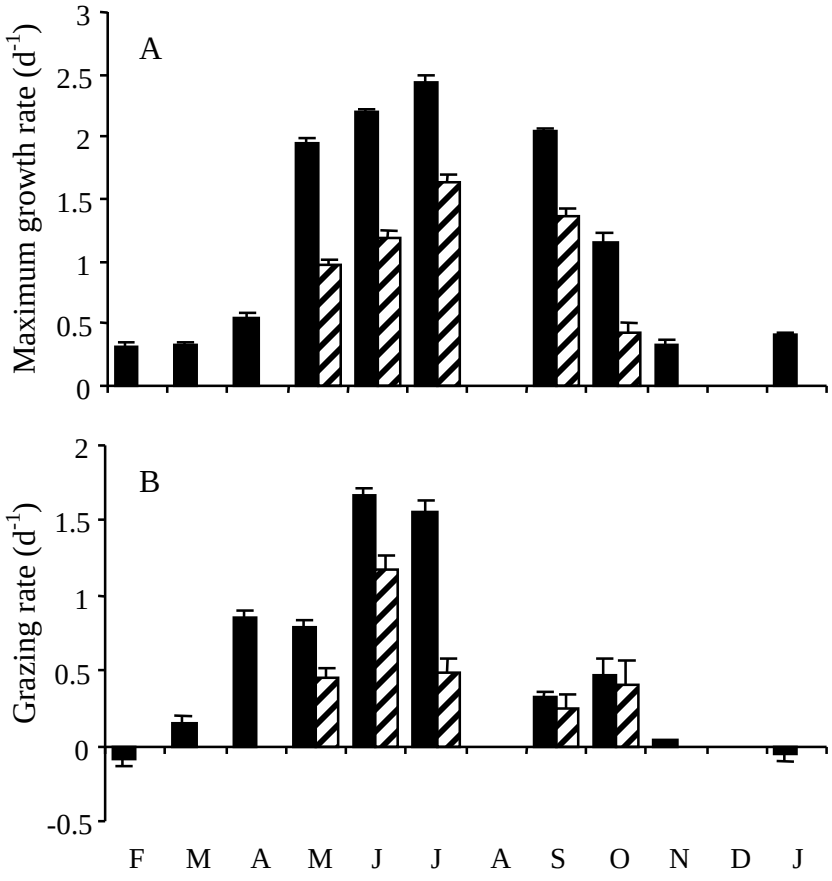

A
Maximum growth rate (d-1)
3
2.5
2
1.5
1
0.5
0
B
Grazing rate (d-1)
2
1.5
1
0.5
0
-0.5
F M A M J J A S O N D J

Fig. 5

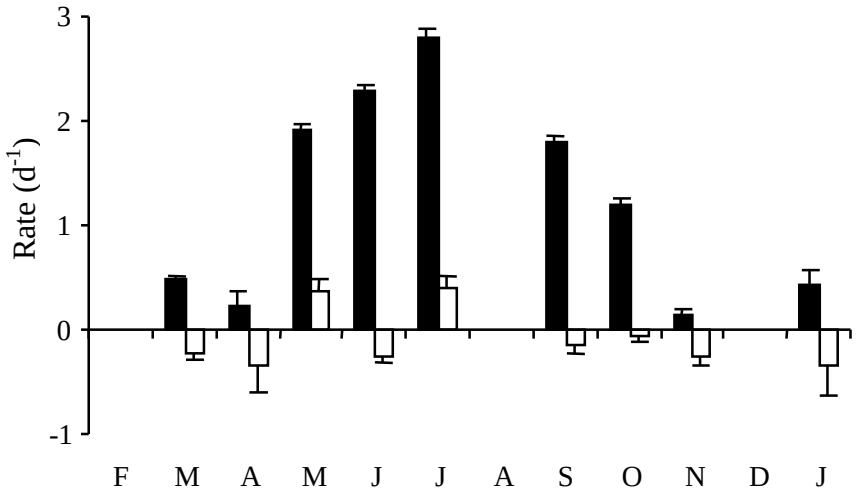


Fig. 6

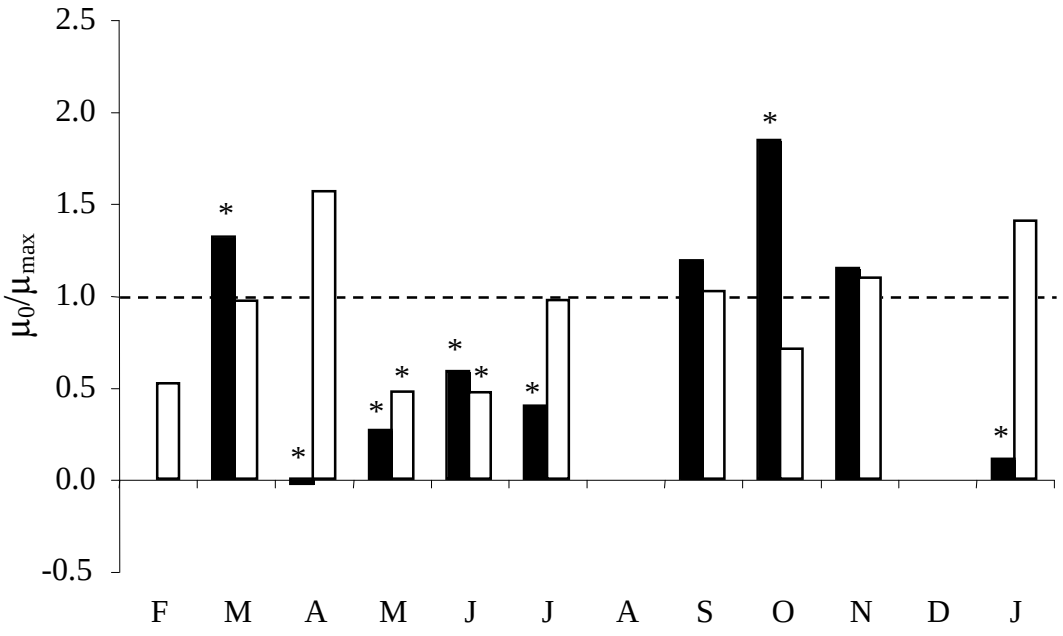
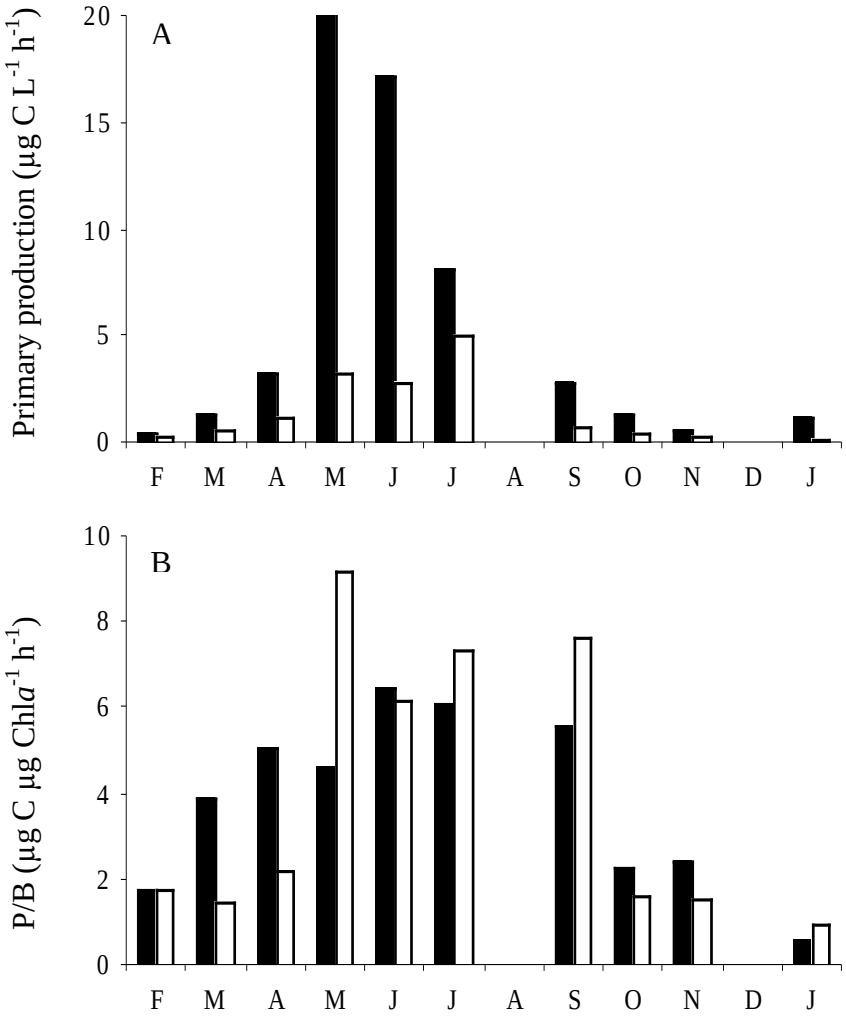


Fig. 7


A
Primary production (µg C L-1 h-1)
20
15
10
5
0
F M A M J J A S O N D J
B
P/B (µg C µg Chla-1 h-1)
10
8
6
4
2
0
F M A M J J A S O N D J