Feeding rate of the oyster Crassostrea gigas in a natural planktonic community of the Mediterranean Thau Lagoon

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Bivalve, oyster, food source, Thau Lagoon, microbial food web, heterotrophic protist, picophytoplankton, trophic link

Abstract

The Mediterranean Thau Lagoon is an important European oyster farming area. Oyster growth levels are among the highest in France, although chlorophyll *a* concentration is low. Previous studies have demonstrated that picophytoplankton, nano-microphytoplankton, dinoflagellates and loricate ciliates such as tintinnids are abundant in the Thau Lagoon. Moreover, heterotrophic flagellates and aloricate ciliates have not been investigated. The aim of this study was to assess picophytoplankton, protist and zooplankton abundances in water columns of the Thau Lagoon and to understand the particular structure of the Thau microbial food web, which may explain such a paradoxical oyster growth. In oligotrophic waters in the Thau Lagoon, the picoeukaryote *Ostreococcus tauri* is the dominant autotrophic picoplankter with a maximum Summer abundance. On 17 August 1998, the picophytoplankton and nanophytoplankton abundances were not as high as expected and we observed the development of large diatoms. At this time, available carbon resources arose from microphytoplankton (84.5 %) and picoplanktonic cells represented only 1.27 % in terms of carbon. The heterotrophic cells were few in abundance and constitued only < 14 % of carbon resources. In order to evaluate the importance of the "protozoan trophic link" for energy transfer from "microbial food web" to large benthic suspension feeders, the oyster *Crassostrea gigas* was offered a planktonic community as potential prey. In the grazing experiment, all > 5 µm flagellates, microphytoplankton, dinoflagellates, ciliates and large zooplankton were retained by the oyster gills. Only < 5 µm flagellates and picoeukaryotic cells, *Ostreococcus tauri*, were not very well retained (45 % and 2 %). The high clearance rates of *Crassostrea gigas* found in this experiment can be explained by a low suspended particulate matter (0.65 mg l^{-1}). Oysters adapted their retention mechanism when they lived in oligotrophic waters. These results indicate that, under the given experimental conditions, picophytoplankton did not represent a valuable trophic resource for farmed oysters because (1) *Crassostrea* can not retain picoparticles and (2) the picoplankton represented a poor available carbon resource to be transferred *via* a weak heterotrophic protist community. In the oyster pends of the Thau Lagoon during this study, which followed a rainfall event, microphytoplanktonic primary producers, in particular diatoms, could be considered as the main food sources for bivalve suspension feeders.

INTRODUCTION

Oysters obtain energy resources by filtering particles from sea water and their growth depends upon the nutritive value of the retained seston (Berg & Newell 1986), related to the trophic capacity of coastal waters (Héral 1987). Marine lagoons appear as original entities, compared to adjoining ecosystems. The Mediterranean Thau Lagoon is an important European oyster farming area, with standing stocks of *Crassostrea gigas* estimated at 40, 000 tons. Oyster growth levels are among the highest in France (Goyard 1995), although chlorophyll *a* concentration is low (< 2 µg l^{-1}, Frisoni 1984) relative to other coastal ecosystems. The particular structure of the Thau microbial food web may explain such a paradoxical oyster growth. In the oceans, more than 50 % of the primary production is due to unicellular organisms less than 3 µm in size (Platt et al. 1983; Li et al. 1983; Glover et al. 1986), which constitute a nutrient source of particulate and dissolved organic matter for heterotrophic organisms. In the Thau Lagoon, *Ostreococcus tauri*, a picoeukaryote, whose size is less than 1 µm, is responsable for most of the primary production in the summer (Courties et al 1994). Furthermore, high levels of dissolved organic matter (DOM) in the Lagoon provide a potential for high bacterial production. However, such small-sized picoparticles are not efficiently retained by gills of bivalves, particularly oysters (< 20 % for picoparticles, Shumway et al. 1985; Héral 1987; Riisgård 1988; Barillé et al. 1993).

Protists, which consume bacteria and phytoplankton, are abundant in coastal ecosystems (Revelante & Gilmartin 1983; Sherr et al. 1986 a; Fenchel 1988; Leakey et al. 1992). They are preyed upon by numerous organisms of zooplankton, particulary copepods (Berk et al. 1977; Jonsson & Tiselius 1990; Gifford & Dagg 1991; Hartmann et al. 1993) and thus have been suggested as a major trophic link between picoplankton and micro or macroplankton (Porter et al. 1979; Conover 1982; Sherr et al. 1986 b; Stoecker & Capuzzo 1990). Likewise, protists may represent a trophic link between picoplankton and filter-feeding bivalves. Some data support this assumption. Tintinnids were observed

in oyster's stomachs (Paulmier 1972). Moreover, filter-feeding benthic molluscs retain protists, as shown by contaminations of bivalves by toxic flagellates (Sournia et al. 1991). In a mixed cell suspension of phytoplankton and dinoflagellates, six different species of bivalves were able to selectively clear and digest dinoflagellates (Shumway et al. 1985). The mussel *Geukensia demissa* removed microbiota from a salt-marsh water column with different effectiveness according to the cell type (Kemp et al. 1990). *Crassostrea gigas* easily consume a non-toxic microdinoflagellate (Bardouil et al. 1996). Ingestion and assimilation of bacterial carbon *via* heterotrophic flagellates were demonstrated in mussels (Kreeger & Newell 1996). Similarly, the oyster *Cr. gigas* retained and ingested a cultured bacterivorous ciliate, *Uronema* sp. (Le Gall *et al.* 1997). In an Atlantic coastal oyster pond, the field community of hetero/mixotrophic protists was 90 % retained and represented the main energy resource for oysters (Dupuy et al. 1999). Heterotrophic protists may thus constitute an alternative or complementary food resource for oysters, allowing indirect recovery of DOM and picoplanktonic production, otherwise not accessible to them.

In the Thau Lagoon, loricate ciliates such as tintinnids are abundant (Lam-Hoai et al 1997); but, heterotrophic flagellates and aloricate ciliates had not yet been studied. The aim of this study is to assess protist and zooplankton abundances in the water column of the Thau Lagoon and investigate their potential use by oysters as trophic resource. Specific questions to be addressed were: 1) do the different microbiota from the planktonic community represent a substantial energy resource in the Thau Lagoon water columns? 2) is oyster filtration effective to account for the removal of a sufficient trophic resource from cleared microbiota? Our experimental design to study the effect of oyster filtration on a field planktonic community was to monitor changes in the abundance of the different microbiota in a 1300 ml tray, on a time scale of 30 minutes.

MATERIALS AND METHODS

The Thau Lagoon (France, 3° 36' E, 43° 24' N) is spread over 75 km^2, with a mean depth of 5 m and is connected to the sea by 3 narrow channels. Shellfish breeding is carried out in 3 areas off the northern-western shore (fig. 1).

Oyster collection and acclimation

Oysters were collected in the middle of August 1998 from the NW farming area (station Z, fig. 1) of the Thau Lagoon. Fifty adult *Crassostrea gigas* (1 year old, shell length about 5 cm) were transported to the laboratory, removed of epibionts and acclimatized overnight to the ambiant field temperature of 25°C, in GF/C (1.2 µm, Whatman) filtered water. Just before the experiment, 10 active filtering oysters were selected and only 3 oysters were used to perform the retention experiments.

Planktonic community collection

The planktonic community devoted to assess protist abundance in the Thau Lagoon and provided as potential food to the experimental oysters, came from station Z (fig. 1), in the middle of the northern farming area, near the NW bank of the Lagoon (5 m in depth). Water samples were collected on 17 August 98, from the subsurface (50 cm), using a sampling 5 l "Niskin" bottle whose central latex cord had been replaced with silicone tubing and held at field temperature (25°C) in an opaque carboy until use for enumeration of protist and for oyster retention experiment.

Pigments determination

Sea water aliquots (from 30 to 50 ml) were filtered under a 10 mm Hg vacuum through a 2.5 cm GF/F filter, which was kept in Corning glass tubes at -20°C until extracted. Triplicate filters were ground in 4 ml of 90 % acetone. Samples were stored in the dark at 4 °C for 24 h; at the end of the extraction period, the tubes were centrifugated for 10 min at 1 200 x g. Concentrations of chlorophyll *a* and pheopigment were measured using a Perkin-Elmer LS50 b spectrofluorometer and pigment amounts calculated according to Neveux & Lantoine (1993).

Phytoplankton: taxonomy, enumeration and gross growth rates

Sea water triplicates were fixed by formaldehyde (4% final concentration) for microscopic determination of nano-microphytoplankton and enumerated in Utermöhl settling chambers. For flow cytometry analysis of picophytoplankton (< 2 µm), samples were formaldehyde-fixed (0.5% final concentration) and stored in liquid nitrogen, according to Troussellier et al. (1995). Subsamples (200-500 µl) were analyzed with an ACR-1400-SP flow cytometer (Brucker Spectrospin, Wissembourg, France). Picoplanktonic cell sizes were defined by using fluorescent beads (2 µm diameter PolySciences) and controlled by filtration through Nuclepore polycarbonate membranes (2 µm porosity). Cells > 2 µm group together nano and microphytoplankton up to 50 µm diameter (upper boundary set by the cytometer). Numeration variability was evaluated from triplicate sample measurements. Standard deviations (SD) were between 1 and 7 % for picoplanktonic numerations and between 5 and 41 % for the largest phytoplanktonic cells, a high value related to their low abundance in the sample volume. This variability is similar or slightly inferior to that obtained from 5 samples during each experiment: either 4 to 11 % for picoplankton in control and grazing experiments and 20 to 35 % for cells greater than 2 µm in control experiments.

Phytoplankton gross growth rates and mortality by grazing were determinated *in situ* at station Z and simultaneously over 24 h using the dilution technique of Landry & Hassett (1982) as modified by Landry et al. (1995). No prefiltration was performed, as large diatoms (size greater than 200 µm) were present. Flow cytometry was used to distinguish size classes. The variability was estimated from duplicate samples by the software of Statview 4, i.e. 3 % for the gross growth rate and 21 % for the grazing mortality.

Ciliates and flagellates: taxonomy and enumeration

Ciliates, dinoflagellates and flagellates were fixed, stained and enumerated according to methods modified from Haas (1982), Caron (1983) and Sherr et al. (1994) modified by Dupuy et al (1999). Samples were made in triplicate. Sizes of all cells (length and width) were measured through a

calibrated ocular micrometer. 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 0.17 pg Carbon (C) μm^{-3} (Putt & Stoecker 1989), corrected for glutaraldehyde fixative (Leakey et al. 1994). For Tintinnina protists, besides enumerations according to Utermöhl, counts of individuals retained by a 40 µm mesh-size collector were proceeded by image analysis (Lam-Hoai et al. 1997).

Zooplankton

The large zooplankton was sampled from triplicate mixed vertical tows from the 2 m deep water column were performed, using a 40 µm mesh-sized net (input volume: 356 l). Zooplankter counts, size measures and biovolume estimations were made using an image analysis technique (Lam-Hoai et al. 1997). Cell volume was converted into carbon units as reported above. Zooplankton abundances at station Z were evaluated by means of two sampling methods: mixed vertical tows from the 2 m deep water column with a 40 µm mesh-sized net and sampling with 5 l "Niskin" bottle filtered through the same mesh size. Similar results were obtained: 22 identical taxa out of 30 were identified in both sample types.

Experimental protocol for the study of protist retention by oysters

The possible influence of oyster filtration upon the natural planktonic community was studied by comparing the changes in plankton abundances in triplicate suspensions with or without filtering oysters during 30 min at ambient temperature (25°C). At the start of the feeding period, 3 replicate incubations , each containing one oyster (mean dry tissue weight 1.86 ± 0.15 g) were assessed: each oyster was transferred into an individual 1500 ml pyrex round tray containing 1300 ml of natural unfiltered Lagoon water from station Z, gently homogeneized with a magnetic rod to prevent sedimentation. Two experimental treatments were performed, each in triplicate: suspensions were (1) allowed to evolve as controls, or (2) delivered to actively filtering oysters. Water samples were collected every 5 min to assess picophytoplankton, nano-microphytoplankton, flagellate,

dinoflagellate and ciliate abundances. Diatoms were enumerated at t_0 and t_{15} min and large zooplankton abundances at t_0 and t_{30} min. It is to be noticed that at the very low natural food concentration used in this study (seston load = 0.65 mg l^{-1}), there was no visible production of pseufaeces. Dry tissue weight of each oyster was recorded at the end of the experiment to express the carbon resource from retained particles per g of oyster dry tissue.

Calculation of specific retention percentages, clearance rates and retained resource

To investigate the occurence of a differential grazing by the oyster among the various taxons of picophytoplankton, nano-microphytoplankton, flagellate and ciliate protists and large zooplankton, the percentage of cells retained between 0 and 15 min was calculated. Clearance rates were estimated for each planktonic type, as previously reported (Dupuy et al. 1999) between 0 and 15 min. During the first five minutes of the experiment, individual variations in the settling of a regular oyster filtration prevented any reliable study of the protist abundance evolution in the triplicate suspensions: therefore, we selected the subsequent sampling time (15 min) as the most appropriate "standard" time in our clearance experiment. Defined as the theoretical water volume entirely cleared from particles per unit time (l h^{-1}) (Bayne & Widdows 1978), the clearance rate was calculated according to Coughlan (1969), for the main taxonomic groups from planktonic suspension. Taking into account that weight specific filtration decreases with increasing body size, standardized clearance rates were calculated according to Riisgård (1988).

Specific contribution of the various planktonic taxa to the particulate resource retained by oysters was expressed as particulate organic carbon (POC) retained per unit time and per g of oyster dry tissue (µg C h^{-1} g^{-1}). It was calculated by multiplying the field carbon resource of each taxon (µg C l^{-1}) by the corresponding standardized clearance rate (l h^{-1} g^{-1}). Initial picophytoplankton, nano-microphytoplankton, diatom, flagellate, dinoflagellate, ciliate and large zooplankton abundances from the triplicate experiments with or without a filtering oyster were compared using a t-Student test (data were previously tested for normality using the Kolmogorov-Smirnov test). Changes in these microbial

abundances were followed in triplicate controls during the 30 min experiment by comparing with regression test aliquots sampled at 5 time points (0, 5, 10, 15 and 30 min).

RESULTS

1. Planktonic resource available in the Thau Lagoon in August 1998

Taxonomic composition and standing stocks of field microbiota were evaluated in order to estimate the relative contribution of micro-organisms to the particulate organic carbon (POC) resource.

Picophytoplankton

Picophytoplankton was the most abundant autotrophic plankter at the time of our investigation in the oyster breeding area. It was mainly constituted of picocells exhibiting the same flow cytometric characteristics as the eukaryotic *Ostreococcus tauri*, which is the dominant autotrophic taxon in this < 2 µm size-class of the Thau Lagoon community during the summer period (Vaquer et al. 1996). Mean abundance of this eukaryotic picophytoplankter was $2.5 \pm 0.3 \times 10^7$ cells l^{-1}, which represents more than 97 % of the total autotrophic community density. However, these tiny cells accounted for a very low biovolume (0.52 μm^3) and a subsequent low carbon cell content (0.089 pg C $cell^{-1}$).

Diatoms

The microphytoplankton was mostly constituted in August 1998 by large diatoms, such as *Rhizosolenia setigera*, *Lioloma pacificum*, *Nitzschia longissima* and colonial diatoms, such as *Pseudo-Nitzschia seriata*, *Thalasionema nitzschioides*, *Rhizosolenia striata*, *Skeletonema costatum*, *Leptocylindricus sp.* and several species of *Chaetoceros*. The average abundance of these algae was $4 \pm 0.8 \times 10^5$ cells l^{-1}, mostly represented by *Skeletonema costatum* and *Chaetoceros* sp. Other taxons (*Hemiaulus* and *Pleurosigma*) were scarce. Diatom sizes (table 1) ranged from 15 µm for *Skeletonema costatum* up to a maximum value of 541 µm for *Lioloma pacificum.* Biovolumes were high and carbon cell contents fluctuated between ≈ 25 pg C $cell^{-1}$ for *Leptocylindrus* and *Nitszchia longissima* and ≈ 5 000 pg C $cell^{-1}$ for a large diatom, *Guinardia striata*.

Flagellates, dinoflagellates

Flagellate abundance in the oyster farming area was 2.9 ± 1.2 x 10^5 cells l^{-1}, among which 20 % were heterotrophic. Cell lengths ranged from 3 to 19 µm. Dinoflagellate abundance was 1.1 ± 0.3 x 10^4 cells l^{-1}, with cell sizes ranging from 35.5 µm for an unidentified dinoflagellate up to 146 µm for *Ceratium* sp. (table 2).

Ciliates

Aloricate ciliates (total abundance 7.2 ± 2.1 x 10^3 cells l^{-1}) were dominated by the subclass Choreotrichia, mainly represented by the order Haptorida (5.1 ± 2.2 cells l^{-1}) and dominant type was *Mesodinium* sp. Common taxa from the order Oligotrichida (*Strombidium* sp., 1.8 ± 1 x 10^3 cells l^{-1}) were also observed. Ciliate sizes ranged from a minimal 12.5 µm length for a *Mesodinium* sp., up to a maximum value of 209 µm for *Favella* sp. (table 3). Loricate ciliates were mostly represented by Tintinnina with abundance of 137 ± 66 cells l^{-1} among which 13 large cells l^{-1} were retained by the 40 µm mesh of the zooplancton collector. Tintinnina biovolumes, as well as carbon cell contents, were much higher than for other assemblages (table 3).

Large zooplankton

In the farming area (station Z), 68 zooplanktonic organisms from 27 identified taxa could be grouped into 5 major groups, arranged in a decreasing abundance order (table 4). The total biovolume, estimated from image analysis was 47 x 10^6 μm^3 l^{-1}. Meroplankton, such as Polychaeta larvae, Cirripeda nauplii and nectobenthic taxa were almost absent.

Potential planktonic carbon resource

In order to estimate the potential planktonic resource available for oysters in the farming area, the particulate organic carbon (POC) relative to each taxonomic group was estimated. By multiplying for each microbiota the specific carbon cell content by the field abundance, we estimated the relative

contribution of each taxon to the total carbon resource of the planktonic community (table 5). Microplanktonic diatoms, which have large biovolumes, constituted the first trophic resource (161.5 µg C l^{-1}), in spite of their low abundance in the Thau Lagoon on 17 August 1998. Dinoflagellates, represented the second carbon resource (16.6 µg C l^{-1}). On the contrary, picoeukaryotic cells, with a very small biovolume (0.52 μm^3), accounted for a minimal carbon biomass (2.4 µg C l^{-1}), despite their high field density. Ciliates, flagellates and large zooplankton were not important in terms of biomass in Thau waters at the time of our experiment.

Gross growth rates and grazing mortality of autotrophic plankton was estimated *in situ* using incubations after dilution manipulations: the subsequent reduction of grazing pressure thereby uncoupling growth and mortality. The small nanophytoplankton exhibited the highest gross growth rate: 3.27 day^{-1} (table 6). The gross growth rate of other phytoplankters averaged 2.6 day^{-1}. The grazing mortality of picoplankton in a size range between 0.7 and 0.9 µm was the highest, with 3.32 day^{-1}. Thus, the whole daily picoplanktonic production was consumed by heterotrophic planktonic grazers (G/Ke = 1.29, table 6).

2. Retention by oysters of trophic resource cleared from the planktonic microbiota of Thau Lagoon in August 1998

Grazing experiments

The planktonic suspension used for oyster grazing experiments, which was collected in subsurface at station Z, exhibited a complex taxonomic composition: 2.5 x 10^7 picophytoplankters l^{-1}, 4 x 10^5 diatoms l^{-1} from nano-microplankton, 2.2 x 10^5 ± 2 x 10^4 flagellates l^{-1}, 1.2 x 10^4 ± 3.2 x 10^3 dinoflagellates l^{-1}, 7.4 x 10^3 ± 2 7 x 10^3 ciliates l^{-1}, 68 large zooplankters l^{-1}.

Since all experimental suspensions originated from the same water sample, initial microbial abundances showed no significant difference between suspensions without (control) or with filtering oysters (t Student test, n= 6, p >> 0.05). In the same way, the chlorophyll *a* concentration was similar at t_o in control suspensions (1.86 µg l^{-1}) and oyster treatments (1.82 µg l^{-1}), and pheophytin initial

concentrations were identical in control and oyster trays.

In control suspensions gently homogeneized, chlorophyll *a* concentrations remained constant (1.82 µg l^{-1}) until the end of the experiment; similarly, pheophytin concentrations were steady in controls. Abundances of picoeukaryotes, >2 µm phytoplankton, flagellates, dinoflagellates and ciliates remained virtually constant for 30 min (fig. 2). Results of regression tests were $r^2 = 0.204$, $p >> 0.05$ for picoeukaryotes, $r^2 = 0.18$, $p >> 0.05$ for flagellates, $r^2 = 0.12$, $p >> 0.05$ for dinoflagellates and $r^2 = 0.0019$, $p >> 0.05$ for ciliates.

On the contrary, in suspensions with actively filtrating oysters, chlorophyll *a* concentrations dropped to 0.34 µg l^{-1} at the end of the grazing experiment, i.e. only 18.7 % of the initial concentration. The level of pheophytin increased, probably in relation with pigment damage, resulting from oyster grazing. The abundance of picoeukaryotes was never affected by the presence of oysters during our experiments (fig. 2a). Changes in the abundance of nano/microplanktonic particles through time are shown in fig. 2 b-e. Phytoplanktonic cells > 2 µm decreased in triplicate oyster trays, in various ratios according to taxa (mean removal of 80 to 98 %): most of the largest protists in a size between 12.5 and 540 µm, diatoms, ciliates, dinoflagellates and large flagellates were highly retained.

Yield of cells cleared within 15 min in the oyster treatment suspensions was 92 % for microphytoplankton, 94% for dinoflagellates, 93 % for ciliates (fig. 3). Only 44 % flagellates were retained. At the end of the experiment (30 min), approximately 90 % of the large zooplankton, all dinoflagellates and ciliates and 55 % of the flagellates had been cleared by the bivalves. Flagellates in a size lower than 5 µm were poorly (45 %) retained (fig. 4).

Clearance rates and carbon resource cleared from planktonic particles

Particle abundances at 0 and 15 min were also used to estimate oyster clearance rates for each microbiota. The calculation was based on the assumption that abundance decreased exponentially through time in an enclosed suspension volume. Specific clearance rates, ie water volume entirely cleared from specific type of particles per unit time and per oyster dry tissue weight (l $h^{-1}g^{-1}$), were compared among particle types (table 7). Maximum clearance rates were typical of the most efficiently

retained microbiota (16.7 l $h^{-1}g^{-1}$for > 5 µm flagellates, 14.8 l $h^{-1}g^{-1}$for dinoflagellates). Conversely, clearance rates were low for poorly retained particles (6.8 l $h^{-1}g^{-1}$ for < 5 µm flagellates) and close to zero for the picoeukaryote, showing that picoparticles are not retained by oysters. The mean clearance rate for all microbiota in a size > 5 µm averaged 11.8 l $h^{-1}g^{-1}$.

The carbon removed as living planktonic particles by oysters was evaluated from the calculated clearance rate and initial biomasses of microbiota in experimental suspensions. Carbon removal was dependent on taxon (table 7). The main resource arose from diatoms (81 %) and dinoflagellates (15 %). Ciliates and flagellates poorly contributed to oyster resources and picoeukaryotic cell contribution was only 0.03 % of the trophic resource in this experiment. The oyster *Crassostrea gigas* retained on average 1 600 µg C h^{-1} g^{-1} from plankton. However, large zooplankton was not taken into account, as the lack of intermediary zooplankton sampling between 0 and 30 min in our protocol precludes a rigorous estimation of the clearance rate.

DISCUSSION

The aim of this study was to assess a possible contribution of picoeukaryotes in food resource intake of the oyster *Crassostrea gigas.* In Thau Lagoon, the picoeukaryote *Ostreococcus tauri* is the dominant autotrophic picoplankter (Vaquer et al 1996) with a maximum summer abundance of 2 x 10^8 cells l^{-1} (Chretiénnot-Dinet et al 1995). As not directly retained by oyster gills, picoeukaryotes might constitute an indirect food resource, transfered towards bivalves *via* heterotrophic protists. Such a trophic link between picoplankton and benthic suspension feeders was already evidenced in oysters (Le Gall et al. 1997; Dupuy et al. 1999). In order to test this assumption, we have evaluated the planktonic resource in Thau lagoon and investigated its utilization by oysters.

Trophic resource available in the Northern farming area of Thau Lagoon in August 1998

Inside the NW oyster farming area of Thau Lagoon (station Z), picophytoplankton abundance was 2.5 x 10^7 cells l^{-1} on 17 August 1998. Most of the autotrophic picocells exhibited the same flow cytometric characteristics as the picoeukaryotic Prasinophyceae, *Ostreococcus tauri* (Courties et al. 1994). Our current abundances are in the range previously observed in Thau Lagoon, ie 3.4 x 10^7 cells l^{-1} (Vaquer et al. 1996), but lower than the maximum 10^8 cells l^{-1} densities at the same station in August 1992. Such high levels of picoeukaryotic abundances have been reported elsewhere in Mediterranean Sea (Magazzu & Decembrini 1995).

Abundance of the nano-microphytoplankton (ie. cells > 2 µm: diatoms, dinoflagellates and phytoflagellates) inside the oyster farming area was 6.5 x 10^5 cells l^{-1}, a value 7.5 times lower than mean abundance (5 x 10^6 cells l^{-1}) previously observed in Thau Lagoon, and 3 times lower than previous summer data (2 x 10^6 cells l^{-1}) at the same station (Vaquer et al. 1996). Diatoms were either isolated or chain associated, in a size range between 15 µm and 541 µm, constituting 62 % of the nano-microphytoplankters and *Skeletonema costatum* (Thalassiosiraceae) was the dominant taxon. Phytoflagellates represented 36.3 % and dinoflagellates only 1.7 % of the nano-microphytoplanktonic community. Among, them, 80 % were auto or mixotrophic and 20 % heterotrophic. Their sizes were between 3 and 19 µm and their total abundance (2.94 x 10^5 ± 1.2 x 10^5 cells l^{-1}) was low compared to data reported in other environments: Saint-Lawrence estuary: 1.9 x 10^6 to 6 x 10^6 cells l^{-1} (Lovejoy et al. 1993); Limfjorden marine shallow-water: 2 x 10^6 cells l^{-1} (Andersen & Sorensen 1986); Parker Estuary, Massachusetts: > 10 x 10^6 heterotrophic flagellates l^{-1} (Wright et al. 1987).

As far as we know, the naked ciliate community has not yet been studied in Thau Lagoon: therefore, we compared our data to results from distant areas. Our estimate of planktonic ciliate abundance (naked ciliates and Tintinninds) in the oyster farming area was 7 194 x 10^4 ± 2 10^4 ciliates l^{-1}. Ciliates were dominated by the order Choreotrichida, with the prevailing Haptorida taxon, *Mesodinium* spp., (5 089 ± 2 211 cells l^{-1}). Oligotrichida, with the dominant taxon, *Strombidium* spp, (1 765 ± 1 054 cells l^{-1}) were in low abundance, compared to high summer densities (9 x 10^4 cells l^{-1}) observed in the

Mediterranean Sea (Rassoulzadegan 1977). Tintinnid abundance was 209 ± 87 cells l^{-1}, a value slightly higher than reported data (75 cells l^{-1}) from Thau Lagoon in 1994 (Lam-Hoai et al. 1997). In Mediterranean waters (Villefranche-sur-mer Bay), ciliates may be much more abundant: 10^4 cells l^{-1} (Rassoulzadegan & Gostan 1976).

Large zooplankton abundance in the oyster farming area was 68 000 cells m^{-3}, a value 20 times higher than mean value in 1994 (Lam-Hoai et al. 1997).

The amounts of potential carbon resources available in the field were estimated for every planktonic microbiota (table 5). Microphytoplankton was the most important resource on 17 August 1998 (161.5 µg C l^{-1}, ie 84.6% of the total organic carbon). Dinoflagellates (16.6 µg C l^{-1}) were the second C resource. Flagellates (≈ 3 µg C l^{-1}), ciliates (≈ 3 µg C l^{-1}) and zooplankton (≈ 5 µg C l^{-1}), were not responsible for trophic richness of Thau Lagoon waters and picophytoplanktonic cells represented only 1.3 % of the carbon resource. The ciliate resource in Thau was much lower than elsewhere: ciliates can represent up to 52 µg C l^{-1} (annual mean) in the Saint-Lawrence Estuary (Sime Ngando et al. 1995) or 63.5 µg C l^{-1} (in June 97) in French Atlantic coastal ponds (Dupuy et al. 1999).

Our estimation of microbiota in Thau Lagoon and the wide range of previously reported data show the natural variability of picophytoplankton, protist and zooplankton abundances in the field. In terms of numerical abundance, the phytoplanktonic community was overnumbered by picoeukarotes, but as regards C resource, microphytoplankton dominated in Thau Lagoon waters. In the last years, the highest picoeukaryote and nanophytoplankton densities were usually found in summer in Thau Lagoon, because of favourable environmental factors (Vaquer et al. 1996). Surprisingly, in August 1998 the picophytoplankton and nanophytoplankton abundances were not as high as expected, and we observed the development of very large diatoms; probably related to terrestrial nutrient inputs, resulting from several rainy days before sampling. However, the retrospective estimation of picophytoplankton C resource in past periods of maximal abundance: (10^8 cells l^{-1} in 1992; Vaquer et

al. 1996) only results in a weak resource of 16 µg C l^{-1}. Even during picoeukaryotic bloom events, the main trophic resource would proceed from microphytoplankton, particularly diatoms.

Utilization by oysters of the planktonic trophic resource of Thau Lagoon in August 1998

Grazing experiments were carried out using a natural planktonic population from the Northern Thau Lagoon oyster farming area. Chlorophyll *a,* pheopigment concentrations and microbial abundances remained stationary in controls. Conversely, chlorophyll *a* decreased in the oyster grazing experiments (from 1.82 to 0.34 µg l^{-1}), due to retention of phytoplanktonic cells by oysters; in the same time, pheopigment concentrations increased, perhaps in relation to a damage of pigments resulting from oyster grazing. Actually, diatoms (even large species such as *Rhizosolenia setigera*, *Lioloma pacificum*), dinoflagellates and ciliates were highly retained by oysters (respectively 92 %, 96 %, 93 %) during grazing experiments. Only 45 % of flagellates (size from 3 µm to 19 µm) were cleared. Their retention depended on their size: the smallest flagellates (< 5 µm), which were also the most abundant, were poorly retained (~ 48 %), compared to flagellates in a size > 5 µm (~ 80 % retained). The maintenance of picophytoplanktonic abundances, either in controls or in oyster grazing experiments, evidenced that those tiny particles (~1 µm size), were not retained by oysters gills. In our experiment, oysters retained efficiently particles larger than 5 µm diameter. Our data agree with previous reports of the known size spectrum of particle retention by oyster filtration: oyster retained less than 10 % of < 1 µm particles, more than 50 % of > 3 µm particles (Deslous-Paoli et al. 1987) and 100 % for 7 µm particles in *Crassostrea gigas* (Héral 1987) and *Crassostrea virginica* (Riisgård 1988); when sestonic load was low, 4 µm particles were 100 % retained (Barillé et al. 1993).

The observation of ciliate retention agrees with the previously demonstrated retention by *Crassostrea gigas* of a cultured ciliate (Le Gall *et al.* 1997) and of a natural protist community from French Atlantic coastal ponds, with retention of 94 % for ciliates and 86 % for flagellates (Dupuy et al. 1999). It also corroborates the observations by Paulmier (1972), who reported Tintinnids to be abundant in the stomachs of wild oysters from the Atlantic coast.

The mean clearance rate for all microbiota (except picoeukaryote) averaged 11.8 l $h^{-1}g^{-1}$, a value similar to the estimation calculated according to Riisgård (1988) for an oyster, whose weight was 1.86 g (10.7 l h^{-1} g^{-1}). Clearance rates of oysters for flagellate, dinoflagellate and ciliate protists were between 12 and 16 liters h^{-1} g^{-1} and for diatoms 8 liters $h^{-1}g^{-1}$. These values were higher than previously reported clearance rates in water with high sestonic load: 5.7 l h^{-1} g^{-1} for *Phaeodactylum* at high concentration (10^7 cells l^{-1}, ie 16 µg C l^{-1}) (Fiala-Medioni et al. 1983), 5.5 l $h^{-1}g^{-1}$ for estuary plankton of Marennes-Oléron (Deslous-Paoli et al. 1987), 4.8 l $h^{-1}g^{-1}$ (Bougrier et al. 1997) and 7.8 l $h^{-1}g^{-1}$ (Dupuy et al. 1999). Conversely, with low sestonic loaded waters, clearance rate was 12 l $h^{-1}g^{-1}$ for *Phaeodactylum* at 10^6 cells l^{-1} (Fiala-Medioni et al. 1983) and 16 l h^{-1} g^{-1} for *Isochrysis galbana* at 1 x 10^5 cells l^{-1}, ie 0.9 mg C l^{-1} (Walne 1972). These last values are in the range of our current data. Oysters may adjust their clearance rate in oligotrophic conditions. Fiala-Medioni et al. (1983) and Deslous-Paoli et al. (1987) noticed that when seston load is low, bivalves increased their effort of filtration to satisfy their energy requirement.

Our current investigation supports the importance of phytoplankton in oyster nutrition (Héral 1987, Pastoureaud et al. 1996). Evaluation of the retained resource from each taxonomic compartment during grazing experiments was calculated. The highest values of retained resource were from the microphytoplankton (1300 µg C $h^{-1}g^{-1}$) at a concentration of 4 x 10^5 cells l^{-1}. Fiala-Medioni et al. (1983) estimated that oyster filtering *Phaedactylum tricornatum* retained about 90 µg C h^{-1} g^{-1} for a phytoplankton concentration of 8 x 10^6 cells l^{-1}. This difference may result from the greater size and carbon content per cell of diatoms present in Thau Lagoon in August 1998 (*Rhizosolenia setigera* contains 4 310 pg C $cell^{-1}$) compared to *Phaedactylum tricornatum* (1.6 pg C $cell^{-1}$). Dinoflagellate represented a resource of 245 µg C $h^{-1}g^{-1}$. For ciliates, the retained resource was 39 µg C $h^{-1}g^{-1}$, a value much lower than the 126 µg C ciliates $h^{-1}g^{-1}$ retained by oysters in Atlantic oyster ponds, where ciliates were abundant (2.3 x 10^4 cells l^{-1}) (Dupuy et al. 1999). From the sum of all planktonic partitions, we can estimate that oysters retained 1 600 µg C h^{-1} g^{-1}. Vaquer et al (1996) previously observed over one year that picophytoplankton abundances were similar inside and outside the oyster farming area.

Conversely, they noticed that densities of nano-microphytoplankton, flagellates and zooplanktonic organisms were significantly higher outside the oyster farming area.

Most studies that have examined the nutritional importance of protist as a "trophic link" have focused on pelagic consumers, such as zooplankton (Berk et al. 1977; Porter et al. 1979; Sherr et al. 1986 b, Jonsson & Tiselius 1990; Gifford & Dagg 1991); similar experiments on benthic filter-feeding consumers are scarce (Kreeger & Newell 1996 ; Le Gall et al. 1997, Dupuy et al. 1999). Trophic coupling between pelagic protists and benthic suspension-feeders is poorly documented in aquatic food models (e.g. see Legendre & Le Fèvre 1995), although such a relationship could be of primary importance for C and N transfer, from the microbial food web to filter-feeder bivalves in benthos (Le Gall et al. 1997). In Thau Lagoon, the picoeukaryote community (average abundance 3.4 x 10^7 cells l^{-1}, Vaquer et al. 1996), constituted the most abundant primary food resource for heterotrophic/mixotrophic flagellates, dinoflagellates and ciliates. However, because of the small biovolume of their cells, picoeukaryotes only represented 3 µg C l^{-1}. Even during summer bloom events (2 x 10^8 cells l^{-1}, Chretiénnot-Dinet et al. 1995), the picoeukaroyte would only supply the trophic system with a weak available biomass of 16 µg C l^{-1}. In addition to the low contribution of picophytoplanktonic carbon in Thau lagoon waters in August 1998, picoeukaryotic cells exhibit growth rates of 2.58 to 2.64 day^{-1} (table 6), which are lower than for nanoplankton (2.65 to 3.27 day^{-1}). These results support the recent speculation of Raven (1994), that for small cells (< 0.9 µm), there is no increase in maximum specific growth rate with decreasing cell size. Bacteria which represent about 20 µg C l^{-1} in Thau lagoon (Trousselier M., pers. com.) were also a potential primary food for heterotrophic/mixotrophic flagellates, dinoflagellates and ciliates. Though ciliates exhibit high gross growth efficiencies of 40 % (Johnson et al. 1982; Ohman & Snyder 1991), relatively low amounts of picoplanktonic C would be recovered by oysters *via* the protist trophic link.

In conclusion, Thau Lagoon picoplankton did not represent an important trophic resource for farmed oysters because 1) *Crassostrea* cannot retain picoparticles and 2) the picoplankton only represented a

limited available carbon resource (in terms of biomass) to be transferred *via* a weak protist community. However this assertion must be moderated because 1) the experiment was only made one time 2) the productivity of these cells is not still well known, except very punctually (for example in 08/98). Measures of very raised growth rate of *O. tauri* (until 4.23 div d^{-1}) were obtained in experimental conditions (Courties et al. 1998). They would suggest an important potential productivity of picophytoplancton. In oyster pens of the Thau Lagoon during August 1998, microphytoplanktonic primary producers, in particular diatoms, could be considered as the main food source for oysters. However, as environmental conditions were particular at the time of our experiments (influence of watershed), additional investigations are needed to further identify the energy fluxes towards farmed oysters in Thau Lagoon.

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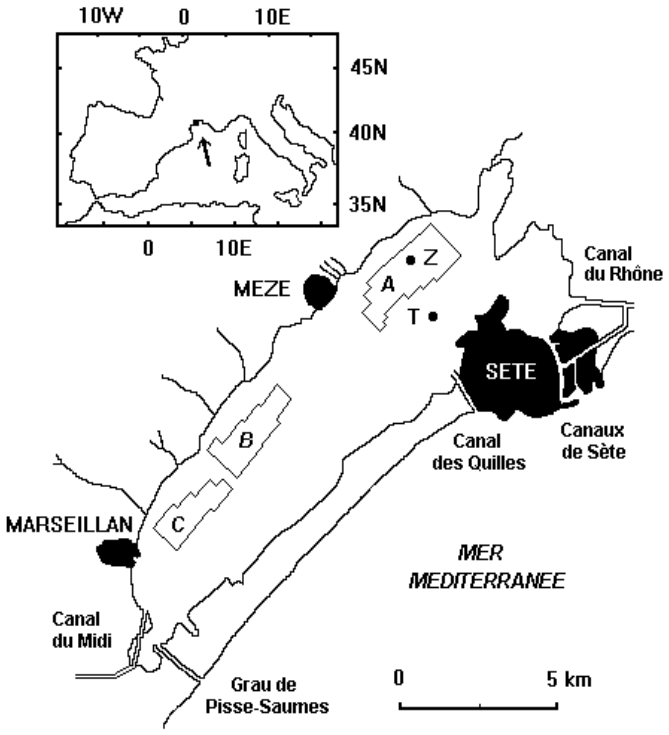
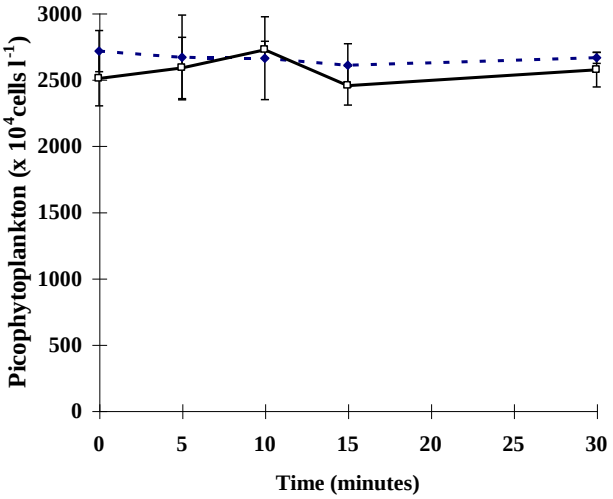
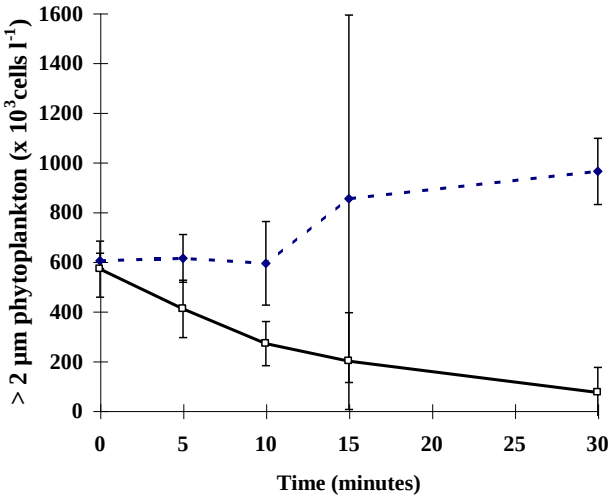


Figure 1: Location of the sampling station in Thau Lagoon: station is inside the northern A oyster farming area (from Thong Lam Hoai).

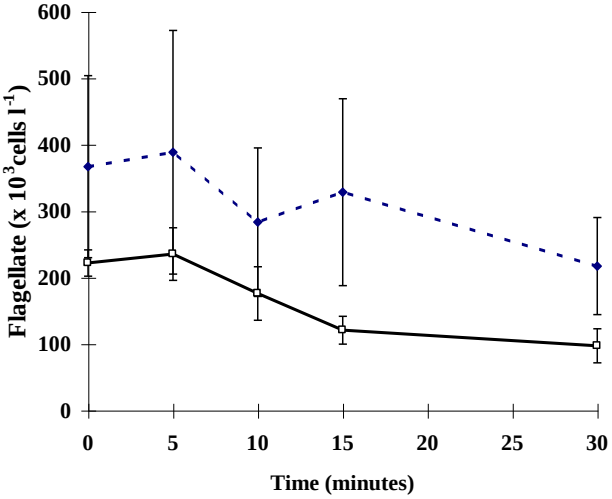
a


Picophytoplankton (x 10^4 cells l^-1)
3000
2500
2000
1500
1000
500
0
0 5 10 15 20 25 30
Time (minutes)

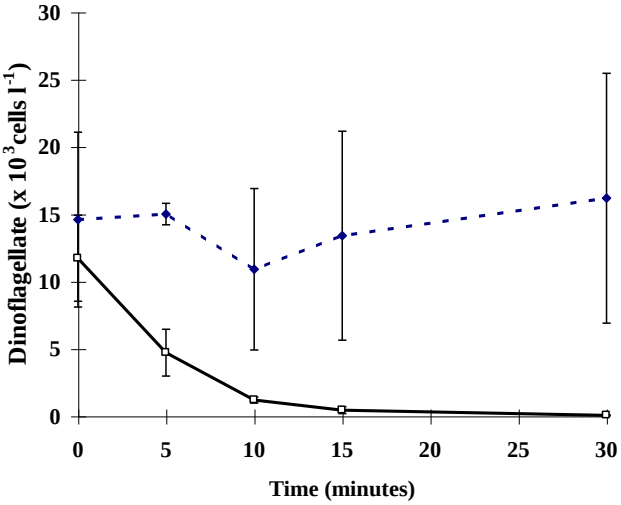
b


> 2 µm phytoplankton (x 10^3 cells l^-1)
1600
1400
1200
1000
800
600
400
200
0
0 5 10 15 20 25 30
Time (minutes)

c


Flagellate (x 10^3 cells l^-1)
600
500
400
300
200
100
0
0 5 10 15 20 25 30
Time (minutes)

d


Dinoflagellate (x 10^3 cells l^-1)
30
25
20
15
10
5
0
0 5 10 15 20 25 30
Time (minutes)

e

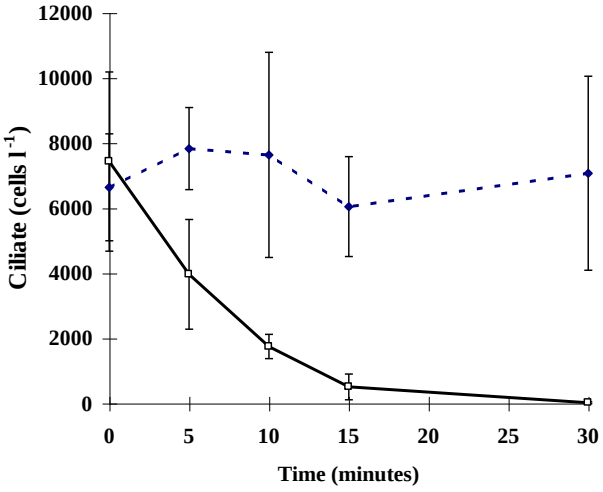

Ciliate (cells l^-1)
12000
10000
8000
6000
4000
2000
0
0 5 10 15 20 25 30
Time (minutes)

Figure 2: Retention of various taxons by the oyster *Crassostrea gigas*: changes in abundances (picophytoplankton (a), > 2 µm phytoplankton (b), flagellates (c), dinoflagellates (d) and ciliates (e), in 3 trays without oyster (◆ and dashed lines) or with a filtering oyster (□ and solid lines). Abundance data (mean ± SD, n = 3) were collected from 3 replicate incubations with an oyster and 3 replicate incubations without an oyster performed in 1300 ml lagoon water suspensions.

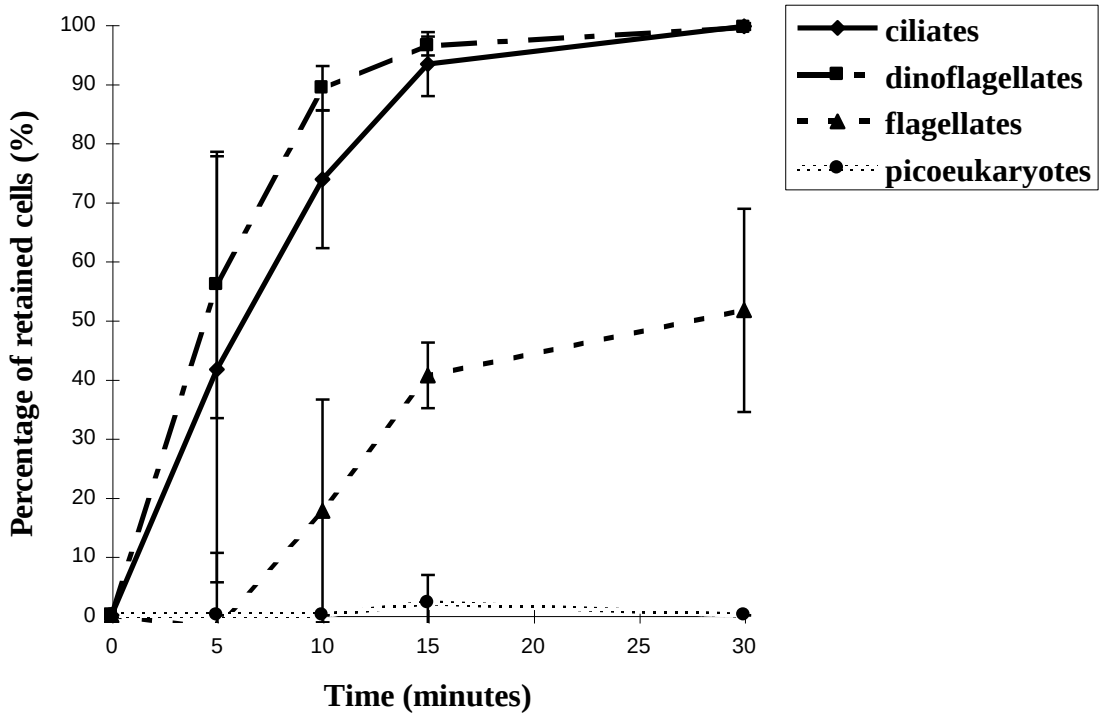


Figure 3: Percentage of cells retained by filtering oysters for ciliates, dinoflagellates, flagellates and picoeukaryotic cells. Abundance data (mean ± SD, n = 3) were collected from triplicate incubations performed in 1300 ml lagoon water suspensions with a filtering oyster.

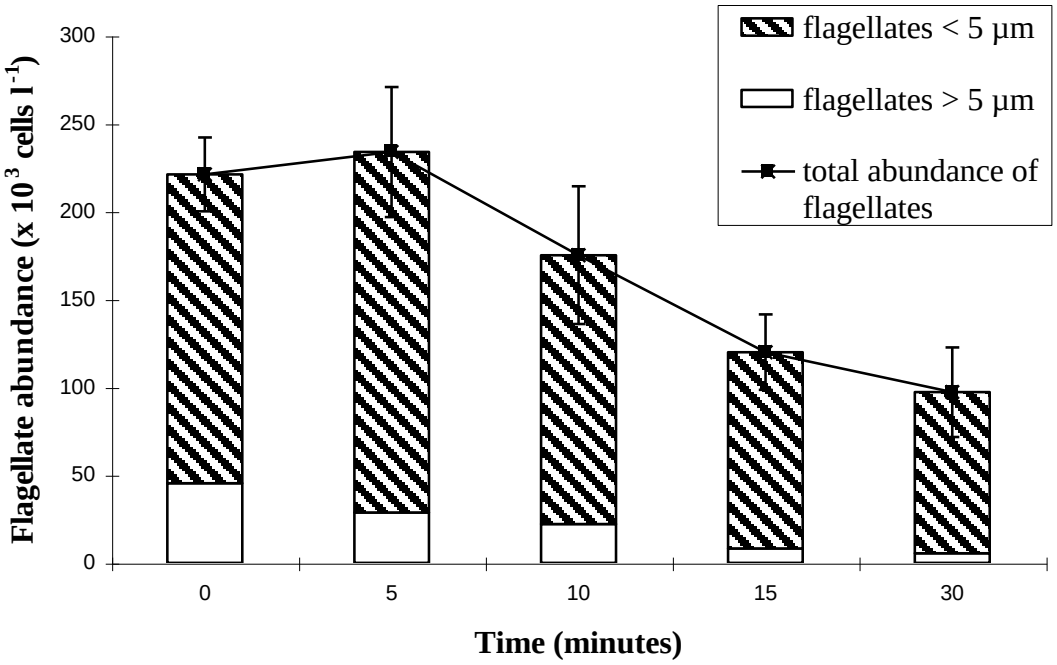


Figure 4: Removal by the oyster *Crassostrea gigas*, of flagellates in a size > 5µm or < 5µm. Flagellate abundance data (mean ± SD, n = 3) were collected from triplicate incubations performed in 1300 ml lagoon water suspensions with a filtering oyster.

Table 1: Taxonomic composition, size, biovolume and carbon content of picoeukaryotic cells and microplanktonic diatoms at Z farming area on 17 August 1998.

Order	Family	Genus	Species	Cell length (µm)	Biovolume ($x10^2$ µm^3)	Carbon per cell (pg C cell^{-1})
Mamiellales	Prasinophyceae	*Ostreococcus*	*tauri*	1	0.005	0.089
Centrales						
	Chaetoceraceae					
		Chaetoceros	spp.	18	27	452
	Rhizosoleniaceae					
		Guinardia	*striata fa.*	111	290	4926
		Rhizosolenia	*setigera fa.*	460	253	4308
	Melosiraceae					
		Leptocylindricus	sp.	24	1	25
	Thalassiosiraceae					
		Skeletonema	*costatum*	15	3	45
Pennales						
		Lioloma	*pacificum*	541	21	363
	Nitzschiaceae					
		Nitszchia	*longissima*	95	1	25
		Pseudonitszchia	sp.	415	21	356
	Fragilariaceae					
		Thalassionema	*nitzschioides*	47	4	73

Table 2: Taxonomic composition, size, biovolume and carbon content of dinoflagellates at Z farming area on 17 August 1998.

	Order	Family	Taxon	Species	Cell length (µm)	Biovolume ($x\ 10^3$ µm^3)	Carbon per cell (pg C cell^{-1})
Dinophyceae							
	Peridiniales						
		Ceratiaceae					
			Ceratium	sp.	146	1665	283025
		Peridiniaceae					
			Protoperidinium	sp.	36	12	1982
			Scripsiella	sp.	32	5	766
	Gymnodiniales						
		Gymnodiniaceae					
			Cochlodinium	sp.	52	8	1402
			Gymnodinium	sp.	48	24	4114
			Gyrodinium	sp.	50	33	5628
	Dinophysiales						
		Dinophysiaceae					
			Dinophysis	sp.	40	6	968
	Ebriales						
			Ebria	*tripartita*	45	12	2115
	Prorocentrales						
		Prorocentraceae					
			Prorocentrum	sp.	41	10	1675
	Dinoflagellates unidentified				35	26	4434
Euglenophyceae							
	Eutreptiales						
		Eutreptiaceae	unidentified		30	1	234

Table 3: Taxonomic composition, size, biovolume and carbon content of loricate and aloricate ciliates at Z farming area on 17 August 1998.

Order	Suborder	Family	Taxon	Species	Cell length (µm)	Biovolume (x 10^3 μm^3)	Carbon per cell (pg C $cell^{-1}$)
Choreotrichida	Tintinnina						
		Codonellidae					
			Tintinnopsis	sp.	37	13	2225
			Tintinnopsis	*corniger*	130	71	
		Tintinnidae					
			Eutintinnus	*fraknoii*	157	116	19777
			Eutintinnus	*fraknoii*	128	56	
		Ptychocyclidae					
			Favella	*serrata*	179	219	37165
			Favella	*serrata*	209	319	
	Strobilidiina						
		Strobilidiidae					
			Lohmaniella	sp.	12	1	174
Oligotrichida							
		Strombidiidae					
			Strombidium	sp.	47	20	3479
Haptorida							
		Mesodiniidae					
			Askenasia	sp.	15	2	348
			Mesodinium	sp.	27	3	536
			Mesodinium	*pulex*	12		
		Didinnidae					
			Didinium	sp.	45	36	6083
Scuticociliatida							
		unidentified			31	6	1080

Table 4: Sizes, biovolumes and corresponding resource of the main zooplanktonic taxa > 40 µm in the farming area on 17 August 98.

Taxons	Biovolume (%)	Length (µm)	Biovolume (x 10^6 µm^3)	Carbon per animal (ng C cell^{-1})	Field C resource (µg C l^{-1})
mostly Bivalvia veligers	21	138	0.608	85	3
Tintinnina: *Favella serrata, Eutintinnus fraknoi, Tintinnopsis corniger, Helicostomella subulata*	7.7	195	0.271	38	1
Copepoda nauplii	9.3	172	0.346	48	0.5
Appendicularia: *Oikopleura dioica*	31.2	518	0.83	116	1.4
Rotatoria: *Synchaeta vorax, Trichocerca marina*	6.7	191	0.454	64	0.3
Total of main taxa	100	247	0.64		6.2

able 5: Contribution of various taxonomic groups to the planktonic carbon resource available for oysters at the Thau lagoon Z farming area from triplicate samples on 17 August 98.

	Abundance (x 10^3 l^{-1})	Carbon ressource (µg C l^{-1})	Contribution to total POC %
Picophytoplankton	25 000	2.4	1.3
Microphytoplankton	400	161.5	84.4
< 5 µm flagellates	207	0.6	0.3
> 5 µm flagellates	87	2.3	1.2
Dinoflagellates	11	16.6	8.7
Ciliates	7.2	3	1.6
Zooplankton	0.07	5	2.6
Sum	25 712	191	100

Table 6: Phytoplankton gross growth rate and microzooplankton grazing rates. Data were collected from duplicate experiments.

Size class	Gross growth rate Ke (day^{-1})	Grazing mortality G (day^{-1})	G/Ke
Picoplankton (0.7-0.9 µm)	2.58	3.32	1.29
Picoplankton (1-2 µm)	2.64	1.6	0.61
Nanoplankton (2-4µm)	3.27	0.93	0.28
Nanoplankton (> 4µm)	2.65	0.83	0.31

Table 7: Contribution of various taxonomic groups to the particular resource retained by oysters, in the Z farming area of Thau Lagoon in triplicate samples on 17 August 1998. Resource is expressed as POC (µg C l^{-1}). Standardized clearance rate is the theoritical water volume entirely cleared from particles per unit time and standardized per oyster dry tissue weight (l $h^{-1}g^{-1}$) (Riisgård, 1988). Resource and Standardized clearance rate were multiplyed to estimate the POC resource retained by oyster (µg C h^{-1} g^{-1}).

	Standardized clearance rate (l $h^{-1}g^{-1}$)		POC resource in the field (µg C l^{-1})	POC resource retained by oysters (µg C $h^{-1}g^{-1}$)
	Mean	SD		
Picophytoplankton	0.02	0.003	2.4	0.05
Diatoms	8.1	1.3	161.5	1307.3
< 5 µm flagellates	6.8	6.2	0.63	4.3
> 5 µm flagellates	16.7	4.8	2.32	38.7
Dinoflagellates	14.8	0.8	16.6	245.5
Ciliates	12.7	6.1	3	38.6
Sum			186	1634