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Christine Dupuy [a,*], Agathe Talarmin [b,c], Hans J. Hartmann [a], Daniel Delmas [d], C. Courties [e], Elise Marquis [f]

[a] Laboratoire LIENSs, UMR 6250, Université de La Rochelle, Bâtiment ILE, 2 rue Olympe de Gouges, 17000 La Rochelle, France
[b] Université Aix-Marseille II- CNRS/INSU UMR 6117, LMGEM, Centre d'Oceanologie de Marseille, Case 901, Campus de Luminy, F-13288 Marseille, France
[c] UPMC Université Paris 06-CNRS, UMR 7621, LOMIC, Observatoire Océanologique, F-66651 Banyuls/mer, France
[d] IFREMER/Centre de Brest, DYNECO, Laboratoire Pelagos, BP 70, 29280 Plouzané, France
[e] Université Paris VI/ CNRS, Laboratoire Arago, BP 44, 66650 Banyuls-sur-mer, France
[f] Institute of Oceanography, National Taiwan University, No. 1, Section 4, Roosevelt, 8 Road, Taipei 10617, Taiwan

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ABSTRACT

In order to investigate the parameters controlling the heterotrophic protists (nano-microzooplankton) on the continental shelf of the southern Bay of Biscay, plankton communities and their physico-chemical environment were studied 4 times in February, April, June and September–October 2004 at three stations in the euphotic zone in the Bay of Biscay. The abundance and carbon biomass of heterotrophic protists (ciliates, heterotrophic dinoflagellates and nanoflagellates) as well as all the others groups of plankton (picoplankton, nanophytoplankton, diatoms, autotrophic dinoflagellates, metazoan microzooplankton and mesozooplankton), the environmental parameters and the primary and bacteria production were evaluated at each sampling period. Microzooplankton grazing experiments were undertaken at the same time. Ciliates and heterotrophic dinoflagellates accounted for the main major component of nano- and microzooplankton communities in term of biomass. The total carbon biomass of heterotrophic protists was highest in spring and lowest at the end of summer. The development of heterotrophic protists started after a winter microphytoplankton bloom (principally large diatoms), the biomass was lower in June and was low in September (through inappropriate prey). The carbon requirement of microzooplankton ranged from 50 to more than 100% of daily primary, bacterial and nanoflagellate production. The heterotrophic protist community was predominantly constrained by bottom-up control in spring and at the end of summer via food availability and quality.

1. Introduction

In pelagic environments, primary production is transferred to higher levels through two main pathways, according to the size of the main primary producers (Azam et al., 1983; Sherr et al., 1986; Sommaruga, 1995; Thingstad and Rassoulzadegan, 1999). Classically, the grazing food chain transfers energy directly from large diatoms to metazoans (herbivorous chain, Pomeroy, 1974). However, most of the primary production is due to phytoplankton less than 3 μm in size (Waterbury et al., 1979; Fenchel, 1988) not efficiently retained by mesozooplankton such as copepods (Sherr et al., 1986) but highly grazed by microzooplankton (Calbet and Landry, 2004; Landry and Calbet, 2004), with energy being transferred through the microbial food web (Azam et al., 1983). Ciliates (phylum Ciliophora) are a dominant component of microzooplankton in marine waters (Pierce and Turner, 1992). They consume a wide range of prey, such as bacteria (Sherr and Sherr, 1987), pico- and nanophytoplankton (Bernard and Rassoulzadegan, 1993), heterotrophic nanoflagellates (Jürgens et al., 1996), dinoflagellates (Stoecker et al., 1984), diatoms (Sime-Ngando et al., 1995) and also other ciliates (Dolan, 1991). Large heterotrophic dinoflagellates (>20 μm), another component of the nano-microzooplankton community, have been found to feed on mainly chain-forming diatoms, dinoflagellates, other flagellates and ciliates (Jacobson and Anderson, 1986; Gaines and Elbrachter, 1987; Hansen, 1991a,b; Jeong and Latz, 1994). Heterotrophic nanoflagellates (HNF) are assumed to feed on picoplankton (Fenchel, 1988) and maintain picoplankton populations at relatively stable concentrations in seawater (Pernthaler, 2005). Heterotrophic protists in turn are common prey for many consumers such as copepods (Vincent and Hartmann, 2001) and fish larvae (Fukami et al., 1999). Thus, nano-micozooplankton is recognized as a trophic intermediary in pelagic food webs, permitting the transfer of carbon from pico- and nanoplankton to the metazoans (microbial

* Corresponding author.
E-mail address: cdupuy@univ-lr.fr (C. Dupuy).

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food web, Sherr et al., 1986; Pierce and Turner, 1992). Nanomicozooplankton occupied an important trophic node in food webs and their dynamics may be either controlled by predation or by resource availability as well as hydrography (e.g. Sanders, 1987; Cowlishaw, 2004).

The Bay of Biscay in the North East Atlantic Ocean has temperate climatic conditions where the hydrographic conditions of the water column on the continental shelf are seasonally governed by freshwater runoff from the Loire and the Gironde rivers. These discharges create a longshore flow which may dominate a wide part of the continental shelf at the end of winter (Lazure and Jegou, 1998; Labry et al., 2001). Haline stratification combined with an increase in solar radiation may induce late-winter phytoplankton blooms (Labry et al., 2001). Such blooms have been observed in both the Loire (March 2000, Gohin et al., 2003; Lunven et al., 2005; Guillaud et al., 2008; Maguer et al., 2009) and the Gironde estuarine plumes (March 1998, Labry et al., 2001). They considerably reduce the amount of nutrients available within the surface layer, in particular phosphorus. Phosphate limitation is enhanced by an excess of nitrate in both rivers (Labry et al., 2002; Loyer et al., 2006). As a result, small-sized cells tend to dominate in spring due to their high competitiveness in such an environment (Legendre and Rassoulzadegan, 1995). Consequently, spring represents a change from a diatom-dominated winter community (Herbland et al., 1998) to one of small phytoplankton cells that are mainly grazed by microzooplankton such as ciliates or heterotrophic dinoflagellates (Sautour et al., 2000; Marquis et al., 2007).

Components of the nano- and microzooplankton community were scarcely documented and especially in the Bay of Biscay (Verity, 1987; Kamiyama and Tsujino, 1996; Modigh, 2001; Urrutxurtu et al., 2003). It is important to consider all components of the nano- and microzooplankton when attempting to elucidate their function in planktonic food web processes. To our knowledge, this is the first investigation into all the major components of nano- and microzooplankton in the Bay of Biscay (temperate coastal waters). We hypothesize whether the heterotrophic protists community is controlled by hydrography, or/and resource availability. To answer this question, we investigated the structure and the grazing impact of heterotrophic protists (ciliates, heterotrophic nanoflagellates and heterotrophic dinoflagellates) as well as the environmental factors (abiotic and biotic factors) over the continental shelf of the Bay of Biscay at different months (February, April, June and September 2004) for three contrasting sites (river plume, coastal and offshore areas).

2. Material and methods

2.1. Study site and hydrography

The continental shelf of the Bay of Biscay (Fig. 1) is up to 200 km wide with a surface area of 223,000 km^2. The hydrological structure over the shelf is principally influenced by the seasonal dynamics of the Loire and the Gironde river plumes (Lazure and Jegou, 1998). Moreover, the shelf ecology is highly variable in time related to temperate zone climatic fluctuations (Koutsikopoulos et al., 1998). Three stations of the Bay were studied in 2004 on board of R/V Thalia: 08 to 10 February, 23 to 25 April, 09 to 11 June, and 30 September to 02 October. They were located in front of the Gironde River estuary on a coast offshore triangle: "Gironde" (01°30W, 45°30N), "Coast" (01°30W, 45°01N) and "Offshore" (2°20W, 45°10N) (Fig. 1). Salinity and temperature profiles were measured using a CTD (Conductivity–Temperature–Density) probe (Sea-Bird SBE 9). Concurrently, irradiance and fluorescence profiles of the water column were measured in order to obtain the depths of the photic zone and of maximum *in situ* fluorescence and to adapt the plankton sampling accordingly.

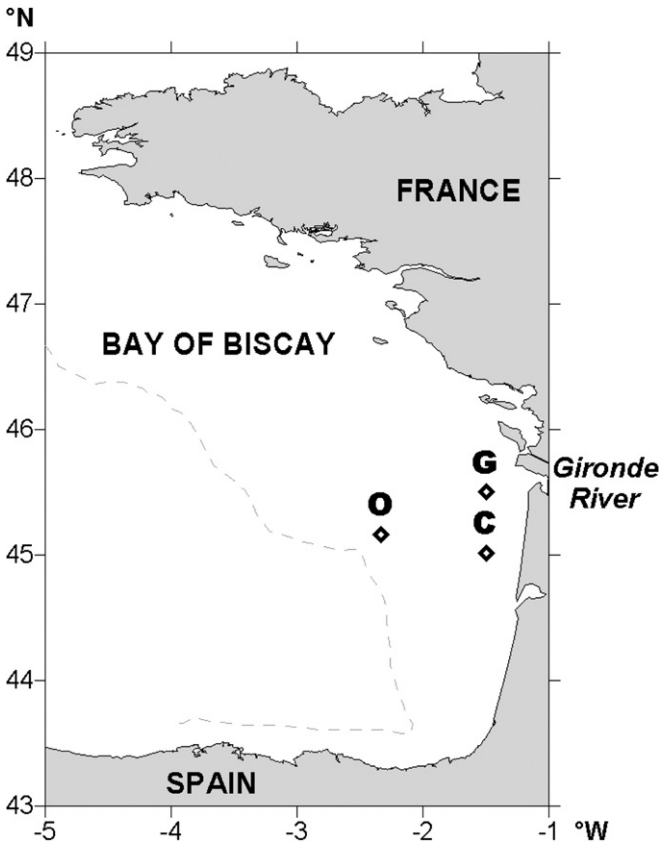

Fig. 1. Map of the Bay of Biscay with the location of the three study sites. Dashed line is a schematic representation of the continental shelf limits.

2.2. Plankton sampling and analysis

Water samples from subsurface (2 m), depth of maximum fluorescence and bottom of the photic zone were collected using 12-L Niskin bottles. Samples for dissolved inorganic nutrients [nitrate (NO_3), nitrite (NO_2), silicate ($Si(OH)_4$) and phosphate (PO_4)] were filtered immediately through Whatman GF/F filters (47 mm diameter) and the filtrate was stored at −20 °C until analysis with a Skalar autoanalyzer (Strickland and Parsons, 1972). Size-fractionated (<3 μm, 3–20 μm, >20 μm) primary production was assessed by *in situ* incubation of 300 ml seawater with ^{14}C-bicarbonate (10 μCi) from sunrise to sunset and filtered through different mesh and filter sizes, according to Labry et al. (2002). The incubations were processed on board under *in situ*-like irradiance conditions. Filters were placed in scintillation vials with 200 μl of 1 N HCl, dried overnight at 60 °C then recovered with 4 ml of scintillation cocktail before laboratory counting. Time zero filtration served as control for non-biological ^{14}C-adsorption onto the filters.

The bacterial production was estimated by the [^{3}H] methylthymidine (TdR) incorporation method (Fuhrman and Azam, 1982). Control and triplicates (20 ml) were incubated for 60 min with 20 nM final concentration of [^{3}H] TdR at *in situ* temperature. The incubations were stopped by addition of 0.75 ml de TCA à 50% and then stocked at 4 °C. In the laboratory, the precipitation of DNA was made into ice during 30 min. DNA was collected by filtration onto 0.2 μm polycarbonate membrane. Tubes were rinsed twice with cold TCA (5%), and samples were rinsed 4 times with cold TCA (5%), to eliminate all non-incorporation of [^{3}H] methylthymidine. Filters

were placed in 5 mL of scintillation fluid and analyzed by scintillation coulter (Perkin–Elmer). Rates of ^{3}H-thymidine incorporation were transformed to cell production using a conversion factor of 2.18×10^{10} cells produced per mole of thymidine incorporated (Fuhrman and Azam, 1982). Because of on-board technical problems, bacterial and primary productions were not measured in February and in April at the Offshore station at two sampling depths (maximum of fluorescence and bottom of the photic zone).

Bacteria and picophytoplankton (cyanobacteria, picoeukaryotes) were fixed with formaldehyde (final concentration 2%), frozen in liquid N_2. Flow cytometry analyses (FCM) were run with a FACSCan flow cytometer (BD-Biosciences) equipped with an air-cooled argon laser (488 nm, 15 mW). Picophytoplankton cells were detected according to their right-angle light scatter (SSC; related to cell size and structure) and their orange (FL2, 585/42 nm) and red (FL3 > 670 nm) fluorescence emissions, due to phycoerythrin and chlorophyll pigments, respectively. Beads of 1 mm (Polysciences) were used as an internal standard and simultaneously analyzed with the sample in order to normalize and to compare the cytometric signatures defined as cell fluorescence emission and apparent cell size of each different discriminated cell type. During analysis, accurate volume analyzed (generally between 250 and 500 µl) and subsequent estimations of cell numbers were calculated by measuring the remaining volume and subtracting it from the initial subsample volume (1 ml). For bacterial counts, 1 mL subsamples were incubated with the nucleic acid stain SYBRGreen I (Molecular Probes) for 15 min at room temperature in the dark (Marie et al., 1997). Total bacterial cells were enumerated measuring green fluorescence of stained bacteria by SyBRGreen I collected at 530/30 nm and their side scatter properties.

Nanoflagellates were fixed with buffered paraformaldehyde (final concentration 1%) then stained with DAPI and counted on 0.8 µm black polycarbonate filters by epifluorescence microscopy (Sherr et al., 1994). Heterotrophic nanoflagellates (HNF) were distinguished from pigmented (autotrophic) nanoflagellates (ANF) by the absence of chlorophyll fluorescence. Microphytoplankton (diatoms and dinoflagellates) was fixed with formaldehyde (final concentration 1%) plus alkaline lugol (final concentration 1%), enumerated and measured by inverse microscopy (Utermöhl, 1958). Heterotrophic and mixotrophic dinoflagellates (HDF) were determined from morphologic species recognition and relevant literature (e.g. Lessard and Swift, 1986). The cell sizes (length and width) were measured on at least 100 cells through a calibrated ocular micrometer. From cell size measurements, the mean cell volume of each taxon was calculated by equating the shape to standard geometric configurations. The cell volume was converted into carbon units (Table 1).

Ciliates were stained with alkaline lugol (final concentration 1%), counted and measured by inverse microscopy. The cell sizes (length and width) were measured for at least 100 cells through a calibrated ocular micrometer. From cell size measurements, the mean cell volume of each taxon was calculated by equating the shape to standard geometric configurations. The cell volume was converted into carbon units (Table 1). Ciliate samples from the surface and bottom of the photic zone collected in February at the Gironde station were not analyzed due to poor preservation.

Samples of metazoan microplankton were obtained by gently filtering 10 L of collected seawater through a 63 µm mesh. The retained organisms were then diluted in filtered (<63 µm) seawater and preserved in buffered formaldehyde (final concentration 2%). They were counted under a binocular microscope. The cell sizes (length and width) were measured for at least 100 cells through a calibrated ocular micrometer. From organism size measurements, the mean volume was calculated by equating the shape to standard geometric configurations. The cell volume was converted into carbon units (Table 1).

All the conversion factors and equations used to convert the abundance of pico-, nano- and microplankton into biomass were obtained from the literature (Table 1).

Mesozooplankton was collected from vertical tows through the entire photic zone using a 200 µm-mesh WP2 net, preserved in buffered formaldehyde (final concentration 2%) and counted under a binocular microscope. A second replicate was used to measure the mesozooplankton dry weight. Mesozooplankton carbon biomass was determined by multiplying dry weights of each sample by a factor of 0.38 (Bode et al., 1998).

The planktonic community was classified by size: bacteria, autotrophic cells <3 µm (cyanobacteria and picoeukaryotes), autotrophic cells between 3 and 20 µm (ANF, and small autotrophic ciliates, *Myrionecta rubra*), autotrophic cells >20 µm (diatoms and autotrophic dinoflagellates), heterotrophic cells <20 µm (very small ciliates, HNF), heterotrophic cells between 20 and 50 µm (small ciliates and heterotrophic dinoflagellates), heterotrophic cells between 50 and 100 µm (ciliates and heterotrophic dinoflagellates) and heterotrophic organisms >100 µm (nauplii, mesozooplankton).

2.3. Microzooplankton grazing experiments

Microzooplankton grazing impact was estimated using the dilution method (Landry and Hassett, 1982) in February, June and September 2004. Water samples from the « Maximum Fluorescence » depth of each station were collected using a 12-L Niskin bottle. Water was filtered through a 200 µm mesh to isolate microzooplankton fraction from larger predators (e.g. copepods). Organisms were fractionated by gravity filtration and reverse flow through nylon screens (Ferrier-Pagès and Rassoulzadegan, 1994). The filtered water was therefore handled as carefully as possible to minimize production of bubbles and physical damage to fragile microplankton organisms. Particle-free water was obtained by

Table 1
Factors and formulas with their reference used to convert biovolume to carbon mass of each plankton organisms.

Plankton organisms	Conversion factor or formulas	References
Bacteria	0.016 pgC cell^{-1}	Labry et al. (2002)
Cyanobacteria	0.104 pgC cell^{-1}	Blanchot and Rodier (1996)
Eucaryotic picophytoplankton	0.22 pgC cell^{-1}	Shinada et al. (2005)
Nanoflagellates	0.125 pgC µm^{-3} = 3.14 pgC cell^{-1} (with mean biovolume of 25.2 µm^3)	Pelegri et al. (1999) and our data of biovolumes
Dinoflagellates	$\mathrm{Log_{10}}$ C (in pgC cell^{-1}) = −0.353 + 0.864 × $\mathrm{log_{10}}$ V	Menden-Deuer and Lessard (2000)
Diatoms	$\mathrm{Log_{10}}$ C (in pgC cell^{-1}) = −0.541 + 0.811 × $\mathrm{log_{10}}$ V	<3000 µm^3
	$\mathrm{Log_{10}}$ C (in pgC cell^{-1}) = −0.933 + 0.881 × $\mathrm{log_{10}}$ V	>3000 µm^3 Menden-Deuer and Lessard (2000)
Naked Ciliates	0.19 pgC µm^{-3}	Putt and Stoecker (1989)
Ciliate Tintinnids	C (in pgC cell^{-1}) = 444.5 + 0.053 × LV	Verity and Langdon (1984)
Copepod Nauplii	pgC ind^{-3} = 0.08 × V	Gowing et al. (2003)

V: biovolume (in µm^3) and LV: Lorica volume (in µm^3).

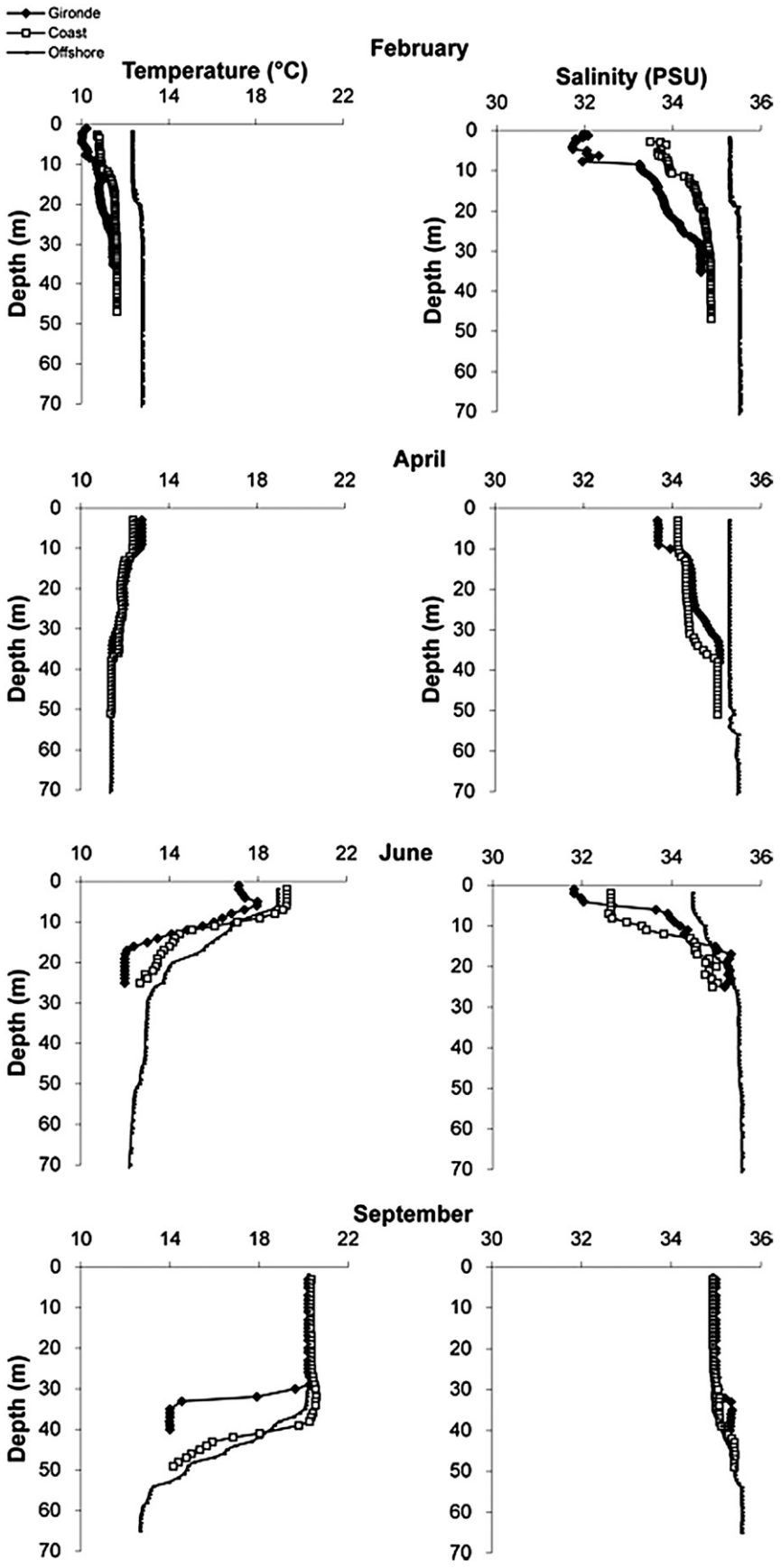

Fig. 2. Temperature (°C) and salinity (PSU) profiles of the water column at the three stations and the four sampling periods.

gently filtering a large volume of collected water, using a peristaltic pump, through a 0.2 μm serial filtration unit. The filtered seawater was diluted with particle-free seawater to obtain the following proportions: 40, 60, 80 and 100% of filtered seawater. The different dilution treatments were distributed into 1.20-L polycarbonate bottles with three replicates for each treatment. All bottles were enriched with inorganic nutrients (100 μM $NaNO_3$ and 3 μM NaH_2PO_4) to minimize nutrient limitation during the incubations (Verity et al., 1996) (see Fig. 3 for *in situ* nutrient concentrations). The bottles were incubated during 24 h on board in a tank with a circulation of surface seawater to keep the temperature constant and the bottle free to move and covered with a screen to mimic *in situ* light conditions. Samples were collected for each experiment at the beginning (t_0, undiluted treatment) and at the end (t_{24}, each treatment) of the incubation to enumerate the pico-, nano- and microplankton composition and estimate the biomass of each group as described above. The prey was classified into five groups: autotrophic cells <3 μm (cyanobacteria and eukaryotic picophytoplankton), autotrophic cells between 3 and 20 μm (ANF and small autotrophic ciliates, *M. rubra*), autotrophic cells >20 μm (diatoms and autotrophic dinoflagellates), bacteria (Bac) and heterotrophic nanoflagellates (HNF).

The grazing rates of the microzooplankton were calculated by the exponential growth model of Landry and Hassett (1982). The instantaneous rate coefficients of microzooplankton grazing (g) and prey growth (k) were estimated from the linear regression (95% of confidence limits) of the apparent growth rate:

$$P_t = P_0 e^{(k-g)t}$$

where P_t is prey carbon biomass at time t and P_0 is the initial prey carbon biomass. Both g and k were used to calculate the grazing loss of potential production, while only g was employed to calculate the daily loss of initial standing stock.

2.4. Statistical analysis

Spearman rank (r_s) correlations were performed to investigate the relationships between heterotrophic biomasses and environmental parameters. All variables were logarithmically transformed to improve the linearity and homoscedasticity of residuals. The missing data were replaced by means calculated on the entire data set in order to minimize their role in the correlation. The following parameters were considered: salinity, temperature, concentrations of N and P, N/P ratio, and biomasses of bacteria, autotrophic cells <3 μm (picophytoplankton: cyanobacteria and picoeukaryotes), autotrophic cells between 3 and 20 μm (nanophytoplankton: ANF, and small autotrophic ciliates, *Myrionecta rubra*), autotrophic cells >20 μm (microphytoplankton: diatoms and autotrophic dinoflagellates), heterotrophic cells <20 μm (very small ciliates, heterotrophic dinoflagellates, heterotrophic nanoflagellates (HNF)), between 20 and 50 μm (small ciliates and heterotrophic dinoflagellates), heterotrophic cells between 50 and 100 μm (ciliates and heterotrophic dinoflagellates) and heterotrophic organisms >100 μm (large ciliates and heterotrophic dinoflagellates). All statistical analyses were run using the statistical software Excel Stat Pro.

3. Results

3.1. Environmental conditions of abiotic parameters, primary and bacterial productions

In winter (February, Fig. 2), the waters of the Gironde and Coast stations were halostratified with a halocline close to 10 m. The halocline was less developed in April, and was the strongest in June with surface salinities of 31.8 and 32.7 PSU respectively at the Gironde and Coast stations. The surface halostratification was absent in September. Except in June, the Offshore water column did not show significant surface halostratification. The water column temperature of the three stations showed progressive warming from February to September, contributing significantly to stratification in June (thermocline at 10–20 m) and September (thermocline at 30–60 m). Overall, at all three stations, the surface mixed layer was shallowest in February and June, followed by April, and deepest in September.

The concentration of total NO (NO_2 and NO_3) ranged from 0.2 to 12.1 μmol L^{-1} on average in the photic zone of the three stations (Fig. 3), reaching the highest values in February (e.g. Gironde, 12.1 ± 1.6 μmol L^{-1}). The average concentrations of $Si(OH)_4$ at the three stations were <6 μmol L^{-1} whereas the concentrations of PO_4 were even lower (<1 μmol L^{-1}, Fig. 3). The lowest concentrations of the three studied nutrients were found in April at the Gironde and Coast stations and in June at the Offshore station. The N/P ratio was highest in April at the two coastal stations (77 in Gironde and 173 in Coast) and lowest in September (below the Redfield ratio, i.e.10 and 11, respectively). The highest N/P ratio at the Offshore station was found in September but never exceeded 13. The highest N/Si ratios were found in April at the three stations (6 at Gironde and Coast, 156 Offshore).

Depth-integrated primary production varied from 7.6 ± 1 to 27.5 ± 5.3 × 10^2 mg C m^{-2} d^{-1} (Fig. 4a), being lowest in September, and peaking either in June (Coast, Gironde) or April (Offshore). In April, microphytoplankton (>20 μm) was an important primary producer at all three stations, largely dominating at the Coast station (73% of the total integrated production, Fig. 4a). On the contrary, picophytoplankton was the main producer in September,

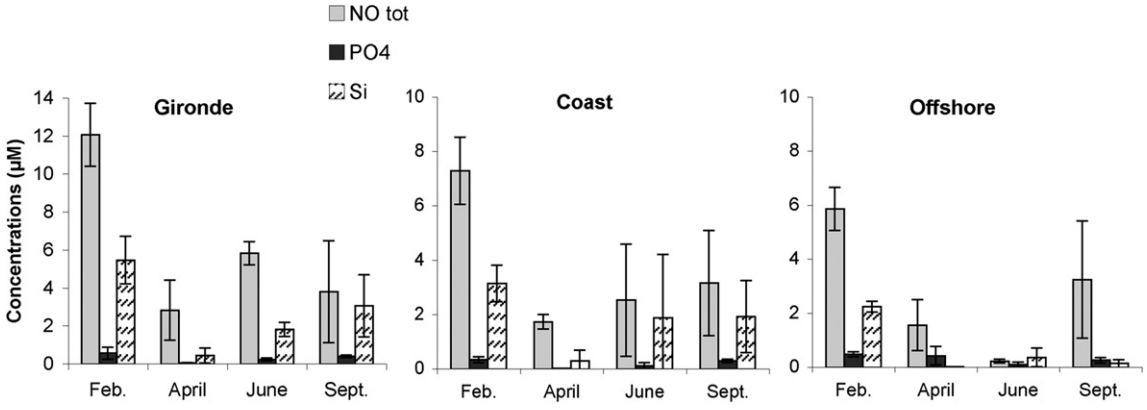

Fig. 3. Seasonal variations of nutrient concentration averages (μM) over the photic zone of the three stations: Nitrites and Nitrates (N tot), Phosphates (PO4) and Silicates (Si).

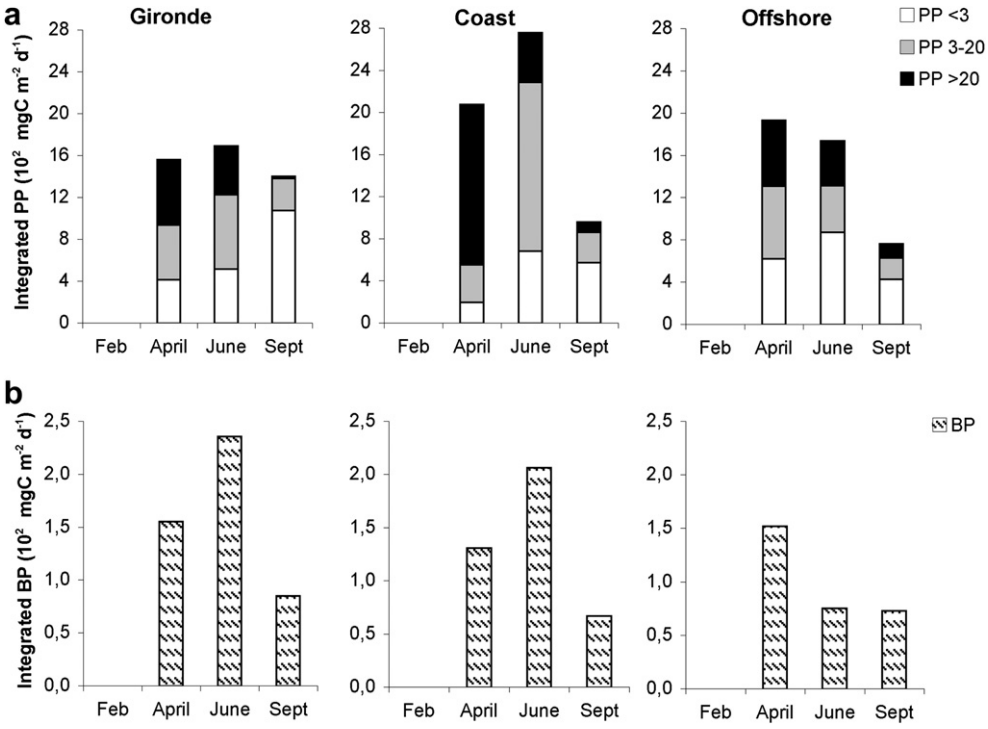

Fig. 4. Seasonal variations of the primary production by size-classes (a) and the bacterial production (b) integrated over the photic zone at the three stations. PP<3 for primary production <3 μm, PP 3–20 for primary production 3–20 μm, PP > 20 for primary production >20 μm and BP for bacterial production.

representing more than 56% of the total production at each station (Fig. 4a). Integrated bacterial production followed the pattern of primary production and was lowest in September at all three stations (66.7 ± 8.3 to 84.7 ± 7.3 mg C m^{-2} d^{-1}, Fig. 4b). Earlier in the year, it was considerably higher, with maxima in April (151.6 ± 6.2 mg C m^{-2} d^{-1} Offshore) and June (235.6 ± 14.8 and 206.1 ± 34.5 mg C m^{-2} d^{-1} respectively at Gironde and Coast).

3.2. Variation in plankton abundance and biomass

3.2.1. Autotrophic community abundance and biomass

Integrated phytoplankton biomass was maximal in February at Gironde (82.1 × 10^2 mg C m^{-2}, Fig. 5a) or in April at the Coast and Offshore (respectively 33.1 and 59.5 × 10^2 mg C m^{-2}), and low in June and September over the entire shelf (<5.8 × 10^2 mg C m^{-2} in

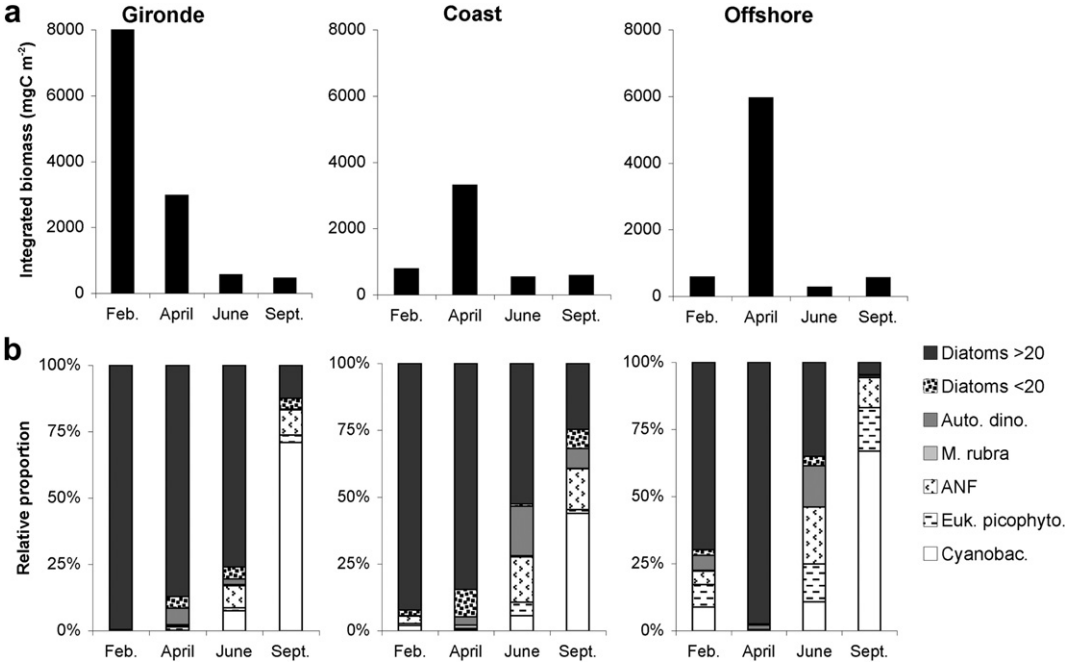

Fig. 5. Seasonal variations of integrated autotrophic biomass (a) and its relative composition (b) over the photic zone at the three stations. Diatoms <20 or >20 for diatoms <20 μm or >20 μm, Auto. Dino. for autotrophic dinoflagellates, *M. Rubra* for *Myrionecta rubra*, ANF for autotrophic nanoflagellates, Euk. Picophyto. for eukaryotic picophytoplankton, Cyanobac. for cyanobacteria.

September, Fig. 5a). Large diatoms were the main component of the autotrophic community in February, April and June at the two coastal stations and in February and April at the Offshore station (Fig. 5b). In February, diatoms were dominated by large chain-forming cells (e.g. *Thalassiosira*), illustrated by the high biomass averages (371 μg C L^{-1}, Table 2) combined with relatively low abundance (4.8×10^4 cells L^{-1}, Table 2). In contrast, smaller diatoms (e.g. *Leptocylindrus minumus*) were very abundant in April at the Coast station (biomass: 104.3 μg C L^{-1}; abundance: 50×10^4 cells L^{-1}, Table 2) and at the Offshore station (biomass: 152.7 μgC l^{-1}; abundance: 46×10^4 cells l^{-1}, Table 2). The biomass of smaller autotrophs (ANF, cyanobacteria and picoeukaryotes) proportionally increased from April to September at all three stations and dominated in September (>60% of integrated biomass, Fig. 5b).

3.2.2. Heterotrophic community abundance, biomass and composition

At the three stations, integrated biomasses of heterotrophic plankton peaked in April (23.2 and 28.2 and 15.7×10^2 mg C m^{-2} at Gironde, Coast and Offshore, respectively, Fig. 6a) and were the lowest in February (less than 9.2×10^2 mg C m^{-2}) except at the Coast station (lowest biomass in September: 17.2×10^2 mg C m^{-2}, Fig. 6a). The Offshore biomasses were consistently the lowest of the three stations.

The biomass of bacteria ranged from 5 to 21.5 μg C L^{-1} (Table 2). The biomass was highest in June and September at Gironde and Coast. Finally, the concentrations were higher at the Gironde and Coast stations than at the Offshore station. Bacteria dominated in September (>57% of the integrated biomass, Fig. 6b).

Heterotrophic nanoflagellates (HNF) were <10 μm in size and typically ranged from 2 to 5 μm. The biomass of HNF ranged from 0.02 to 0.5 μg C L^{-1} (Table 2), and was relatively higher in February and September. The biomass of heterotrophic dinoflagellates (HDF) ranged from 0.3 to 17 μg C L^{-1} (Fig. 6b; Table 2). The biomass of HDF was highest in April and June 2004, and showed a small peak in February and September. Finally, the concentrations were higher at the Gironde and Coast stations than at the Offshore station (Fig. 6b). HDF community was mainly composed of athecate dinoflagellates with the most abundant genus *Gymnodinium* spp.

Integrated ciliate biomass was highest in April at all three stations with a maximum of 15.6×10^2 mg C m^{-2} at the Coast station. Ciliate abundances, averaging from 0.2 to 9.4×10^3 cells L^{-1} over the sampling period, and the biomass followed the same pattern (Table 2). Finally, except for February, the concentrations were higher at the Gironde and Coast stations than at the Offshore station (Table 2). The ciliate community was mainly composed of naked ciliates (>82% of the total abundance). The most abundant were the genus *Strombidium*. The highest relative abundances of loricate ciliates (Tintinnina) were found in February at the Gironde and Coast stations (respectively 13 and 18% of the total ciliate abundance).

The biomass of copepod nauplii (microzooplankton metazoan) ranged from 0.2 to 3.8 μg C L^{-1} (Table 2). The values were high in February and April, decreased in June, to enhance in September. Finally, the concentrations were higher at the Gironde and Coast stations than at the Offshore station.

The biomass of mesozooplankton varied from 1.5 to 51.5 μg C L^{-1} (Table 2). The values were high in February at the Gironde and Coast stations and June at three stations and decreased in September. In fact, close to the coast, mesozooplankton dominated in winter (>65% of the total heterotrophic biomass at Gironde and Coast). In June, it dominated (>47%) at all three stations (Fig. 6b). At the coast, mesozooplankton was mainly composed of appendicularians in winter and of small copepods (e.g. *Oithona* sp.) in June. In Offshore, mesozooplankton was dominated by large copepods (e.g. *Calanus* sp.).

Table 2
Seasonal abundances (bold font in cells L^{-1}) and biomasses (in μgC L^{-1}), with their standard deviations, of the different plankton components in average over the photic zone at the three stations.

Station	Month	Ciliates (10^3 cells L^{-1})	Bacteria (10^8 cells L^{-1})	Cyanobacteria (10^6 cells L^{-1})	Picoeukaryotes (10^6 cells L^{-1})	HNF (10^4 cells L^{-1})	ANF (10^4 cells L^{-1})	Dinoflagellates (10^3 cells L^{-1})	Diatoms (10^4 cells L^{-1})	Metazoan microplankton (ind L^{-1})	Mesozoo. (ind L^{-1})
Gironde	February	0.18 ± 0.00	6.55 ± 0.19	6.38 ± 0.65	1.48 ± 0.08	12.75 ± 1.56	21.19 ± 5.88	2.01 ± 1.16	4.82 ± 2.85	66.12 ± 15.58	4.55
		(0.2 ± 0.0)	(10.5 ± 0.3)	(0.7 ± 0.1)	(0.3 ± 0.0)	(0.4 ± 0.0)	(0.7 ± 0.2)	(4.9 ± 3.5)	(370.9 ± 378.2)	(3.4 ± 1.5)	(25.7)
	April	4.67 ± 1.47	5.17 ± 0.92	1.68 ± 0.18	4.72 ± 5.73	5.48 ± 2.12	19.26 ± 2.43	21.56 ± 10.56	16.91 ± 1.59	81.52 ± 13.11	6.61
		(12.1 ± 7.2)	(8.3 ± 1.5)	(0.2 ± 0.0)	(1.0 ± 1.3)	(0.2 ± 0.1)	(0.6 ± 0.1)	(21.6 ± 4.6)	(72.7 ± 48.6)	(3.5 ± 0.3)	(33.6)
	June	3.83 ± 3.00	11.75 ± 3.63	16.06 ± 14.25	3.53 ± 2.98	2.66 ± 3.41	57.64 ± 35.57	10.43 ± 8.80	17.27 ± 17.96	52.37 ± 23.07	29.88
		(10.9 ± 7.9)	(18.8 ± 5.8)	(1.7 ± 1.5)	(0.3 ± 0.1)	(0.1 ± 0.4)	(1.8 ± 1.1)	(16.4 ± 12.9)	(17.3 ± 11.9)	(2.5 ± 1.1)	(41.8)
	September	0.64 ± 0.51	13.42 ± 2.13	74.34 ± 23.17	2.59 ± 1.20	16.24 ± 11.64	59.75 ± 32.65	0.45 ± 0.36	1.12 ± 0.75	115.02 ± 57.10	7.04
		(1.8 ± 0.8)	(21.5 ± 3.4)	(7.7 ± 2.4)	(0.3 ± 0.0)	(0.5 ± 0.4)	(0.9 ± 0.4)	(0.3 ± 0.2)	(1.9 ± 1.4)	(3.8 ± 1.9)	(13.4)
Coast	February	1.80 ± 0.87	6.65 ± 0.90	5.00 ± 1.80	0.90 ± 0.38	14.64 ± 6.98	25.42 ± 11.60	0.52 ± 0.31	2.58 ± 0.81	38.63 ± 44.08	4.00
		(5.4 ± 5.0)	(10.6 ± 1.4)	(0.5 ± 0.2)	(0.2 ± 0.1)	(0.5 ± 0.2)	(0.8 ± 0.4)	(0.7 ± 0.7)	(33.3 ± 11.8)	(1.6 ± 1.7)	(40.8)
	April	9.44 ± 5.48	3.12 ± 0.58	1.91 ± 0.51	1.82 ± 1.66	3.01 ± 0.69	12.21 ± 1.99	15.25 ± 8.15	49.90 ± 4.21	119.35 ± 57.93	3.94
		(42.8 ± 37.5)	(5.0 ± 0.9)	(0.3 ± 0.1)	(0.4 ± 0.4)	(0.1 ± 0.0)	(0.4 ± 0.1)	(16.6 ± 9.7)	(104.3 ± 19.5)	(3.2 ± 0.4)	(29.0)
	June	4.26 ± 3.28	11.40 ± 3.51	12.55 ± 4.47	2.31 ± 1.76	1.55 ± 0.74	125.17 ± 66.94	10.52 ± 5.96	1.99 ± 1.10	25.94 ± 32.73	14.17
		(9.5 ± 4.4)	(18.2 ± 5.6)	(1.3 ± 0.5)	(1.2 ± 0.5)	(0.0 ± 0.0)	(3.9 ± 2.5)	(9.3 ± 8.1)	(13.1 ± 12.7)	(1.4 ± 1.8)	(51.5)
	September	0.51 ± 0.55	9.23 ± 2.76	41.59 ± 43.94	1.01 ± 0.09	2.48 ± 1.98	59.41 ± 12.19	1.57 ± 0.85	0.95 ± 0.59	46.12 ± 15.77	4.03
		(1.0 ± 1.1)	(14.8 ± 4.4)	(4.3 ± 4.6)	(0.2 ± 0.1)	(0.1 ± 0.1)	(1.9 ± 04)	(1.5 ± 1.2)	(5.0 ± 2.9)	(1.9 ± 1.5)	(14.4)
Offshore	February	2.60 ± 1.39	5.57 ± 0.37	14.07 ± 4.43	6.20 ± 1.41	15.36 ± 3.43	27.90 ± 1.72	8.53 ± 1.78	1.87 ± 1.09	40.08 ± 12.92	0.30
		(3.5 ± 2.4)	(8.9 ± 0.6)	(1.5 ± 0.5)	(1.4 ± 0.3)	(0.5 ± 0.1)	(0.9 ± 0.1)	(4.5 ± 2.0)	(11.7 ± 6.3)	(2.4 ± 0.8)	(1.5)
	April	4.30 ± 1.74	4.90 ± 0.93	0.24 ± 0.10	0.19 ± 0.11	1.28 ± 0.86	7.34 ± 1.94	11.37 ± 3.72	46.05 ± 13.57	44.91 ± 25.38	1.69
		(19.2 ± 14.0)	(7.8 ± 1.5)	(0.0 ± 0.0)	(0.0 ± 0.0)	(0.0 ± 0.0)	(0.2 ± 0.1)	(9.8 ± 5.5)	(152.7 ± 34.2)	(1.0 ± 0.4)	(5.1)
	June	0.47 ± 0.10	5.43 ± 2.41	13.50 ± 10.27	6.67 ± 6.44	1.68 ± 1.15	86.93 ± 3.55	6.45 ± 3.57	1.08 ± 0.94	19.75 ± 14.55	5.81
		(2.0 ± 2.2)	(8.7 ± 3.9)	(1.4 ± 1.1)	(1.8 ± 0.1)	(0.1 ± 0.0)	(2.7 ± 0.1)	(7.7 ± 4.5)	(3.7 ± 3.6)	(1.0 ± 0.7)	(32.9)
	September	0.49 ± 0.32	6.55 ± 2.83	45.19 ± 25.22	0.68 ± 0.54	0.63 ± 0.49	40.77 ± 17.75	2.67 ± 2.35	0.21 ± 0.25	3.43 ± 2.82	0.97
		(1.6 ± 1.7)	(10.5 ± 4.5)	(4.7 ± 2.6)	(1.4 ± 0.3)	(0.0 ± 0.0)	(1.0 ± 0.5)	(1.7 ± 1.3)	(0.9 ± 1.2)	(0.2 ± 0.1)	(4.9)

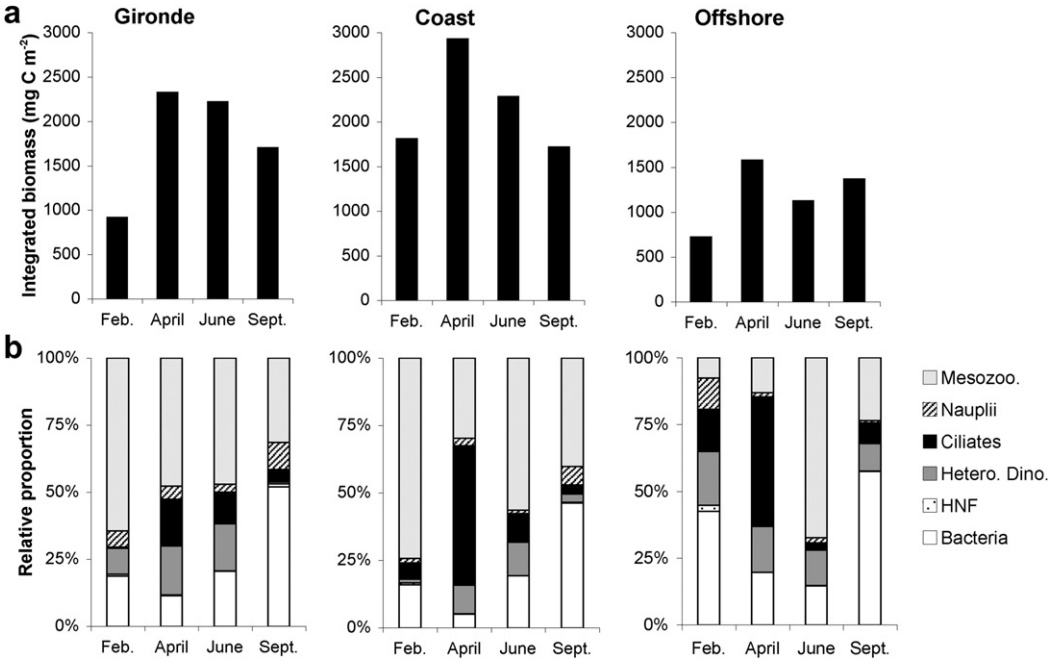

Fig. 6. Seasonal variations of integrated heterotrophic biomass (a) and its relative composition (b) over the photic zone at the three stations. Mesozoo. for mesozooplankton, HNF for heterotrophic nanoflagellates, Hetero Dino. for heterotrophic dinoflagellates.

Ciliates and heterotrophic dinoflagellates dominated the integrated heterotrophic protist biomass in April particularly at the Coast and Offshore stations (Fig. 6b) with high percentage of ciliates. Heterotrophic nanoflagellates were consistently present, but never dominant (<2% of integrated biomasses, Fig. 6b).

3.2.3. Ratio heterotrophy/autotrophy

The heterotrophy/autotrophy ratio was calculated using the biomass data of every group (Fig. 7). In February, the heterotrophy/autotrophy ratio was close to 1 at Coast and Offshore sites which corresponded to an equilibrium between biomass of heterotrophic and autotrophic organisms. The ratio was very low at Gironde site, corresponding to the high bloom of diatoms in post-winter. In April, the ratio was lower at Coast and Offshore (0.5 and 0.3 respectively), corresponding to the large development of diatoms. In Gironde, the ratio was superior to 1 (1.5), in relation to a diminution of the biomass of diatom and a presence of ciliates and HDF. In June and September, the ratio was largely >1 at every site (higher in Gironde, 6.5 to 10), due to the bloom of heterotrophic organisms (bacteria, microzooplankton and mesozooplankton) and the bloom of small autotrophic cells. The HNF were abundant but the biomass was so low that it did not impact the heterotrophy/autotrophy ratio.

3.3. Correlation relationships between heterotrophic nano-microplankton, and size-fractionated phytoplankton and environmental factors

Correlations indicate potential relationships between organisms and give the direction of relationships. For the entire data set (all depths, stations and sampling periods combined), temperature showed no correlation with any heterotrophic nano-microplankton group (Table 3). Salinity was negatively correlated with HDF. NO_3 concentration was correlated with HNF biomass. PO_4 concentration was positively correlated with HNF and negatively correlated with ciliate biomass. Ciliates were significantly correlated with N/P ratio, microphytoplankton production and biomass and nanophytoplankton biomass (Table 3). HDF were correlated to microphytoplankton production and bacterial production, and negatively correlated to picophytoplankton biomass. The correlations were also calculated with size-class of heterotrophic protists (HNF, HDF and ciliates). All the size-classes of heterotrophic protists were correlated with >20 μm primary production. The <20 μm size-fraction of heterotrophic protists was positively correlated with nitrites, N/P ratio and microphytoplankton biomass and negatively correlated with PO_4 concentration. The 20–50 μm size-fraction of heterotrophic protists, dominant in this study, was positively correlated with N/P ratio and bacterial production and negatively correlated with salinity, PO_4 and picophytoplankton biomass. The 50–100 μm size-fraction was correlated by all size of primary production and bacterial production and negatively correlated with salinity. The >100 μm size-fraction was correlated with N/P ratio and microphytoplankton biomass.

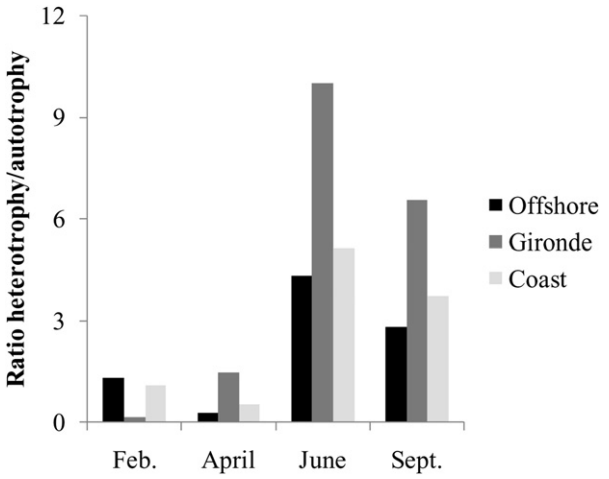

Fig. 7. Heterotrophic/autotrophic biomass ratio over the photic zone at the three stations.

Table 3
Simple (rs, Spearman rank) correlation coefficients between heterotrophic protists biomasses and environmental parameters (physical and chemical conditions and phytoplankton and bacteria biomass and production).

A (Community structure)			
	HNF	Ciliates	HDF
Temp			
Sal			−0.555**
Nitrites			
Nitrates	0.466*		
Phosphates	0.447*	−0.417*	
Nitrogen/phosphorus		0.562**	
Microphyto		0.604**	
Nanophyto		0.449*	
Picophyto			−0.423*
Bacteria	0.426*		
<3 μm primary production			
3–20 μm primary production			
>20 μm primary production		0.684***	0.582**
Bacterial production			0.721***

B (size structure)				
	Heterotrophic protists			
	<20 μm	20–50 μm	50–100 μm	>100 μm
Temp				
Sal		−0.588*	−0.764***	
Nitrites	0.484*			
Nitrates				
Phosphates	−0.474*	−0.428*		
Nitrogen/Phosphorus	0.776***	0.512*		0.506*
Microphyto	0.605**			0.542**
Nanophyto				
Picophyto		−0.465*		
Bacteria				
<3 μm primary production			0.482*	
3–20 μm primary production			0.421*	
>20 μm primary production	0.452*	0.694***	0.628**	0.537**
Bacterial production		0.702***	0.907***	

Significant correlations were defined as ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$. Temp.: temperature; Sal: salinity; Microphyto: microphytoplankton; Nanophyto: nanophytoplankton; Picophyto: picophytoplankton.

3.4. Grazing experiment

Phytoplankton growth and grazing coefficients (Table 4) show that the relationship between the apparent growth rate and the dilution factor was linear ($p < 0.05$). Phytoplankton growth rate ranged from 0.12 to 4.58 d^{-1}, while microzooplankton grazing ranged from 0.10 to 3.25 d^{-1}. This level of grazing corresponds to a daily loss varying between 50 and more than 100% of the daily potential primary production.

Bacteria and heterotrophic nanoflagellates growth and grazing coefficients are shown in Table 5. Bacteria growth rate ranged from 0.3 to 1.96 d^{-1}, while microzooplankton grazing ranged from 0.73 to 1.98 d^{-1}. This level of grazing corresponds to a daily loss varying between 62 and >100% of the daily potential bacteria production. Heterotrophic nanoflagellates growth rate ranged from 1.06 to 3.72 d^{-1}, while microzooplankton grazing ranged from 0.62 to 4.95 d^{-1}. This level of grazing corresponds to a daily loss varying between 48 and more than 100% of the daily potential heterotrophic nanoflagellates production.

4. Discussion

During our study period (i.e. 2004), the hydrography of the continental shelf of the Southern Bay of Biscay followed seasonal variations that appeared to be largely influenced by the Gironde plume, in a pattern similar to that described by Lazure and Jegou (1998). The seasonal runoff variation influenced both density stratification and photic zone depth (through turbidity). Our data showed that, across the shelf, the photic zone deepened as the runoff signal decreased, between February and April (Figs. 2 and 8), and then was shallower again in June after the increasing runoff in May (Fig. 8), while finally showing typical offshore summer conditions (deep photic zone with deep thermal stratification) in September. Furthermore, the thermohaline profiles suggest that the photic zone depth exceeded the depth of the surface pycnoclines throughout the study period at all three stations.

4.1. Heterotrophic protists abundance, biomass and composition in the Bay of Biscay

The variations of the heterotrophy/autotrophy ratio were similar to typical patterns found in temperate ecosystems: a low ratio in winter and spring corresponding to the bloom of primary producers, and a high ratio in June corresponding to the bloom of grazers, such as mesozooplankton (Fig. 6) (Legendre and Rassoulzadegan, 1995; Lunven et al., 2005; Maguer et al., 2009).

Heterotrophic protist abundance and biomass showed strong variations at the three stations with highest values in mid-spring (April) and lowest in late summer (September, Fig. 6). This pattern is comparable to that observed in other temperate areas (e.g. Nielsen and Kiorboe, 1994; Johansson et al., 2004).

The range of measured ciliate abundances and biomass is similar to ciliate abundance commonly found in coastal and oceanic ecosystems (e.g. Montagnes et al., 1988; Dolan, 1991; Leakey et al., 1992; Chiang et al., 2003; Johansson et al., 2004) and encompasses concentrations observed previously in the Bay of Biscay (Vincent and Hartmann, 2001). The range of measured HDF abundances and biomass is a little higher than HDF abundance found in Northern Arabian Sea (Garisson et al., 1998). The range of measured HNF abundances and biomass is 10x higher all the year

Table 4
Summary of seasonal dilution experiment data for microphytoplankton (Micro, diatoms and autotrophic dinoflagellates), nanophytoplankton (Nano, autotrophic nanoflagellates and *Myrionecta rubra*) and picophytoplankton (Pico, eukaryotic picophytoplankton and cyanobacteria). The data include growth (Growth coef.), grazing rates (Grazing coef.), correlation coefficient (R^2), percentage of primary production consumed per day (PP consumed).

			Growth coef (d^{-1})	Grazing coef (d^{-1})	R^2	PP consumed (%)
February	Gironde	Micro	–	–	0.58*	–
		Nano	1.16	1.23	0.42*	105.4
		Pico	0.18	0.13	0.73*	74.6
	Coast	Micro	1.07	0.97	0.69*	90.4
		Nano	1.65	1.63	0.64*	99
		Pico	0.15	0.11	0.73*	69.1
	Offshore	Micro	1.47	1.49	0.51*	101.2
		Nano	–	–	0; 47*	–
		Pico	–	–	0.45*	–
June	Gironde	Micro	0.77	0.82	0.37	106.4
		Nano	1.26	2.36	0.91**	187
		Pico	1.11	1.58	0.79**	141.9
	Coast	Micro	1.77	0.88	0.45*	49.6
		Nano	–	1.18	0.58*	–
		Pico	–	–	0.38*	–
	Offshore	Micro	4.58	3.25	0.48*	70.9
		Nano	–	–	0.68*	–
		Pico	–	–	0.5*	–
September	Gironde	Micro	2.97	2.04	0.45*	68.7
		Nano	1.09	1.47	0.65*	135.8
		Pico	0.23	0.47	0.75**	204.6
	Coast	Micro	–	–	0.08	–
		Nano	1.26	1.12	0.48*	89.4
		Pico	–	–	0.52*	–
	Offshore	Micro	1.97	2.15	0.32*	109.3
		Nano	0.51	1.66	0.33*	328.2
		Pico	0.12	0.10	0.62*	90.8

Table 5
Summary of seasonal dilution experiment data for bacteria (Bac) and heterotrophic nanoflagellates (HNF). The data include growth (Growth coef.), grazing rates (Grazing coef.), correlation coefficient (R^2), percentage of production consumed per day.

			Growth coef (d^{-1})	Grazing coef. (d^{-1})	R^2	Production consumed (%)
February	Gironde	Bac	1.41	1.3	0.73*	92.6
		HNF	–	–	0.31	–
	Coast	Bac	0.97	1.05	0.41*	108.3
		HNF	1.06	2.07	0.69*	196.1
	Offshore	Bac	1.96	1.33	0.46*	67.6
		HNF	2.97	3.29	0.7*	111
June	Gironde	Bac	0.87	–	0	
		HNF	3.72	4.95	0.56*	133.1
	Coast	Bac	0.81	0.73	0.29	90
		HNF	2.41	1.15	0.27	47.9
	Offshore	Bac	1.78	1.11	0.35*	62.5
		HNF	1.28	0.62	0.13	48.4
September	Gironde	Bac	0.3	–	0.42*	
		HNF	1.49	1.5	0.84**	101
	Coast	Bac	1.45	1.98	0.87**	137.2
		HNF	1.52	1.25	0.67*	81.9
	Offshore	Bac	0.77	–	0.34	–
		HNF	3.64	3.72	0; 91**	102.3

The significance of the regression is showed with ** for $p < 0.001$, * for $p < 0.05$ and italic font for non-significant correlation. – stands negative values.

for the zones of Bay of Biscay compared to data of 6 oceans (Massana et al., 2006).

The composition of heterotrophic protists showed that naked oligotrichs dominated the ciliate abundances at the three stations over the sampling periods, as found in open waters (Leakey et al., 1992; Nielsen and Kiorboe, 1994; Fileman and Leakey, 2005). Tintinnids were not numerous in the Bay of Biscay (0–318 cells L^{-1}) and are more concentrated in estuaries than in open waters (e.g. Urrutxurtu et al., 2003). HDF were dominated by the genus *Gyrodinium* which are common in temperate coastal waters (Yang et al., 2008).

4.2. Heterotrophic protists community in relation to environmental parameters

In many previous studies, temperature, food availability and predator density seemed responsible in structuring the heterotrophic protist distribution, abundance and composition (e.g. Sanders, 1987; Nielsen and Kiorboe, 1994; Urrutxurtu et al., 2003). This leads to the question of which parameters are the most likely to structure the community of heterotrophic protists in the Bay of Biscay. Although the correlations do not necessary mean directly causal relationships such as trophic links, they can give information on the biological parameters that influenced the heterotrophic protists. In addition, the microzooplankton grazing experiments, using the dilution method, allow determination of the grazing pressure on different size-classes of prey.

4.2.1. Abiotic factors influence

Several authors have identified temperature as a key parameter influencing the rate of numerical response of heterotrophic protists to increases in phytoplankton due to temperature-enhancement growth and feeding (e.g. Sherr et al., 1988; Montagnes, 1996). However, in this study, we did not find significant correlations between heterotrophic protist biomass and temperature (Table 3). In June at Offshore, the water column was thermostratified (Fig. 2) which prevented subsurface-water nutrient injection and thus prohibited the development of resources for heterotrophic protists (low primary and bacterial productions). However, one may consider that the increase of temperature during spring (due to increasing thermostratification between April to June, Fig. 2) may have facilitated a rapid assimilation of primary production by heterotrophic protists especially ciliates (James and Hall, 1995). However, as shown previously, freshwater runoff, and thus salinity, is a major factor structuring the depth and development of the pyknocline over the shelf (Gironde and Coast). Sanders (1987) described a negative correlation with salinity and microzooplankton that may illustrate the higher abundances in waters where river runoff is significant. Our study showed that this is the case for HDF and 20–100 μm size-class heterotrophic protists. However, ciliate biomasses and 2–50 μm and >100 μm size-class heterotrophic protists were not correlated to salinity but were negatively and significantly correlated to PO_4 concentrations or positively correlated to N/P ratio, conditions of river runoff (Table 3). In this part of the Bay of Biscay (coast and continental shelf), Labry et al. (2002) showed that phosphate limitation is provoked by the development of a winter diatom bloom in the Gironde plume. Therefore, lower concentrations of phosphate or a high N/P ratio in winter-spring could indicate a recent development of microphytoplankton (mainly diatoms), as was observed in our study through high diatom biomasses in winter in Gironde (Figs. 3 and 5). In addition, ciliate biomass was correlated the nano-microphytoplankton (Table 3). Our results thus suggest that the conditioning of the shelf water mass dynamics through freshwater runoff influenced the development of heterotrophic protist biomass together with other biological variables, with temperature being a secondary, non-significant factor.

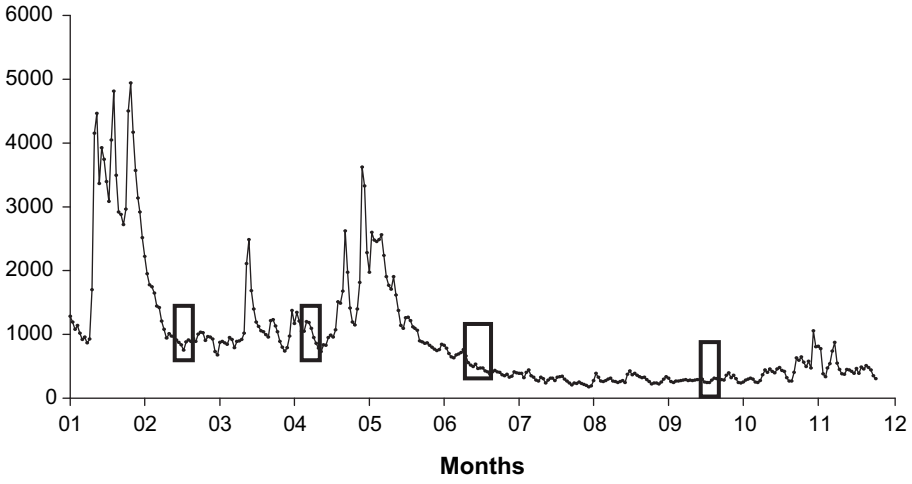

Fig. 8. Gironde river discharge (m^3 s^{-1}) over the year 2004. Red square: sampling date.

4.2.2. Biological influences

4.2.2.1. Resource availability. Heterotrophic protists consume a wide range of prey items from bacteria (Sherr and Sherr, 1987), to nanophytoplankton (e.g. Burkill, 1982; Dolan and Coats, 1990) and diatoms (Sime-Ngando et al., 1995). In our study, the number of biomass doublings per day was lower than 3 in most of the cases for phytoplankton, bacteria and HNF. The very elevated numbers of biomass doublings per day found for the microphytoplankton in Offshore in June and in Gironde in September (6.6 and 4.3) were due to a very low initial biomass and high growth rates. Those situations may be more due to a bottle effect (e.g. addition of nutrients) than to *in situ* reality. In fact, phytoplankton growth is around 0.7 d^{-1} in temperate habitats, and higher (0.72 d^{-1}) in tropical/subtropical habitats (Calbet and Landry, 2004) but always lower in comparison to our data.

In our study, heterotrophic protists started to develop immediately after or during the peak of the microphytoplankton bloom, i.e. in April, when a mix of small and large sized-food began to develop (positive correlation with ciliates and nano-microphytoplankton biomass, Table 3). The dilution experiment confirmed that in general there was a high predation pressure on nanophytoplankton and a moderate one on microphytoplankton (Table 4). In addition, the entire set of heterotrophic protist (except HNF) biomasses were positively correlated to microphytoplankton production. Verity (1987) and Lynn and Montagnes (1991) argued that the distribution of ciliates generally shows a close association with nanophytoplankton in temperate coastal ecosystems. Such a pattern was also described in the Northwestern Indian Ocean (Leakey et al., 1992), in Lake Erie (Hwang and Heath, 1997) and in Gyeonggi Bay, Yellow Sea (Yang et al., 2008).

In late summer (September), when the water column was deeply stratified over the shelf and primary and bacterial productions were minimal, heterotrophic protist biomasses and abundances were also the lowest. Despite the availability of picoplankton, especially cyanobacteria at this time (i.e. peak of cyanobacteria biomass and abundance, Fig. 5b), the picoplankton was little grazed, confirmed by dilution experiment (Table 4), the absence of correlation with picoplankton biomass and production, and a negative correlation with HDF and major fraction 20–50 μm of heterotrophic protists (Table 3). We argue that this potential prey was not sufficiently grazed to maintain a high heterotrophic protist production in late summer on the shelf. In winter, heterotrophic protist biomass may have been low due too to the lack of suitable food (biomass dominance of large chain-forming diatoms and insufficient HDF to graze the bloom, Table 2). In many previous studies, heterotrophic protist abundance has been described as a function of food availability (e.g. Dolan and Coats, 1990).

Heterotrophic bacteria appear to be potentially important prey for heterotrophic protists. HNF are generally considered to be the main consumers of bacteria (e.g. Hagström et al., 1988; Shinada et al., 2003). Our data indicate this trophic link by a significant correlation between HNF and bacterial biomasses. However, ciliates may also be able to consume bacteria in high quantity (e.g. Sherr and Sherr, 1987). However, in Chesapeake Bay, the microphagous ciliate abundance (mostly bacterivorous species) was not related to bacterial biomass despite an obvious trophic link (Dolan, 1991). In such a situation, the heterotrophic protist biomass would be more related to bacterial productivity than to biomass. This is the case in our study: HDF and 20–100 μm size-class heterotrophic protists were correlated with bacterial production (Table 3). The observed correlation between heterotrophic protists and bacterial production may then reveal a double link: first, a direct feeding link (driven by bacterivorous ciliate species) and secondly by a trophic link between ciliates and HNF. These conclusions were confirmed by our grazing experiment. The predation of microzooplankton on HNF led to lower grazing rates on bacteria in the dilution experiments with microzooplankton. However, the growth rates of bacteria were always superior to the grazing rates (Table 5, Fonda Umani and Beran, 2003). That situation corresponded to a lower relationship between grazing activity of microzooplankton and growth of bacteria and heterotrophic nanoflagellate populations. These results may demonstrate a trophic cascade operating in the incubation bottles, with the grazing of bacteria by HNF.

4.2.2.2. Predation. Predation is an important potential structuring factor of the heterotrophic protists (Sanders, 1987). Although we did not study the predation pressure of metazoans on heterotrophic protists, we showed that in winter (February) along the Bay's inner shelf (Gironde plume and Coast), mesozooplankton density was relatively important (maxima up to 66 ind L^{-1}) and ingestion rates ranged between 0.22 and 0.33 μg C of ciliates ind^{-1} d^{-1} (Vincent and Hartmann, 2001). The consumption of heterotrophic protits by mesozooplankton on heterotrophic protists can be high. In June, when heterotrophic protists biomass declined, mesozooplankton density peaked (maxima up to 100 ind L^{-1}). As a consequence, we can predict the predation rates on heterotrophic protists could be high and greater than the winter values (Hartmann and Nejstgaard, pers. comm.) (Table 2). By contrast, in April, resource availability may have been the dominant structuring factor for the heterotrophic protists, since their biomass peaked while mesozooplankton biomass was less important (Table 2).

5. Conclusions

Overall, heterotrophic protists community did not differ from the typical pattern found in temperate areas where Gironde runoff influence is the highest (Gironde station) until the limits of the continental shelf. Among the three stations studied, heterotrophic protist followed a succession with highest biomass in spring, and lowest biomass under strong summer conditions. Heterotrophic protist community was predominantly constrained by bottom-up control in spring and in the end of summer (food availability and quality).

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