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To cite this version:

Christine Dupuy, Annie Pastoureaud, Mireille Ryckaert, Pierre-Guy Sauriau, Hélène Montanié. Impact of the oyster Crassostrea gigas on a microbial community in Atlantic coastal ponds near La Rochelle. Aquatic Microbial Ecology, 2000, 22 (3), pp.227-242. 10.3354/ame022227 . hal-01248027

HAL Id: hal-01248027

https://hal.science/hal-01248027v1

Submitted on 26 Dec 2016

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Impact of the oyster *Crassostrea gigas* on the microbial community in Atlantic coastal ponds near La Rochelle

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ABSTRACT

To assess the *in situ* impact of oysters, *Crassostrea gigas*, on planktonic protist and bacteria communities and the potential contribution of protozoa to their food resource intake, the abundance and the diversity of protists and bacteria were followed in 2 Atlantic coastal ponds, with and without oysters. The protist biomass in such ponds was high, with a maximum in spring of 982 µg C l^{-1} and a minimum in winter of 179 µg C l^{-1}. Whatever the season, the presence of oysters (20 m^{-2} corresponding to on average 23 mg dry weight m^{-2}) induced a significant decrease in > 5 µm protist abundance. On the contrary, planktonic organisms < 5 µm, such as Chlorophyta flagellates and bacteria, developed similarly in both ponds. It can be assumed that such a depletion in micro-sized protists was especially related to the grazing activity of *C. gigas*, which efficiently retains > 5 µm particles. In spring, oyster grazing triggered dramatic changes in the protist community by lowering the taxonomic diversity. In autumn and winter, the presence of oysters deeply influenced the taxonomic structure of the protist communities: > 5 µm protists could only develop in the control pond, whereas they were removed by filtration into the oyster pond; on the contrary, < 5 µm protists that were not retained, were favoured in the oyster pond. The set of results showed that hetero/mixotrophic protists represent an important potential resource in coastal ponds: flagellates > 5 µm were the main protist resource for *C. gigas*; ciliates represented the second resource with a substantial contribution in autumn; diatoms and dinoflagellates, though efficiently removed represented a weak carbon resource. Our study supports the hypothesis that oysters may access to the strong bacterioplanktonic production through hetero/mixotrophic protists, which would thus allow the transfer of carbon from the microbial loop towards *C. gigas.*

Key-words: Bivalve, oysters, food source, coastal pond, microbial food web, protists, bacteria, trophic link

INTRODUCTION

Charente-Maritime, along the French Atlantic coast, is the most important oyster farming area in Europe: shellfish cultures extend over lower parts of tidal flats (4,800 ha) where oysters are grown from larvae to adult size. They are also located in land-based oyster ponds (3,000 ha, mainly in the Marennes-Oléron area) traditionally used for fattening and greening marke table-sized oysters (Korringa 1976). Tidal flats and coastal ponds are two hydrodynamically contrasting systems. Broad muddy bays suffer high and variable levels of turbidity, due to the influence of tidal currents and wind-induced resuspension. Seasonal and spatial variations of seston quantity and quality, together with their effects on bivalve nutrition, have been studied on numerous occasions in the Marennes-Oléron Bay (Héral et al. 1987, Zurburg et al. 1994, Pastoureaud et al. 1996, Hawkins et al. 1998).

In contrast, ponds are earth basins with a clay bottom and renewal of sea water occurrs for a few days at each spring tide. Once a pond has been filled with new coastal waters, which is loaded with silts, nutrients and coastal planktonic assemblages, it functions like a closed-system during the subsequent neap-tide period: silts settle, so that shallow waters become clear again and phytoplanktonic and/or microphytobenthic species can develop. Fattening of oysters has been achieved through feeding on rich phytoplankton and resuspended microphytobenthic communities, as previously reported by studies mainly focused on the phytoplanktonic growth potential of ponds (Robert et al. 1979, Robert 1983, Zanette 1980, Chrétiennot-Dinet & Guillocheau 1987, Turpin et al. 1999) and the bacterial biomass and production (Delmas et al. 1992). However, in semi-closed systems, such as ponds, where nutrients are quickly exhausted, phytoplankton and phytobenthos cannot entirely satisfy energy requirements of oysters (Héral 1987). Bacterioplankton accounts for between 17 and 50 % of the planktonic organic carbon biomass in coastal ponds (Frikha et al. 1987, Delmas et al. 1992), but oysters cannot feed on such picocells, which are not retained by their gills (Barillé et al. 1993). Besides phytoplanktonic diatoms (Fiala-Médioni et al. 1983), phytoflagellates (Barillé et al. 1993) and toxic dinoflagellates (Bardouil et al. 1996), oysters retain *in vitro* cultured ciliates (Le Gall et al. 1997) or a natural ciliate and flagellate planktonic community (Dupuy et al. 1999): accordingly, protozoa may transfer carbon and probably nitrogen from the microbial food web towards upper trophic levels.

This grazing activity may deeply influence the abundance and composition of water-column microbiota (Kemp et al. 1990, Riemann et al. 1990, Baker et al. 1998). Oysters obtain energy resources by filtering suspended particles from sea water and their growth depends upon the nutritive value of the retained seston (Berg & Newell 1986) and the trophic capacity of coastal waters (Héral 1987). Oysters use a wide variety of living cells as well as protists (e.g. phytoplankton, protozoa) and detritus (Riera & Richard 1996). One of the main feeding processes that can be involved in this impact is the differential particulate uptake dependent on their size (Valh 1972, Riisgård 1988, Stenton-Dozey & Brown 1992) and their quality (Shumway et al. 1985, Newell et al. 1989).

The current study investigated the *in situ* influence of *Crassostrea gigas* (Thunberg) grazing on the diversity and abundance of water-column protists (diatoms, dinoflagellates, flagellates and ciliates) in Atlantic coastal ponds. Our aim was to assess the *in situ* impact of oysters on planktonic microbiota, together with a possible contribution of protozoa in food resource intake by oysters, in order to control the occurrence of a realistic trophic link between bacterioplankton production, protozoa and the benthic suspension feeder *C. gigas*.

Our experimental design was to monitor the weekly changes in bacteria and protist community abundances during 3 water sequestration cycles, in spring, autumn and winter. This follow-up was carried out in a pond with oysters, shown at a density used by farmers, compared to a pond with no oysters.

Materials and methods

Experimental procedure

The study was performed in two experimental coastal ponds, in the oyster rearing area "Marais du Plomb" (L'Houmeau, near La Rochelle, French Atlantic coast). They were dug in clay sediments 15 years ago and their flat bottom is constituted of thin layers of silt resulting from sedimentation of marine particles brought by turbid coastal waters and by destabilisation of surrounding terrestrial banks. The two experimental coastal ponds, dug at the same time, are located side by side and have the same limited surface area (200 m²) and depth (≈ 1 m). Owing to the closeness of these 2 ponds, they have undergone the same climatic conditions with similar temperature and salinity of water and wind regimes.

To estimate the impact of oysters on protist and bacteria planktonic communities, the temporal changes in planktonic assemblages were monitored in spring, autumn and winter during 3 sequestration cycles, by means of weekly water sampling in a pond with oysters (oyster pond) compared to a pond with no oysters (control pond). A mean density of 20 oysters per m² was used and corresponded to a density used by farmers for fattening and greening oysters (Goulletquer & Héral 1997). Oysters were put together in plastic nettings (1 m length, 0.5 m wide, mesh size averaged 1 cm), which were placed on iron tables at 0.5 m off the bottom of the pond at low tide at the beginning of the experiments. Then, both coastal ponds were supplied with coastal water, via channels, at high spring tides. Two days after the arrival of the seawater in the ponds, turbidity levels were low and the first sampling was made. It was monitored by 4 weekly samplings. Sea waters were sequestrated in the ponds for 3 to 4 weeks, which resulted in regular modifications of ecological conditions in these semi-closed systems. At the end of the sequestration period, the ponds were emptied at ebb tide and filled again with new sea water during the next high tide.

Preliminary tests were performed for defining an accurate sampling strategy of the pond water column in order to estimate average values of both seston parameters and abundances of microbiota over the whole surface of each pond: each pond was divided into 12 squares (3 m wide) and 1 sub-

surface water sample was taken in each square at each sampling date. These 12 samples were collected with a 2.5 l "Van Doorn" bottle (Wildco) and from each sample, 500 ml was mixed in a unique opaque carboy, taken rapidly to the laboratory and treated. The final 6 liters sample was assumed to represent a spatial average estimate of water column parameters within each pond and sampling date.

Pigment determination

To perform fluorimeter and HPLC analyses, triplicate sea water samples (from 30 to 150 ml) extracted from the previous whole sampling bottle content, were filtered in a 10 mm Hg vacuum onto a 2.5 cm GF/F filter, which was kept at -80°C in Corning glass tubes until extraction. For fluorimeter analyses, filters were put into 7 ml of 99 % methanol and stored for 1 h in the dark at 4°C. Concentrations of extracted chlorophyll *a* and pheopigment were measured using a Turner fluorimeter N°10 037, equiped with a Corning CS 5-60 filter for excitation light and a Corning CS 2-64 filter for emitted light. Pigment amounts were calculated according to Neveux & Lantoine (1993). For HPLC analyses of chlorophylls and carotenoids, pigments were extracted from filters in 2 ml of 100% methanol, by crushing and stirring with a glass rod and placed in a bath sonicator for 30 s. Vials were then stored for 1 h in the dark at 4°C. The extract was filtered over a GF/F filter and diluted just before injection with ammonium acetate buffer (0.5M) to 80 % methanol. Pigments were analysed by HPLC according to Wright et al. (1991). The system consisted of a Kontron liquid chromatograph equiped with 3 HPLC 422 pump, a diode array detector 440 and a spectrofluorometer SFM 25. The reverse phase column used was Allsphere ODS2, 25 cm x 4.6 mm ID, 5 µm particle size (Alltech). Pigments were identified by diode array spectroscopy during elution and quantified by injection of standard pigments.

Measurements of total particulate matter (TPM)

TPM was measured according to Aminot & Chaussepied (1983). The whole sampling bottle content was mixed and triplicate sub-samples (from 300 to 1 000 ml) were filtered within 1 h onto a Whatman GF/C glass fibber filter (47 mm in diameter) under a vacuum pressure <10 mm Hg. Filters had been previously combusted at 490°C for 2 h to eliminate their subsequent organic carbon content and then weighed. After sample filtration, each filter was rinsed twice with ammonium formiate (Osi, 68 g l^{-1}) to remove salt, dried at 60°C for 12 h and weighed to measure TPM. To determinate the proportion of particulate inorganic matter (PIM) *versus* particulate organic matter (POM), filters were combusted at 490°C for 2 h and then weighed. Knowing that the particulate organic matter (POC) contributed to half of the POM, we can estimate the POC in the water for the 3 periods and calculate the proportion of protist biomass in the 3 cycles of water sequestration in the pond.

Enumeration of bacteria

Triplicate samples (10 ml) were fixed with 0.2 µm-filtered formaldehyde (final concentration 1 %). A subsample was stained according to Porter & Feig (1980) with DAPI (final concentration 2.5 µg ml^{-1}) for 15 min at 5°C in the dark, filtered onto a black Nuclepore filter (0.2 µm pore size) and

examined under UV excitation with an epifluorescence microscope (Leitz Dialux 22 EB) to enumerate heterotrophic bacteria.

Taxonomy and enumeration of microphytoplankton community

An aliquot of pond water was fixed with formaldehyde (final concentration 1 %) and stained with alkaline lugol. Microphytoplanktonic cells were enumerated in Utermöhl settling chambers (Hydro-Bios combined plate chambers) under a reverse microscope. Diatoms were identified up to the genus and the sizes of all cells (length and width) were measured 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, using a theoretical carbon/volume ratio of 0.14 pg Carbon (C) μm^{-3} (Putt & Stoecker 1989).

Autotrophic and hetero/mixotrophic protist community: taxonomy and enumeration

Flagellates, dinoflagellates and ciliates were fixed, stained and enumerated according to methods by Haas (1982), Caron (1983) and Sherr et al. (1994), modified by Dupuy et al. (1999). Cells were enumerated in Utermöhl settling chambers (Hydro-Bios combined plate chambers), using a reverse epifluorescence microscope (Zeiss Axiovert, 100 W mercury lamp and blue light excitation). Dinoflagellates and ciliates were identified up to the genus. Dinoflagellates are heterotrophic or autotrophic cells: we considered this group as mixotrophic cells. Ciliates were considered as heterotrophic cells. The method of flagellate enumeration excluded the taxonomic determination of flagellates due to the presence of a black Nuclepore filter. Only the size and the cell outlines of flagellates were estimated. Moreover, the method used in this study permitted us to distinguish autotrophic from heterotrophic flagellates via repeated interchange of the filter sets (Caron 1983): phototrophic cells (crimson under UV 365 nm excitation and red colored under green 450-490 nm excitation) and heterotrophic cells (blue under UV excitation and invisible under green excitation) were separately enumerated. However, it was not possible to differenciate autotrophic cells and mixotrophic cells. In fact, flagellates were characterized according to their shape, size and acquisition mode of food. Each flagellate, which had a different size or shape or which had a different acquisition mode of food was regarded as a new group.

From replicate cell size measurements of all autotrophic and hetero/mixotrophic protists, the mean cell volume of each group was calculated by equating the shape to standard geometric configurations. The cell volume was converted into carbon units, using a theoretical carbon/volume ratio of 0.14 pg Carbon (C) μm^{-3} for flagellates and 0.17 pg C μm^{-3} for dinoflagellates and ciliates, (Putt & Stoecker 1989 corrected for glutaraldehyde fixative, according to Leakey et al. 1994).

Diversity and structure of protist communities

To assess the impact of oysters on the diversity and abundance of the planktonic protist, rank species abundance curves were used. This method has been widely used in marine ecology (Pielou 1975,

Frontier 1985, Warwick & Clarke 1994). Warwick (1986) proposed to use the ABC method (abundance/biomass comparison) for describing changes in macrozoobenthic communities in response to disturbance or pollution. However, when applied to tidal flat assemblages (Beukema 1988) or estuarine polluted communities (Dauer et al. 1993), application of the ABC method may result in unacceptable misclassifications of disturbance status of a given benthic assemblage. According to Warwick & Clarke (1994), the ABC- method should be used with caution in the case of organisms other than macrobenthos, but remains efficient in detecting substitutions of species within polychaetes, known to be a good pollution indicator species. An alternative to these problems, in the case of planktonic community with short life span and small-sized species, is to use rank-frequency diagrams as proposed by Frontier (1985). Rank-frequency diagrams (RFD) are similar to " simple dominance curves " (see Clarke 1990), but are based on the log-log plot of ranked abundances, expressed as percentages of the total abundance of all species in the sample, against the relevant species rank. As indicated by Frontier (1985) from zooplanktonic and phytoplanktonic studies, changes in the shape of RFD characterize temporal changes in community structure: an S-shaped curve indicates the predominance of one or two species in a low species richness assemblage (stage 1, pioneer community). An increase of the species richness may induce a more even distribution of abundance between species and thus, the curve becomes more convex (stage 2, mature community). At the end of the ecological succession (stage 3), first ranked species are more dominant and the curve becomes more linear; the species richness is also lower than in the previous stage. In some cases, e.g. a recolonization phase after a disturbance or after an intense nutrient input, a few species can quickly develop and the RFD appears irregular and coarsely rectilinear (stage 1' intermediate between stages 1 and 2, Frontier, 1985). In our study, rank-frequency diagrams were carried out on the total abundances of planktonic protists, excluding bacteria (size < 1µm), which are not protist and not retained by oyster gills.

Estimation of protist carbon removal by oysters and contribution of each various protist to the carbon resource removed by oysters

To estimate the living protist carbon removed from each type of protist (diatoms, ciliates…) by *Crassostrea gigas* filtration, the field carbon biomass of each type of protist in the control pond (see above for calculation) was multiplied by the corresponding oyster clearance rate, defined as the theoretical water volume entirely cleared from particles per time unit (Bayne & Widdows 1978) and reported to the total oyster biomass in the pond (20 oysters m^{-2}). The clearance rate (l h^{-1} g^{-1}), standardized per g of oyster dry weight according to Riisgård (1988), was taken from Deslous-Paoli et al. (1987) for diatoms and Euglenophyceae, from Dupuy et al. (1999) for ciliates, dinoflagellates and > 5 µm flagellates and from Barillé et al. (1993) for < 5 µm flagellates. The contribution of each type of protist to oyster food was expressed as a percentage of the total protist resource.

Statistical treatment

A 2-way partially nested ANOVA was used to estimate differences in water column variables and protist and bacteria abundances due to seasons and treatments (Sokal & Rohlf 1981). Both seasons and treatments represent fixed factors, with 3 levels (spring, autumn and winter) and 2 levels (oyster pond versus control pond), respectively. Dates of observation (5 dates per season) are hierarchical to the season factor and are assumed to be a random factor, whose mean squares will be used for testing effects of seasons, treatments and their interaction. This is a mixed-model ANOVA (Sokal & Rohlf 1981). Since there were no replicates of experimental units within each sampling date, i.e. treatment ponds and/or 12 point spatial survey within a pond, triplicate measurements made within a sampling date only represent the variation within hydrological samples with the error term which was used for testing the level of variation between sampling dates within seasons.

The logarithmic transformation ($\log_{10}$) was used in the case of microbiota abundance because it is assumed that increase of microbial population size during a given time is proportional to its abundance (multiplicative effect). Tests for homogeneity of variances (Bartlett's test) of untransformed and $\log_{10}$ transformed data were performed and results confirmed this assumption, except in the case of dinoflagellates and flagellates (size < 5 µm), whose abundances were extremely high during blooms that occurred on 9th June and 22 nd June, respectively. According to Underwood (1981), when data are not normally distributed, as observed in these two groups, Bartlett's test is too conservative and could cause rejection of the assumption of homoscedasticity, due to lack of normality.

The a posteriori S.N.K. procedure (Student-Newman-Keuls test) was used for testing differences in means between treatments and seasons (Underwood 1981).

Results

Biotic groups and proportions of auto/mixotrophic and heterotrophic > 5 µm and < 5 µm flagellates

Large autotrophic cells were mostly diatoms, large hetero/mixotrophic cells mainly dinoflagellates and ciliates. Small auto/mixotrophic and heterotrophic cells were represented by nanoflagellates. The size was between 3 and 19 µm. The percentage of > 5 µm and < 5 µm auto/mixotrophic nanoflagellates was always higher compared to the percentage of > 5 µm and < 5 µm heterotrophic nanoflagellates (Table 1). Picoplankton was exclusively constituted of heterotrophic bacteria.

Sources of variation in seston, protist and bacteria temporal changes.

There were always significant differences in date-to-date variability within seasons and treatment whatever the variables (Table 2). There was no evidence of a significant difference in particulate organic matter (POM) between oyster and control ponds. However, due to significantly higher POM values in spring (in both oyster and control ponds) and autumn control pond, as indicated by the SNK test, this accounted for a season X pond interaction (figure and results not shown). Seasonal

changes in chlorophyll *a* were similar in both oyster and control ponds, despite higher values of chlorophyll *a* in the control pond in spring ($p<0.001$ for the season X pond interaction). However, chlorophyl *b* pigments, significantly varied with seasons, but were higher in the oyster pond during the spring than in the control pond (SNK test, significant season X pond interaction, Table 2).

The presence of oysters significantly reduced the abundance of diatoms which were assessed by fucoxanthin, dinoflagellates, ciliates and > 5 µm flagellates ($p<0.001$, Table 2), but there was no evidence of any oyster impact on the abundance of both < 5 µm flagellates and bacteria ($p> 0.05$) whatever the season. Significant interactions between seasons and ponds were mainly due to higher values of a given biotic group within a season. For instance, 1) diatom abundances were lower in winter in both controls than in other seasons and oyster ponds (Fig. 3A, SNK test); 2) significant higher abundance of dinoflagellates occurred in the control pond only during the spring and autumn (Figs. 1B, 2B, SNK test) and 3) abundances of > 5µm flagellates were 10 times higher in the control pond in spring than in the other ponds whatever the season.

Comparative temporal changes of bacteria and protist abundances in ponds with and without oysters

Temporal changes in abundance of the main protist and bacteria were comparatively followed in 2 coastal ponds with and without oysters, located side by side, during 3 sequestration cycles.

Spring cycle (May-June)

Two days after the water sequestration (first sampling date, 27/05), diatom, > 5 µm flagellate, dinoflagellate and ciliate abundances were already much lower in the oyster pond than in the control pond (Fig. 1 A, B, C & D), whereas bacteria and < 5 µm flagellate abundance were similar in both (Fig. 1 E & F). Bacteria were highly abundant (ca. 5 x 10^9 cells l^{-1}). A bloom of diatoms soon developed in the control pond during the first and third weeks (500 & 630 x 10^4 diatoms l^{-1}), which was never observed in the oyster pond (ca. 7 x 10^4 diatoms l^{-1}). Similarly, dinoflagellates were dominant in the control pond (87 x 10^4 dinoflagellates l^{-1}) and even when they decreased after the third week, they still remained much more abundant in the control pond (16.4 x 10^4 dinoflagellates l^{-1}) than in the oyster pond (0.01 x 10^4 dinoflagellates l^{-1}). In the third week, large > 5 µm flagellates only developed in the control pond (ca. 70 x 10^6 cells l^{-1}). At the end of the cycle, a bloom of ciliates grew in the sole control pond (ca. 15 x 10^4 ciliates l^{-1}), simultaneously with a strong decrease in diatoms and dinoflagellates. The presence of oysters strongly lowered the abundance of diatoms, > 5µm flagellates, dinoflagellates and ciliates (Tables 2 and 3 and Fig. 1 A, B, C & D: ca. 0.4 x 10^4 diatoms l^{-1}, 0 dinoflagellate l^{-1}, 0.9 x 10^4 ciliates l^{-1} and 4.7 x 10^6 > 5µm flagellates), whereas it increased the development of bacteria and small flagellates (Tables 2 and 3 and Fig.1 E: 3690 x 10^6 flagellates l^{-1} in the oyster pond *versus* 90 x 10^6 < 5 µm flagellates l^{-1} in the control pond).

HPLC analyses of pigments revealed the dominance of chlorophyll *b* and lutein in the oyster pond throughout the cycle; this pigmentary signature, characteristic of Chlorophyta was confirmed by the microscopic observation of a small Chlorophyta flagellate (ca. 3 µm), which developed strongly in the oyster pond (Table 3 and Fig. 1 E).

To summarize, the abundance of large planktonic protist cells was always higher in the control pond, though it fluctuated during the cycle: diatoms mainly developed during the first and third week, dinoflagellates and > 5 µm flagellates during the second week and ciliates at the end of the cycle. Only < 5 µm auto/mixotrophic flagellates (Chlorophyta) and bacteria developed more intensely in the oyster pond (Table 3).

Autumn cycle (September-October)

Within 2 days of water sequestration (first sampling date, 23/09), abundance of diatoms, dinoflagellates, ciliates and flagellates were already much lower in the oyster pond than in the control pond (i.e. 0.3 *versus* 5 x 10^4 dinoflagellates l^{-1}, 1.8 *versus* 8 x 10^4 ciliates l^{-1} and 5 *versus* 15 x 10^6 flagellates l^{-1}). Only bacteria were similarly abundant in the 2 ponds (ca. 4 x 10^7 cells l^{-1}). During the second week of the cycle, small flagellates increased in both ponds (Fig. 2 E). Conversely, most protist solely developed in the control pond: diatoms in the third week (20 x 10^4 cells l^{-1}), dinoflagellates and large flagellates at the end of the cycle (6 x 10^6 large flagellates l^{-1} and 15 x 10^4 dinoflagellates l^{-1}). The ciliate abundance soon dropped in both ponds and thereafter weakly fluctuated. Bacterial abundance evolved similarly throughout the cycle with the presence or absence of oysters (Table 3).

HPLC analyses of pigments resulted in a higher value of fucoxanthin during the second and third week, pointing out that diatoms only developed in the control pond. Alloxanthin (specific of Cryptophycae) became dominant in the control pond in the last week, simultaneously with the large flagellate bloom and the increase in chlorophyll *a* which solely developed in this pond (Fig. 2). Conversely, chlorophyll *b* (specific of Chlorophyta) was higher in the oyster pond during the first 3 weeks.

To summarize, most protist dominated in the control pond: diatoms developed during the third week, > 5 µm Cryptophycae flagellates and dinoflagellates in the last week. Though there was no development of ciliates in any pond during this cycle, their abundance was lower in the oyster pond. Small < 5 µm flagellates increased similarly in both ponds during the second week. Among them, Chlorophyta were dominantly represented in the oyster pond. Bacterial abundance evolved similarly in both ponds (Table 3).

Winter cycle (November-December)

During the winter cycle, protist abundance was much lower in ponds than in spring (Figs. 1 & 3): 6 *versus* 600 x 10^4 diatoms l^{-1}, 0.7 *versus* 87 x 10^4 dinoflagellates l^{-1}, 0.9 *versus* 15 x 10^4 ciliates l^{-1}

and 4 *versus* 3 600 x 10^6 small flagellates l^{-1}. Only large > 5 µm flagellates developed in a similar abundance between winter and autumn (8.7 *versus* 6 x 10^6 flagellates l^{-1}). During the winter cycle, several protist communities fluctuated in both ponds (Fig. 3): dinoflagellates weakly developed in the second week in the control pond and in the third week in the oyster pond. Conversely, > 5 µm flagellates, strongly developed at the end of the cycle in the sole control pond (9 x 10^6 cells l^{-1} and Table 3). Dinoflagellates were higher in the oyster pond in the third week. Ciliate community fluctuated in both ponds, though their abundance was higher in the control pond than in the oyster pond at the end of sequestration cycle.

HPLC analyses of pigments resulted in a higher value of chlorophyll *a* and alloxanthin (specific of Cryptophyceae) from the second week in the control pond, displaying that the > 5 µm flagellates bloom which developed in the sole control pond was triggered by Cryptophyceae flagellates.

To summarize, most protists were poorly represented in both ponds during the winter cycle and only > 5 µm Cryptophyceae flagellates developed in the sole control pond at the end of the cycle (Table 3).

Impact of oysters on the diversity and structure of the planktonic protist community

Rank-frequency diagrams for protists were performed in both ponds on each of the 5 sampling dates within the 3 seasons.

Spring cycle (May-June)

Two days after complete water renewal, the structure of the protist assemblage was identical in both oyster and control ponds (Fig. 4 spring, Table 4). High diversity (37 groups) and similar convex RFD characterized both systems. However, during the course of the closing period, temporal changes in the structure of the protist assemblages differed significantly.

On the one hand, in the control pond, the number of groups quickly decreased, but remained nearly constant (between 21 and 26, Table 4) during the following 4 weeks. Thus, the structure of the protist assemblage was simplified and slightly dominated by > 5 µm auto/mixotrophic flagellates (ANF) and/or heterotrophic flagellates (HNF) (Table 4). The dominant group at the beginning, the first and third weeks was a > 5µm ANF and the second was a diatom, *Cylindrotheca* sp.. In the second and fourth weeks, the dominant group was a > 5 µm HNF (Table 4) and the second a > 5µm ANF.

On the other hand, in the oyster pond, groups sharply decreased in number and reached only 11 in the last week (Table 4). Two groups were dominant at the beginning, a > 5 µm ANF (58 %) and a > 5 µm HNF (28 %). < 5 µm ANF dominated the other protists during the last 3 weeks (more than 99 % of total abundance). Consequently, RFDs were typical of a pioneer planktonic assemblage (stage 1), which characterized the spring bloom (Fig. 1E). It should be noted that this structural analysis is consistent with ANOVA analysis performed on < 5 µm flagellates (Table 2): there was significantly

higher abundance of < 5 µm flagellates in the oyster pond in spring (SNK test and Fig. 1E) than in either control and seasons, accounting for the highly significant season X ponds interaction (Table 2) together with the non significant effect of both season and pond factors (p = 0.076 and 0.082, respectively, Table 4).

Autumn cycle (September-November)

In the control pond, temporal changes in the protist assemblage structure were characterized by shifts in the dominance between a < 5 µm ANF and > 5 µm Cryptophycae. At the beginning of the autumnal cycle, a < 5 µm ANF dominated (67 %) and was followed by a > 5 µm Cryptophycae (18 %). During the second and third weeks, > 5 µm Cryptophycae flagellate became the most abundant protist (70 % and 39 % respectively) before being replaced by < 5µm autotrophic flagellates during the last week (61 % of total abundance, Table 4). RFDs remained similar in shape with an even proportion of species (stage 2). Diversity of biotic groups decreased from 37 to 27 during the whole closing-period, but remained higher than spring diversity.

In the oyster pond, the structure of protist asssemblages significantly changed during the seasons. A bloom of < 5 µm ANF (86 % of total abundance) occurred during the first week (Fig. 2E, Table 4). RFDs evolved from convex at the beginning to slighty concave during the first week, then turned convex from the second week onwards. At the beginning of the closing-period, the number of groups was similar in both ponds, but it increased in the oyster pond during the first week (44 versus 32, Table 4). The initially dominant taxon was a diatom (*Chaetoceros* sp., ca 50%), but a < 5 µm autotrophic flagellate of group of Chlorophyta soon dominated until the end of the period (Table 4). The second group was a < 5 µm autotrophic flagellate at the beginning and in the first week, with 42 and 3% of total abundance, respectively. From the second onwards, Cryptophyceae flagellates were the second dominant group (17 to 25 % of total abundance), even though they were dominant in the control pond (Table 4).

Winter cycle (November-December)

In the control pond, RFDs were concave at the beginning and during 2 weeks, displayed the dominance of one group (abundance higher than 80 %). During the last 2 weeks, RFDs turned convex, showing a more even distribution of proportions among the top 4 first groups out of the 25 groups which constituted the winter protist assemblage (Table 4). The initial number of groups was lower in winter, ca 25, than in spring and autum, but remained constant during the whole period. A < 5 µm ANF was dominant during the first week; from the third week onwards, a Cryptophyceae flagellate dominated (48 and 62 % at the end of the period).

In the oyster pond, RFDs showed similar profiles as in the control pond (Fig. 4). At the beginning of the period, the number of groups was close to that in the control pond, ca 24, but it decreased at the end of the period, ca 19 (Table 4). The dominant taxon during the whole cycle was a <

5 µm ANF. From the third week onwards, the second taxon was a Cryptophyceae flagellate (26 and 38%, for the third and fourth week, respectively), which developed well in the control pond and was never the first dominant group in the oyster pond (Table 4).

DISCUSSION

The aim of this study was 1) to assess the *in situ* impact of *Crassostrea gigas* on planktonic bacteria and protists in Atlantic coastal ponds and 2) the relative contribution of autotrophic, heterotrophic and mixotrophic protists to oyster food resource intake. Such interaction would support the potential role of protozoa as a realistic trophic link between the high bacterioplankton production in coastal ponds and benthic suspension feeding bivalves.

Impact of oysters on the protist and bacteria communities

Two complementary technical approaches were simultaneously used to assess the abundance and taxonomic identification of the protist community: (1) microscopic enumeration and observation and (2) HPLC analysis of pigment signature.

Pond protists and bacteria were in a size range from 1 µm to 250 µm, mainly represented by large autotrophic diatoms (5 to 230 µm); large hetero/mixotrophic dinoflagellates (8-70 µm) and ciliates (8 to 250 µm); small auto/mixotrophic nanoflagellates (3 µm) and hetero/mixotrophic microflagellates (> 5 to 19 µm). Picoplankton was exclusively constituted of bacteria (1 µm).

Whatever the season, after 2 days of isolation of the pond and during the whole cycle, significant changes in protist community abundance of large > 5 µm flagellates, diatoms, dinoflagellates and ciliates were weaker in the pond with 20 oysters m^{-2} than in the control pond. Even though protist abundance was much lower in winter than in May-June and September-October, the large Cryptophycae flagellate, which bloomed at the end of December in the control pond, could never develop as much in the presence of oysters. It can be assumed that such a general depletion in micro-sized protists was related to the grazing activity of *Crassostrea gigas*. Cryptophytes are known to be well consumed by bivalves (Shumway et al. 1985, Loret 1999). However, other species such as micro-mesozooplanktonic cells (bivalve and crustacean larveas, copepods) could be fed on protist community: in the control pond, protists were food for these species. On the contrary, in the oyster pond, oysters fed on larvae species (Dupuy et al. in press) and only the copepods, non retained by oysters, actually too large size, were directly in competition with oysters. However, given with the density of oysters (20 oysters m^{-2}) and copepods (up to 1 000 l^{-1}, Crottereau com. pers.), the protist depletion, whose size was accessible to them, was mainly due to the predation of oysters.

However, planktonic protists < 5 µm, such as Chlorophyta flagellates and bacteria, developed similarly or more intensely in the oyster pond, as if they were either not grazed or even favoured by the presence of oysters. In May-June, the strong development of < 5 µm Chlorophyta flagellates in the

oyster pond (Fig. 1) resulted from the inability of *Crassostrea* to retain them efficiently. The absence of Chlorophyta at the same time in the control pond can probably be explained by the fact that this tiny flagellate was here actively consumed by hetero/mixotrophic protists, which developed abundantly in the absence of oysters and perhaps through micro-mesozooplankton. Conversely, in the oyster pond, protists being grazed by oysters, the small flagellate could develop without any predation pressure. Moreover, oysters excretory products, or nutrient release related to active grazing might have stimulated the development of bacteria and small autotrophic flagellates. Such hypotheses have been evoked to explain the dominance of a photosynthetic picoeukaryote, *Ostreococcus tauri* (Courties et al. 1994, Chrétiennot-Dinet et al. 1995) in the marine Mediterranean Thau lagoon where intensive oyster culture has developed.

The current evidence of a depletion in large > 5 µm protists in the presence of oysters reflects *in situ* a substantial capacity of *Crassostrea gigas* to select among particles by size. These results concorded with the retention spectrum of oysters already studied in previous experimental studies (Deslous-Paoli et al. 1987, Barillé et al. 1993).

Previous laboratory studies showed that oysters retained *in vitro* cultured ciliates or field protist like flagellates and ciliates (Le Gall et al. 1997, Dupuy et al. 1999). Moreover, we report here the first *in situ* proof of a significant retention of a protist community by oysters.

Rank-frequency diagrams have been used to investigate the effect of oyster grazing on protist communities. Considering that oysters are the dominant predators in the oyster pond, oyster grazing thus triggers dramatic changes in coastal pond protist communities, by lowering the taxonomic diversity in spring. The most striking difference between protist planktonic communities in ponds with or without oysters lies in the respective dominant taxon: in autumn and in winter, > 5 µm flagellates (pigmentary signature of Cryptophyceae) strongly develop in the control pond during the last weeks, although a < 5 µm flagellate was dominant in the oyster pond. Due to their preferential retention of > 5 µm protists, the presence of oysters deeply influence the taxonomic structure of the pond protist communities.

Estimation of protist carbon removal by oysters and contribution of each various protist to the carbon resource removed by oysters

The calculation of the quantity of carbon removal by oysters based on the multiplication of the organism biomass in the control pond, the oyster biomass and clearance rates for each organism can only be an approximative estimation. Indeed, the presence of oysters in a coastal pond can modify the functioning of the planktonic food web, especially effect to dissolved organic matters (Sornin et al. 1990). Moreover, the predation of the micro-mesozooplankton (copepods, bivalve and crustacean larvaes...) on protists in the control pond has not been taken into account. However, since few data exist in this estimation, this calculation allows us to obtain a first approximation on the use of trophic resources in the oyster pond. This use of organisms varies quantitatively and qualitatively throughout the year. As oysters retain the

organic particles according to their cell-size and their field abundance, the removal of protist carbon from the pond depends upon (1) the importance of the protist resource in the water column, (2) the oyster retention efficiency upon the available protists, and (3) the importance of the oyster population grazing in the pond. The biomass of protist carbon varied in the control pond according to the season (Table 4): it was maximal in spring (982 µg C l^{-1}) and decreased about 4 fold in autumn and was minimal in winter (179 µg C l^{-1}). Subsequently, oysters removed quantitatively much more protist carbon (Table 4) in spring (109 mg C m^{-2} h^{-1}) than in autumn (29 mg C m^{-2} h^{-1}) or winter (12.5 mg C m^{-2} h^{-1}). Moreover, this energy resource was qualitatively different throughout the year: in spring, > 5 µm flagellates were the first energy resource and represented 74 % of the carbon removed by oysters (Fig. 5), whereas diatoms, dinoflagellates and ciliates only contributed 11 %, 6 % and 5% respectively (Fig. 5). In autumn, diatoms represented the most important part of particulate carbon removed by oysters (57 %); ciliates and > 5 µm flagellates were the second resource (16 %) (Fig. 5). In winter, the poor protist community resulted in a weak oyster removal and bloom-forming Cryptophycae flagellates were the only subsequent protist resource at this period (88 %). Ciliates, diatoms and < 5 µm flagellates represented a weak protist carbon resource removed by oysters, 6 %, 2 % and 3 % respectively (Fig. 5). These data indicate a somewhat minor role for < 5 µm flagellates, whereas > 5 µm flagellates are likely to be the primary protist food resource for *Crassostrea gigas*. This is therefore the first time that the importance of flagellates has been proven in this type of environment where diatoms used to be considered the main resource. Ciliates and diatoms represent the second resource with a substantial contribution in autumn. Dinoflagellates are removed efficienctly but represent a weak carbon resource due to their small volume or punctual abundance. However, other trophic resources exist, which have not been included into account in this carbon removal. In this study, the part of non-protist carbon may be high according to the season (Table 5: from 38 to 88 %). The analysis of the POC nature has not been made, but it is known that the detritic matter in these environments can reach very high values, up to 67 % of detritic matter (Crottereau 1999) as well as in the Marennes-Oléron Bay (84 %, Feuillet-Girard et al. 1994). Studies have shown that the oyster uses this organic matter as food and demonstrated opportunist trophic behaviors in the Marennes-Oléron Bay (Riera & Richard 1996).

We can estimate the local impact of *Crassostrea gigas* filtration on the water column protist from the clearance rate and the oyster density in the pond. Taking into account the mean biomass used by oyster farmers in rearing ponds (oysters m^{-2}) and based on the mean clearance rates (Dupuy et al. 1999, Deslous-Paoli et al. 1987), the oyster population could filter on average 93 l m^{-2} h^{-1} in a pond measuring 200 m^3. This estimated population clearance rate could result in the removal of about 10 % of the planktonic particles in the water volume (200 m^3) within 1 h. In spring, the abundance and diversity of the protist community strongly decreased in the oyster pond, which supported the conclusion that at this period, grazing of 20 oysters per m^{-2} may exceed the protist production for large taxon which

are efficiently retained. Conversely, in autumn and winter, the particle removal met the trophic capacity of the pond in term of protists.

Most studies on the importance of protists as a trophic link between the microbial food web and metazoa focused on pelagic consumers as zooplankters (Berk et al. 1977, Porter et al. 1979, Jonsson & Tiselius 1990, Gifford & Dagg 1991, Hartmann et al. 1993, Sime-Ngando et al. 1995) or oyster larvae (Baldwin & Newell 1995, Klaveness 1992). Only a few recent reports have been dedicated to the benthic macroconsumers (Kreeger & Newell 1996, Le Gall et al. 1997, Dupuy et al. 1999). These results support the hypotheses that hetero/mixotroph protists represent an important potential resource, directly accessible to the filter-feeding oyster which retains them. Indeed, in this study, the dinoflagellates can be mixotrophic species or even heterotrophic cells, and the flagellates, whatever their size, are considered as autotrophic cells (Table 1), but which cannot be differentiated from mixotrophic cells (Granéli et al. 1999). Moreover, it must be noted that a flagellate considered as autotrophic cell and which contains chloroplasts can equally feed off bacteria (Havskum & Riemann 1996) or other prey (Jones et al. 1995), and that in certain cases, the mortality rate of bacteria is essentially due to the grazing of pigmented flagellates (Havskum & Riemann 1996). Taking into account that the available diatom production in ponds does not balance its energy schedule, *Crassostrea gigas* may have access to strong bacterioplanktonic production through hetero/mixotrophic protists, which would thus allow the transfer of carbon from the microbial loop towards the upper trophic levels.

ACKNOWLEGMENTS

We gratefully acknowledge M. Bréret * for technical assistance, C. Trichet ** for bacterial enumeration, C. Vérity for HPLC analyses, H. Hartmann ** for his help in field sampling, C. Bacher * for assistance in statistics and S. Le Gall * for critical review of the manuscript. This study was carried out with financial support from the Conseil Général de Charente-Maritime.

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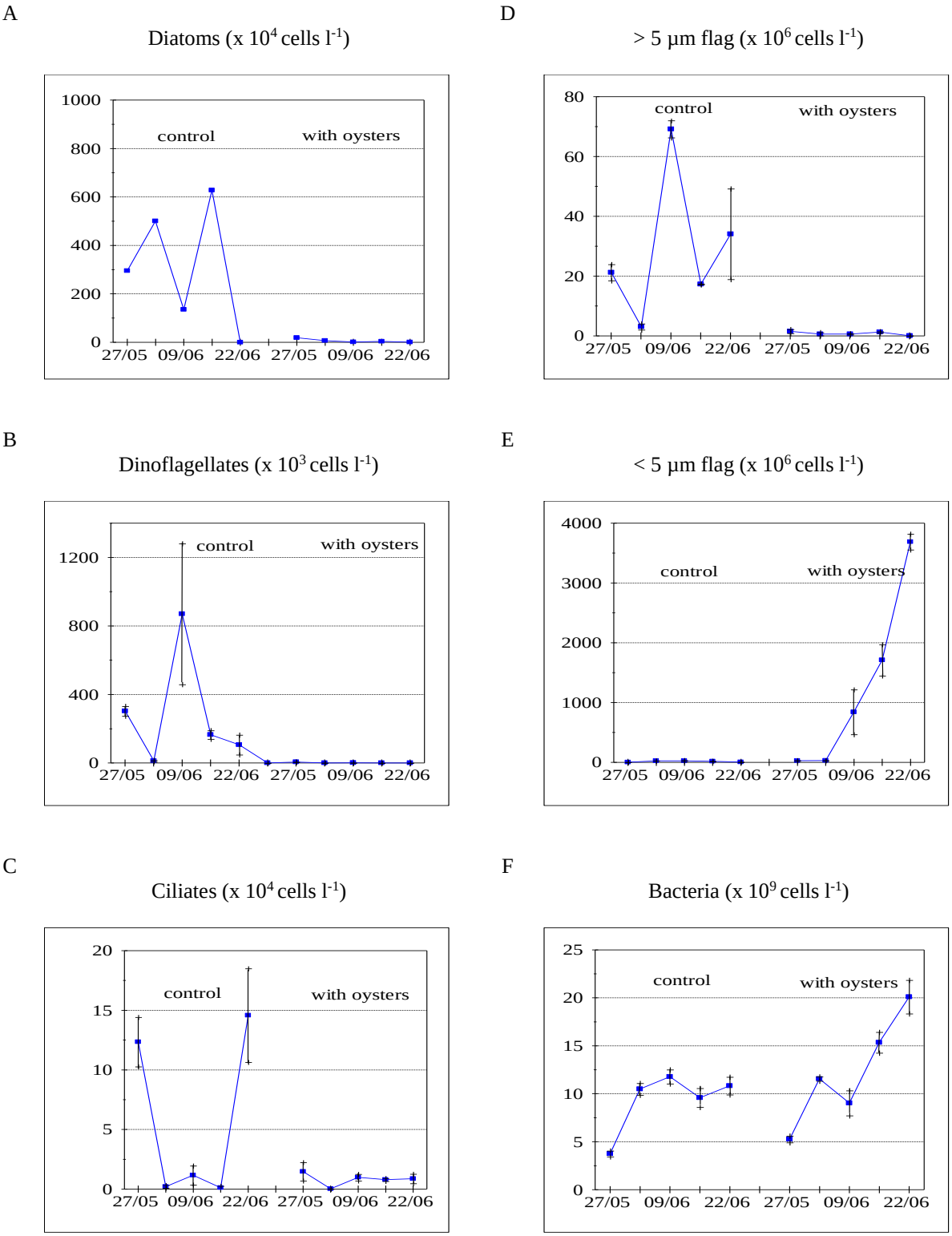

Figure 1: Abundances of planktonic protists and bacteria in both control and oyster ponds during the spring. Abundance data (mean ± SD, n=3, except for diatoms) were collected from 3 subsamples.

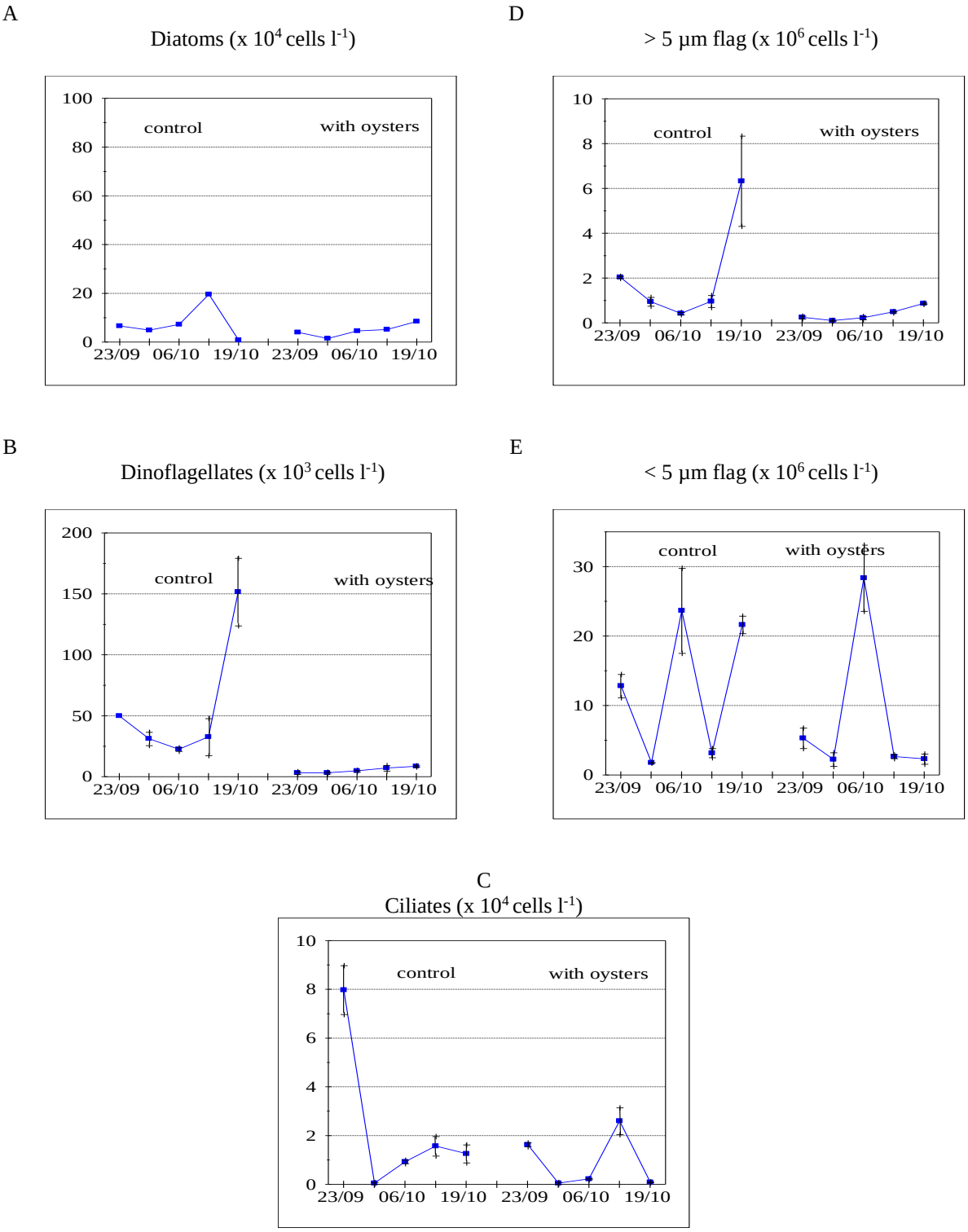

Figure 2: Abundances of planktonic protists in both control and in oyster ponds during the autumn. Abundance data (mean ± SD, n=3, except for diatoms) were collected from 3 subsamples.

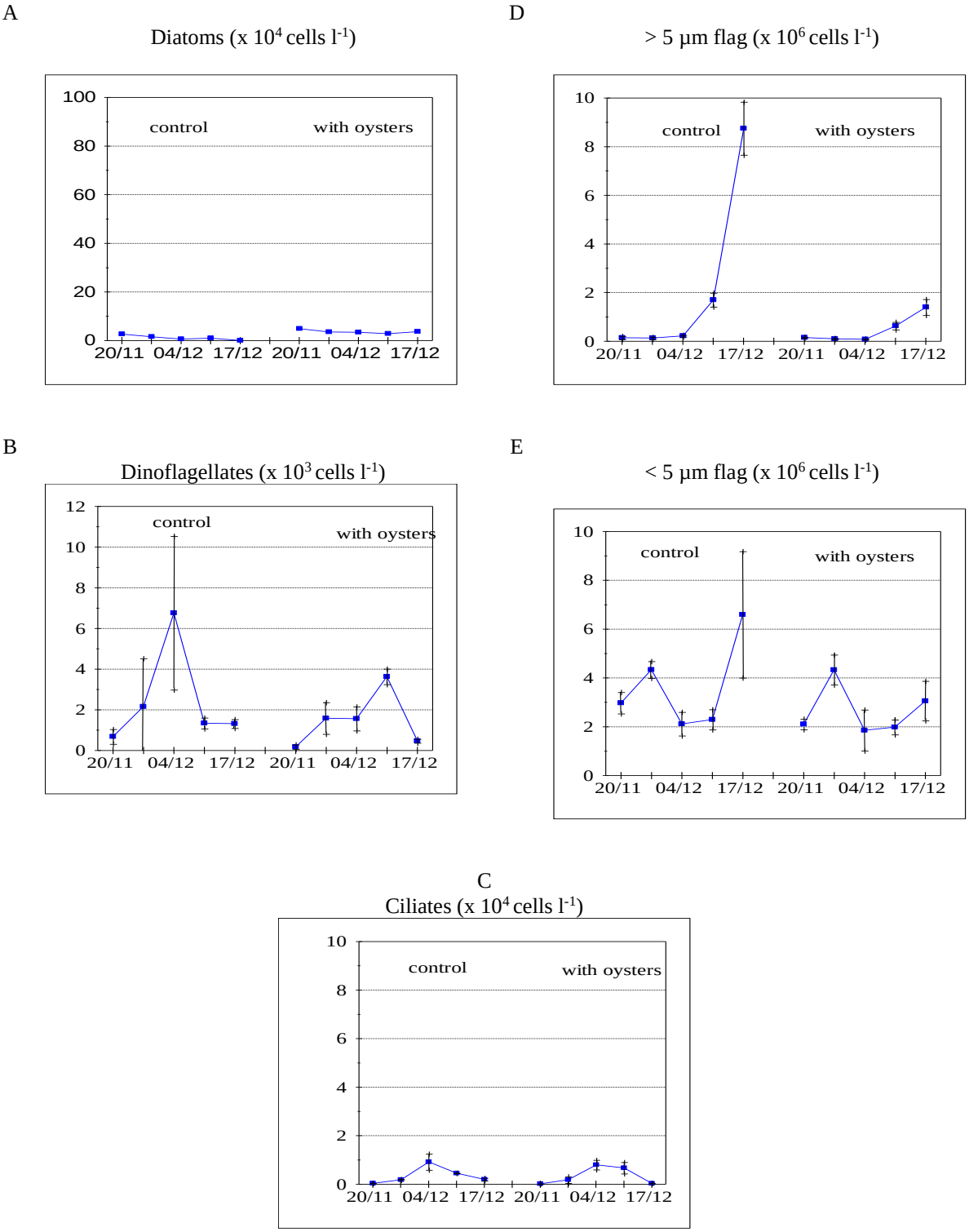

Figure 3: Abundances of planktonic protists in both control and oyster ponds during the winter. Abundance data (mean ± SD, n=3, except for diatoms) were collected from 3 subsamples.

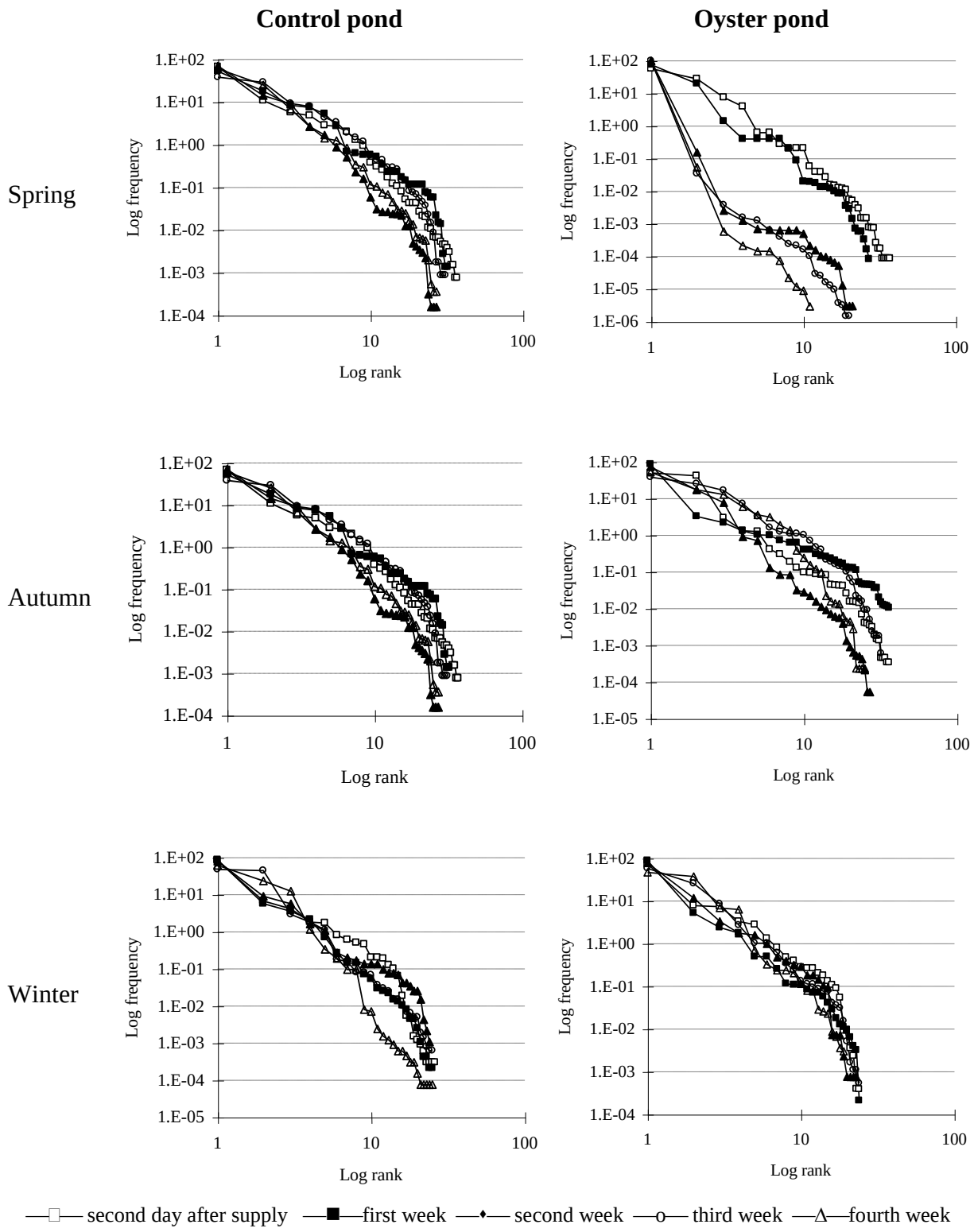

Figure 4: Rank-frequency diagrams in spring, autumn and winter during each sequestration cycle in the control pond (left curves) and the oyster pond (right curves). In the oyster pond during the spring cycle, the last two weeks are at 99 %.

A

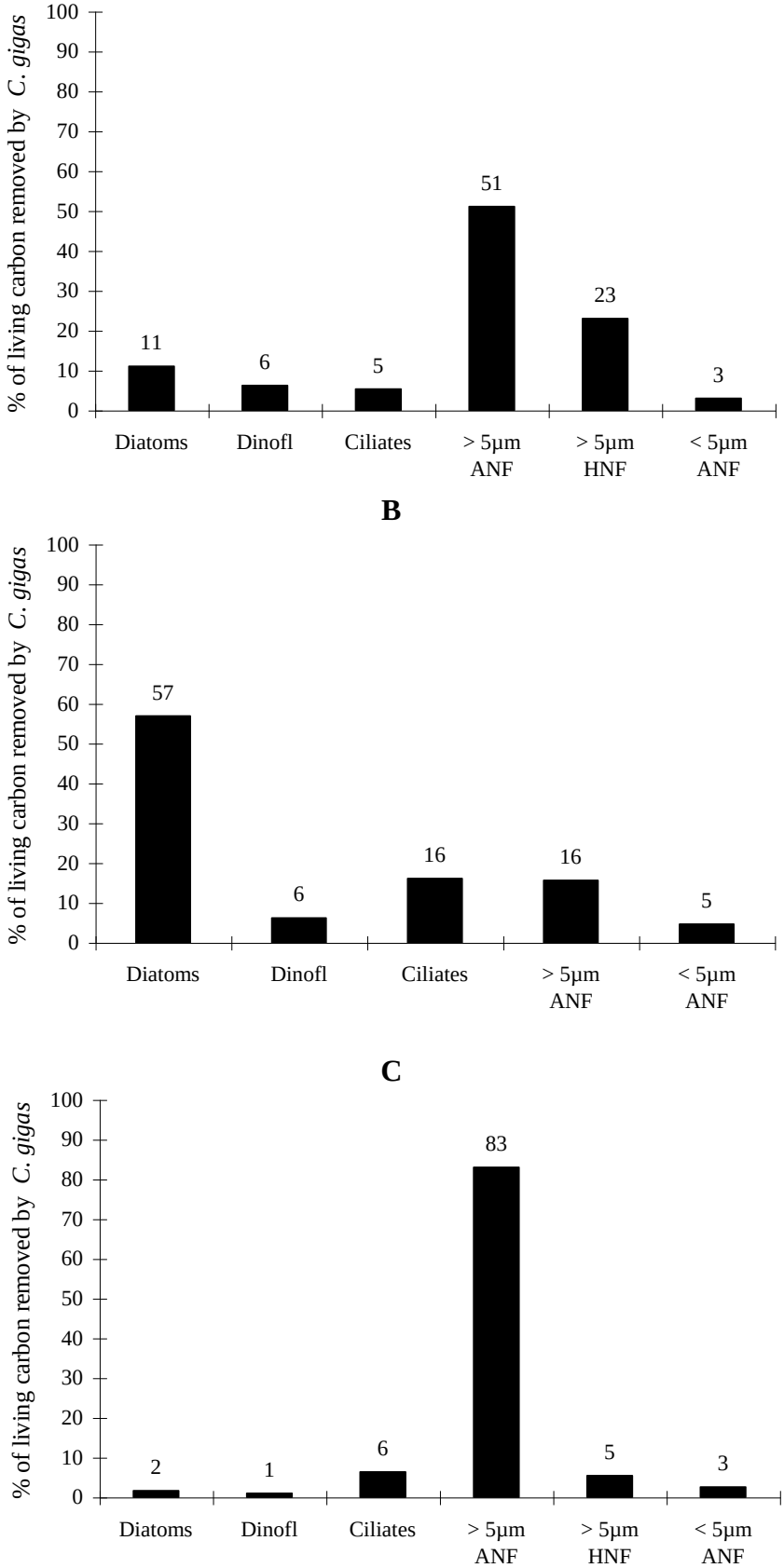

Figure 5: Estimated percentages of living protist carbon removed by *Crassostrea gigas* filtration in spring (A), autumn (B) and winter (C). ANF = Auto/mixotrophic nanoflagellates; HNF = Heterotrophic nanoflagellates, Dinofl = Dinoflagellates.

Table 1: Percentage of > 5 µm and < 5 µm auto/mixotrophic nanoflagellates (ANF) and heterotrophic nanoflagellates (HNF) in the control pond in spring, autumn and winter.

	> 5 µm flagellates		< 5 µm flagellates	
Period	% ANF	% HNF	% ANF	% HNF
Spring	69.2	31	89.6	10.4
Autumn	98.9	1.1	97	3
Winter	94.1	5.9	99.7	0.3

Table 2: Results from 2 way partially nested ANOVA on seasonal changes in water column parameters and microbiota abundances.

Variable	Source of variation	Degrees of freedom	Mean squares	F ratio	P
Chlorophyll *a*	seasons	2	100.584	1.54	0.254
	ponds	1	5.791	0.6	0.440
	seasons X ponds	2	142.688	14.85	< 0.001
	date (seasons)	12	65.248	6.79	< 0.001
	error	72	9.609		
Chlorophyll *b*	seasons	2	7.997	5.28	0.023
	ponds	1	12.3773	55.74	< 0.001
	seasons X ponds	2	8.3994	37.82	< 0.001
	date (seasons)	12	1.5136	6.82	< 0.001
	error	72	0.2221		
Alloxanthin	seasons	2	11.6899	1.32	0.304
(Cryptophyceae)	ponds	1	5.3573	11.41	0.001
	seasons X ponds	2	6.9577	14.81	< 0.001
	date (seasons)	12	8.8821	18.91	< 0.001
	error	72	0.4697		
Fucoxanthin	seasons	2	4.7397	6.38	0.013
(Diatoms)	ponds	1	5.3217	45.81	< 0.001
	seasons X ponds	2	1.8844	16.22	< 0.001
	date (seasons)	12	0.7431	6.4	< 0.001
	error	72	0.1162		
Dinoflagellates	seasons	2	7.4144	3.55	0.061
	ponds	1	39.5745	171.73	< 0.001
	seasons X ponds	2	15.9127	69.05	< 0.001
	date (seasons)	12	2.0857	9.05	< 0.001
	error	72	0.2305		
Ciliates	seasons	2	3.2868	1.08	0.372
	ponds	1	5.2998	23.85	< 0.001
	seasons X ponds	2	0.4066	1.83	0.168
	date (seasons)	12	3.0546	13.75	< 0.001
	error	72	0.2222		
> 5 µm Flagellates	seasons	2	0.387	0.07	0.936
	ponds	1	47.728	29.1	< 0.001
	seasons X ponds	2	19.913	12.14	< 0.001
	date (seasons)	12	5.873	3.58	< 0.001
	error	72	1.64		
< 5 µm Flagellates	seasons	2	7.7438	3.22	0.076
	ponds	1	1.0176	3.11	0.082
	seasons X ponds	2	2.9091	8.88	< 0.001
	date (seasons)	12	2.4056	7.34	< 0.001
	error	72	0.3276		

Table 3: Mean cell abundances of protists and bacteria in both control and oyster ponds in spring, autumn and winter.

	Spring		Autumn		Winter	
	control pond	oyster pond	control pond	oyster pond	control pond	oyster pond
Diatoms ($x10^4$)	312	6	8	5	1	4
Ciliates ($x\ 10^3$)	57	8	24	9	4	3
Dinoflagellates ($x\ 10^4$)	29.1	0.1	5.8	0.5	0.2	0.1
> 5 µm flagellates ($x\ 10^5$)	290	10	21	4	22	5
< 5 µm flagellates ($x\ 10^6$)	15	1260	13	8	4	3
Bacteria ($x\ 10^8$)	93	123	55	61	22	14

Table 4: Number of groups (Nt), frequency (%) of (A) the dominant group and (B) the second group during the three cycles in both control and oyster ponds (ANF = Auto/mixotrophic nanoflagellate; HNF = Heterotrophic nanoflagellate).

A

		Control pond			Oyster pond		
Season	Date	Nt	Dominant group	Frequency (%)	Nt	Dominant group	Frequency (%)
Spring	27/05	37	> 5 µm ANF	44	37	> 5 µm ANF	58
	02/06	23	> 5 µm ANF	55	27	> 5 µm ANF	77
	09/06	21	> 5 µm HNF	46	21	< 5 µm ANF	99.8
	15/06	26	> 5 µm ANF	37	20	< 5 µm ANF	99.9
	22/06	22	> 5 µm HNF	60	11	< 5 µm ANF	99.9
Autumn	23/09	37	< 5 µm ANF	67	36	*Chaetoceros* sp.	50
	29/09	32	< 5 µm ANF	53	44	< 5 µm ANF	86
	06/10	27	Cryptophyceae	70	27	< 5 µm ANF	73
	12/10	31	Cryptophyceae	39	32	< 5 µm ANF	39
	19/10	27	< 5 µm ANF	61	25	< 5 µm ANF	52
Winter	20/11	26	< 5 µm ANF	82	24	< 5 µm ANF	74
	27/11	25	< 5 µm ANF	87	24	< 5 µm ANF	89
	04/12	24	< 5 µm ANF	81	23	< 5 µm ANF	78
	10/12	25	Cryptophyceae	48	24	< 5 µm ANF	59
	17/12	25	Cryptophyceae	62	19	< 5 µm ANF	47

B

		Control pond			Oyster pond		
Season	Date	Nt	Second group	Frequency (%)	Nt	Second group	Frequency (%)
Spring	27/05	37	*Cylindrotheca* sp.	10	37	> 5 µm HNF	28
	02/06	23	*Cylindrotheca* sp.	15	27	> 5 µm HNF	20
	09/06	21	> 5 µm ANF	22	21	< 5 µm ANF	0.2
	15/06	26	*Cylindrotheca sp.*	15	20	< 5 µm ANF	0.03
	22/06	22	> 5 µm ANF	15	11	< 5 µm ANF	0.05
Autumn	23/09	37	< 5 µm ANF	11	36	< 5 µm ANF	42
	29/09	32	Cryptophyceae	18	44	< 5 µm ANF	3
	06/10	27	< 5 µm ANF	14	27	Cryptophyceae	17
	12/10	31	< 5 µm ANF	29	32	Cryptophyceae	25
	19/10	27	Cryptophyceae	25	25	Cryptophyceae	17
Winter	20/11	26	Cryptophyceae	7	24	< 5 µm ANF	8
	27/11	25	< 5 µm ANF	6	24	< 5 µm ANF	5
	04/12	24	Cryptophyceae	9	23	< 5 µm ANF	12
	10/12	25	< 5 µm ANF	45	24	Cryptophyceae	26
	17/12	25	< 5 µm ANF	24	19	Cryptophyceae	38

Table 5: Estimates of living microbial carbon removed by *Crassostrea gigas* filtration. Clearance rate from Dupuy et al. (1999) for ciliates, dinoflagellates and flagellates. Clearance rate from Deslous-Paoli et al. (1987) for diatoms and Euglenophyceae. Particle abundance from present data. Oyster biomass: 27 mg m^{-2} during the spring, 25 mg m^{-2} during the autumn and 17 mg m^{-2} during the winter. Carbon removed (= carbon biomass of particles x clearance rate x oyster biomass). ANF = Auto/mixotrophic nanoflagellate; HNF = Heterotrophic nanoflagellate

Spring	Particle biomass (mg C m^{-2})	Clearance rate (l $h^{-1}g^{-1}$)	Carbon removed (mg C m^{-2} h^{-1})	% of total
Diatoms	90	5	12	11
Ciliates	32	6.8	6	5
Dinoflagellates	63	4	7	6
> 5µm ANF	520	4	56	51
< 5µm ANF	38	3.2	3	3
> 5µm HNF	234	4	25	23
< 5µm HNF	4	3.2	0.3	0
Euglenophyceae	0.3	5	0.04	0
Total	982		110	

Autumn	Particle biomass (mg C m^{-2})	Clearance rate (l $h^{-1}g^{-1}$)	Carbon removed (mg C m^{-2} h^{-1})	% of total
Diatoms	134	5	16.8	57
Ciliates	28	6.8	4.7	16
Dinoflagellates	18	4	1.8	6
> 5µm ANF	46	4	4.6	16
< 5µm ANF	17	3.2	1.4	5
> 5µm HNF	1	4	0.1	0.3
< 5µm HNF	1	3.2	0.1	0.3
Euglenophyceae	0	5	0.0	0.0
Total	245		30	

Winter	Particle biomass (mg C m^{-2})	Clearance rate (l $h^{-1}g^{-1}$)	Carbon removed (mg C m^{-2} h^{-1})	% of total
Diatoms	2	5	0.21	2
Ciliates	7	6.8	0.80	6
Dinoflagellates	2	4	0.12	1
> 5µm ANF	153	4	10.40	83
< 5µm ANF	6	3.2	0.32	3
> 5µm HNF	10	4	0.68	5
Euglenophyceae	0.1	5	0.006	0.05
Total	179		12.5	

Table 6: Percentage of protist carbon compared to total POC in three seasons.

	COP ($\mu g\ l^{-1}$)	Protist biomass ($\mu g\ C\ l^{-1}$)	% of carbon protist/ total POC
Spring	1596	982	62
Autumn	2103	245	12
Winter	801	179	22