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Retention of ciliates and flagellates by the oyster *Crassostrea gigas* in French Atlantic coastal ponds: protists as a trophic link between bacterioplankton and benthic suspension-feeders

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ABSTRACT: In French Atlantic coastal ponds of the Charente, oysters can grow under conditions where phytoplankton production is limited by nutrient exhaustion. Such ponds typically show a high concentration of ciliates and flagellates during the growing season (1×10^4 to 3×10^5 cells l^{-1} in June 1997). In order to evaluate the importance of the 'protozoan trophic link' for energy transfer from the 'microbial food web' to large benthic suspension feeders, we offered a coastal pond community of ciliates and flagellates as potential prey to the oyster *Crassostrea gigas*. Clearance rate, filtered particles and relative retention efficiency were evaluated. In the grazing experiment, 94% of ciliates and 86% of flagellates (size between 4 and 72 µm), were retained by the oyster. Whatever their size, protists were similarly retained by the oyster gills. In terms of carbon, oysters retain on average 126 µg C h^{-1} g^{-1} dry weight, a value over 4 times higher than reported for phytoplankton. These results indicate that a field community of protists can contribute in coastal oyster rearing ponds to the energy requirements of the oyster *C. gigas*. We report here the first experimental evidence of a significant retention of a protist community by oysters, supporting the role of protists as a trophic link between picoplankton and benthic filter-feeding bivalves.

KEY WORDS: Bivalve · Oyster · Food source · Coastal pond · Microbial food web · Protist · Picoplankton · Trophic link

INTRODUCTION

Oysters obtain energy resources by filtering particles from seawater, and their growth depends upon the nutritive value of the retained seston (Berg & Newell 1986) and the trophic capacity of coastal waters (Héral 1987). The natural habitats of the oyster *Crassostrea gigas* are open coastal ecosystems, rocky shores or mud flats. Charente-Maritime, on the French Atlantic coast, is the most important European oyster farming area. Shellfish culture has developed in muddy bays (rearing areas of 4800 ha) and in semi-closed coastal ponds (3000 ha), characterized by relative confinement and low water-renewal rates.

The importance of phytoplankton in the nutrition of oysters is well documented (Héral 1987, Pastoureaud et al. 1996). However, in oyster rearing environments, such as the particularly light-limited turbid estuary of Marennes-Oléron, or in coastal ponds of the Charente where nutrients are quickly exhausted, phytoplankton cannot entirely account for the energy requirements of oysters (Héral 1987).

In the oceans, more than 50% of the primary production is due to unicellular organisms less than 3 µm in size (Li et al. 1983, Platt et al. 1983, Glover et al. 1986), which constitutes a nutrient source of particulate and dissolved organic matter for heterotrophic organisms. Dissolved organic matter (DOM) present in coastal waters (Pomeroy & Wiebe 1993) provides a potential for high bacterial production. Thus, in the

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Atlantic coastal ponds, bacterioplankton constitutes 50% of the planktonic carbon biomass (Frikha et al. 1987). Such heterotrophic bacterioplankters, with typically high growth rates and growth efficiencies, represent a significant energy pathway by recycling DOM into particles potentially available to upper trophic levels (Pomeroy 1974, Azam et al. 1983, Fenchel 1988).

However, small-sized bacteria and autotrophic picoplankton are not retained by gills of bivalves, particularly oysters (Shumway et al. 1985, Héral 1987, Riisgård 1988, Barillé et al. 1993). Flagellate and ciliate protists, which consume bacteria and phytoplankton, are abundant in coastal ecosystems (Revelante & Gilmartin 1983, Sherr et al. 1986a, Fenchel 1988, Leakey et al. 1992) and are preyed upon by the numerous organisms of zooplankton, particularly copepods (Berk et al. 1977, Jonsson & Tiselius 1990, Gifford & Dagg 1991, Hartmann et al. 1993). Protozoa have been suggested as a major trophic link between picoplankton and micro or macroplankton (Porter et al. 1979, Conover 1982, Sherr et al. 1986b, Stoecker & Capuzzo 1990).

Likewise, protists might represent a trophic link between bacteria and filter-feeding bivalves. Some data support this assumption. Tintinnids were observed in the stomachs of oysters (Paulmier 1972). Moreover, filter-feeding benthic molluscs retain protists, as exemplified by contaminations of bivalves by toxic flagellates (Sournia et al. 1991). In a mixed cell suspension of phytoplankton and dinoflagellates, 6 different species of bivalves were able to selectively clear and digest dinoflagellates (Shumway et al. 1985). Recently, Bardouil et al. (1996) showed that *Crassostrea gigas* easily consumes a nontoxic dinoflagellate and Kreeger & Newell (1996) clearly demonstrated in mussels the ingestion and assimilation of bacterial carbon via heterotrophic flagellates. From experimental work, Le Gall et al. (1997) reported significant retention and ingestion of cultured bacterivorous ciliates, *Uronema* sp., by the oyster *C. gigas*. Heterotrophic protists, which are abundant in coastal ecosystems, may thus constitute an alternative or complementary food resource for benthic filter feeders, allowing the indirect recuperation of DOM and picoplanktonic production otherwise not accessible to them.

We present evidence of oyster grazing on protists: a ciliate and flagellate community from a coastal oyster rearing pond was offered to oysters in a laboratory experimental setup. Clearance rate, filtered particles and relative retention efficiency were determined by following the taxonomic composition and relative abundance of the protist community over time in the presence or absence of actively filtering oysters.

MATERIALS AND METHODS

Oyster collection and acclimation. Oysters were collected in June 1997 from our oyster pond research facility 'Marais du Plomb' (L'Houmeau, near La Rochelle, French Atlantic coast). Twenty adult *Crassostrea gigas* (1 yr old, shell length 5 cm and mean dry tissue weight 1.64 ± 0.29 g) were transported to the laboratory, freed of epibionts and acclimated overnight at the ambient field temperature of 18°C, in GF/C (Whatman) filtered coastal pond water. Just before the experiment, 10 actively filtering oysters were selected and placed in 1 l Pyrex rectangular trays containing 800 ml of GF/C (Whatman) filtered coastal pond water.

Protist community: sampling and enumeration. The field planktonic community provided as potential food to the experimental oysters came from the coastal pond. Natural unfiltered oyster pond water was collected, using a 2.5 l 'Van Doorn' sampling bottle (Wildco), and held in the laboratory at 18°C in an opaque carboy until use. Ciliates and flagellates were fixed, stained and enumerated according to methods modified from Haas (1982), Caron (1983) and Sherr et al. (1994). For ciliate examination, 20 ml samples were stained live for 10 min by adding proflavin hemisulfate solution (Sigma, 0.033% w/v, final concentration 0.00066%): preliminary comparative experiments showed that live staining had no deleterious effects on the ciliate community. Ciliates were then preserved by adding glutaraldehyde (Sigma electron microscopy grade, 25% v/v in 0.2 µm filtered seawater, final concentration 1%). The cells were enumerated in Utermöhl settling chambers (Hydro-Bios combined plate chambers), using a reverse epifluorescence microscope (Leitz DMIRB, 100 W mercury lamp and blue light excitation). Ciliate taxa were enumerated and identified under combined epifluorescence and interference contrast illumination (magnification: ×400 or ×630). Sizes of all cells (length and width) were measured through a calibrated ocular micrometer. Mean cell volume of each ciliate 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 C μm^{-3} (Putt & Stoecker 1989), corrected for glutaraldehyde fixative according to Leakey et al. (1994).

For flagellate counting, 20 ml samples were preserved with formaldehyde (paraformaldehyde powder Sigma, 8 w/v in 0.2 µm filtered seawater, final concentration 1%); each sample was concentrated to 10 ml in a filtration tower mounted with a black 0.6 µm pore, polycarbonate membrane (Nuclepore) and a cellulosic backing filter (Whatman 1 µm) and stained by primulin (direct yellow 59 from Sigma; working solution was according to Sherr et al. [1994]: 250 µg primulin in 100 ml of 0.1 M Trizma HCl at pH 4.0; 50 µg ml^{-1} final concentration). The primulin method allows observation of cell outlines and permits distinguishing autotrophic from heterotrophic flagellates by repeated interchange of the filter sets (Caron 1983): phototrophic cells (faint orange under UV 365 nm excitation and red colored under green 450 to 490 nm excitation) and heterotrophic cells (blue under UV excitation and invisible under green excitation) were separately enumerated. Fields were viewed first for primulin fluorescence to locate flagellates, and then for chlorophyll *a* fluorescence (by changing the filter set) to confirm which of these cells were pigmented. Length and width of 100 flagellates were measured (observation under UV 365 nm excitation and magnification ×630) from triplicate samples. However, the presence of the black Nuclepore filter did not allow any observation of the flagellates under light microscopy and thus prevented identification of taxon or species.

Experimental protocol for the study of protist retention. The possible influence of oyster filtration upon the natural protist community was studied for 90 min in an experimental chamber at 18°C by comparing the evolution of protist abundances in triplicate suspensions with or without filtering oysters. At the start of the feeding period, 6 oysters were transferred to individual 500 ml Pyrex rectangular trays containing 400 ml natural unfiltered oyster pond water, gently homogenized with a magnetic rod to prevent sedimentation. As protists are fragile organisms, only a moderate homogenization was carried out in order to avoid cell damage; because of this restriction, the volume of the protist suspension was limited to 400 ml, to maintain a homogenous concentration of living protists.

Two experimental treatments were performed each in triplicate: the natural ciliate and flagellate suspensions were (1) allowed to evolve as controls, in the presence of 3 living but nonfiltering oysters, tightly tied up by a knotted string (controls for physical sedimentation of the suspension), or (2) delivered to 3 actively filtering oysters. It should be noted that, at the natural food concentration used in this study, there was no visible production of pseudofaeces. Dry tissue weight of each oyster was recorded at the end of the experiment, and clearance rates and filtered particles were expressed per gram of oyster dry tissue.

Calculation of clearance rate, filtered particles and relative retention efficiency. In order to control the normality of oyster filtration in our laboratory experiments, the clearance rate was estimated and compared to literature data. Defined as the theoretical water volume entirely cleared from particles (assuming 100% retention) per unit time and per oyster dry tissue weight (l h^{-1} g^{-1}) (Bayne & Widdows 1978), the clearance rate was calculated from the time course of the ciliate or flagellate cell concentration in the triplicate suspensions with filtering oysters. During the first 5 min of the experiment, individual variations in establishing a regular oyster filtration prevented a reliable study of the change in protist abundance in the triplicate suspensions: therefore, we selected the subsequent sampling time (15 min) as the most appropriate 'standard' time in our clearance experiment. Assuming exponential decline of the retained cells, the clearance rate was calculated according to Coughlan (1969) during the first 15 min:

$$F = \frac{\ln C_0 - \ln C_t}{t - t_0} \times V$$

where F is clearance rate (l h^{-1}), V is volume of the suspension (l), C_0 is the initial concentration of the suspension (cells l^{-1}), C_t is the concentration at time t (cells l^{-1}) and $(t-t_0)$ is the time interval (h). Taking into account that weight-specific filtration decreases with increasing body size, standardized clearance rates were calculated according to Riisgård (1988): F/W^b, where F is clearance rate (l h^{-1}), W is dry tissue weight (g) and b equals 0.73 for *Crassostrea virginica* (Riisgård 1988).

The number of filtered particles, which is the number of cells of each protist taxon retained per unit time and per gram of oyster dry tissue (cells h^{-1} g^{-1}), was calculated directly from the difference in the number of cells present between t_0 and t_{15} min.

To investigate the possibility of differential grazing by the oyster among the various protist taxa, we compared the relative retention efficiencies for each ciliate taxon and each ciliate and flagellate order. Defined as the number of a specific cell type retained during 15 min, relative to the initial available number of the same cell type at the beginning of the experiment, each relative retention efficiency (E_r) was calculated as a percentage for the difference in abundances at t_0 and t_{15} min over the abundance at t_0:

$$E_r\,(\%) = 100 \times [(C_0 - C_t)/C_0]$$

where C_0 is the initial particle concentration (cells l^{-1}) at t_0 and C_t is the particle concentration (cells l^{-1}) at 15 min.

Initial ciliate and flagellate abundances from the triplicate experiments with filtering or closed oysters were compared using a Student's t-test (data were previously tested for normality by the Kolmogorov-Smirnov test). The ciliate and flagellate abundances in triplicate controls during the 90 min experiment were followed by comparing the 5 time points sampled (0, 5, 15, 45 and 90 min) with a regression test.

RESULTS

Taxonomic composition and standing stocks of protists in the coastal oyster pond in June 1997

In the summer period of the experiment, the ciliate community of the coastal pond was abundant (23700 ± 3600 cells l^{-1}) and dominated by members of the subclass Choreotrichia, mainly represented by the order Choreotrichida, with *Tintinnopsis* spp. (10000 to 11200 cells l^{-1}), and by the order Oligotrichida, dominated by *Strombidium* spp. (5700 to 8500 cells l^{-1}). Other common taxa from the subclass Haptoria and order Haptorida (*Mesodinium* sp., *Askenasia* sp.) were also representative of the assemblage (3400 to 5700 cells l^{-1}). Ciliate sizes ranged from 8 µm length for a *Mesodinium* sp. to 72 µm for *Strombidium conicum* (Table 1). Prevalant ciliate cell lengths were between 16 and 48 µm.

Flagellate abundances in the coastal pond varied from 4.2 to 6.7 × 10^6 cells l^{-1} and flagellates accounted for about 99.5% of the protists enumerated in water samples. Mean flagellate sizes ranged from 4 µm for heterotrophic to 6 µm for autotrophic flagellates.

Tintinnina biovolumes as well as cell carbon were much higher than those of Oligotrichida and Haptorida (for the most abundant taxon in each order, 19181 µm^3 for *Tintinnopsis* sp. [48 µm by 24 µm], 5579 µm^3 for *Strombidium* sp. [32 µm by 24 µm] and 2145 µm^3 for *Mesodinium* sp. [16 µm by 16 µm]). By multiplying the taxon abundances at the beginning of the experiment by the carbon content per cell for each ciliate taxon, we estimated the quantity of ciliate carbon available to oysters: on average, 63.5 µg C l^{-1}. In this study, the flagellate carbon was not evaluated because flagellate taxonomy and biovolumes could not be determined.

Grazing experiments

The initial concentration in the natural suspension sampled for the grazing experiment was 23000 ± 3900 ciliates l^{-1} and 4.5 × 10^6 ± 1.12 × 10^6 flagellates l^{-1}. Since all suspensions originated from the same coastal pond sample, initial protist abundances in the experimental trays showed no significant difference between controls and oyster treatments (Student's *t*-test, n = 6, $p \gg 0.05$). In the 3 control suspensions, ciliate and flagellate abundances remained relatively constant over 90 min (Fig. 1) according to regression test (r^2 = 0.17,

Table 1. Taxonomic composition, sizes, biovolumes and carbon content per cell of the protists community in the coastal pond in June 1997. Taxa printed in bold type were abundant and represented in all samples. Taxa identified by • were rare and/or not present in all samples. When species were not identifiable, taxa were typified by their size

Order / Suborder	Family	Species	Species length (µm)	Width (µm)	Biovolme (µm^3)	Carbon per cell (pg cell^{-1})
Choreothrichida / Tintinninia	Codonellidae	*Tintinnopsis* sp.	35	24	13994	2379
		Tintinnopsis sp.	40	24	15984	2717
		***Tintinnopsis* sp.**	**48**	**24**	**19181**	**3261**
		Tintinnopsis sp.	48	40	53281	9058
		Tintinnopsis sp.•	51	40	56632	9627
		Tintinnopsis sp.	56	24	22378	3804
	Codonellopsidae	*Stenosemella* sp.•	24	22	7603	1293
Oligotrichida	Halteriidae	*Halteria* sp.	27	19	3024	514
	Strombidiidae	*Strombidium* sp.•	24	19	2617	445
		Strombidium sp.	25	22.5	4307	732
		***Strombidium* sp.**	**32**	**24**	**5579**	**948**
		Strombidium sp.	35	25.6	6863	1167
		Strombidium sp.	40	28.5	9569	1627
		Strombidium conicum	72	32	20642	3509
Haptorida	Didiniidae	*Didinium* sp.•	64	54	97716	16612
	Mesodiniidae	Unidentified	16	16	2145	365
		Unidentified	27	14	2771	471
		Mesodinium sp.•	8	8	268	45
		***Mesodinium* sp.**	**16**	**16**	**2145**	**365**
		Mesodinium pulex	14	10	733	125
		Askenasia sp.	24	16	3217	547
Autotrophic flagellate			**6.2**	**4.2**		
Heterotrophic flagellate			**4.1**	**3.5**		

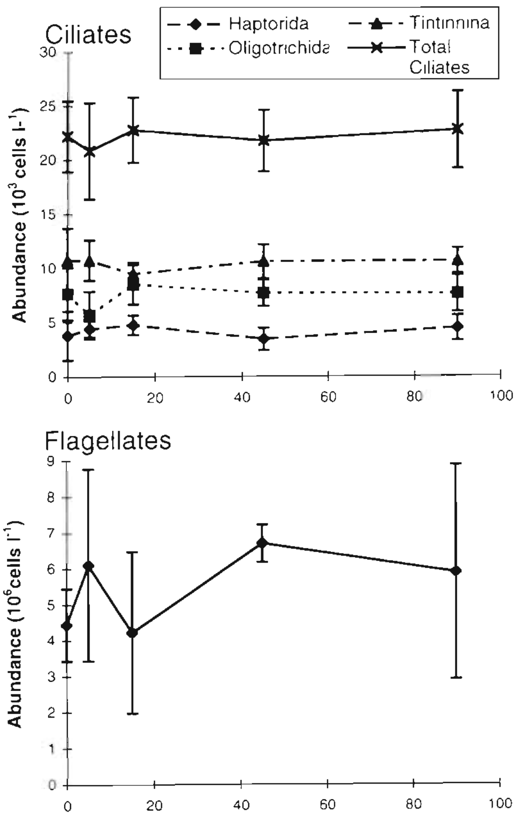

Fig. 1 Time course of ciliate and flagellate abundances in control suspensions. Abundance data (mean ± SD, n = 3) were collected from 3 separate experiments, performed with a closed, nonfiltering oyster in a 400 ml suspension of coastal pond water

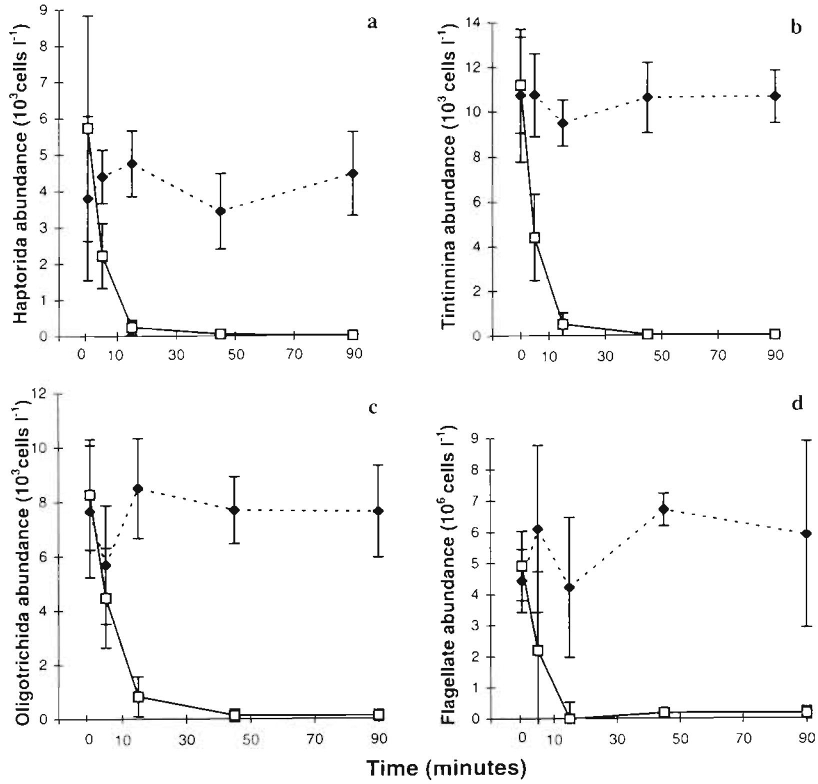

Fig. 2. Retention of various protist taxa by the oyster *Crassostrea gigas*: Haptorida (a), Tintinnina (b), Oligotrichida (c) and flagellates (d). Protist abundance data (mean ± SD, n = 3) were collected from 6 separate experiments performed in 400 ml marine pond water suspensions with a closed, nonfiltering oyster (◆---◆) or with a filtering oyster (□—□)

$p \gg 0.05$ for ciliates and $r^2 = 0.23$, $p \gg 0.05$ for flagellates).

In the 3 experimental trays with filtering oysters, ciliates whose size was between 20 and 40 µm were 100% retained; the relative retention efficiency in the experimental suspension within 15 min was 96% for Haptorida and Tintinnina (Fig. 2a, b) and 91% for Oligotrichida (Fig. 2c). Similarly, flagellates decreased by 86% within 15 min in the trays with the filtering oyster (Fig. 2d). At the end of the experiment (90 min), virtually all ciliates and 96% of the flagellates had been retained by the bivalves.

The relative retention efficiency for each protist taxon, related to the protist sizes present in the suspension, remained constant in the size range dealt with in the study (Fig. 3), except for a slight decrease for the smaller and larger taxa: only 84% of the 4 µm particles and 88% of the 72 µm particles were retained. For concentrations below the pseudofaeces threshold, all protist from 4 to 72 µm were similarly retained by the oyster gills.

Clearance rates, filtered particles

Clearance rates of oysters averaged $4.0 \pm 1.3\ l\ h^{-1}\ g^{-1}$ for flagellates and $7.2 \pm 3.5\ l\ h^{-1}\ g^{-1}$ for Oligotrichida ciliates (Table 2). The number of filtered particles, calculated between 0 and 15 min (Table 3), was dependent on protist taxon. Tintinnina were more readily retained (ca 27500 ± 11500 cells $h^{-1}\ g^{-1}$) than Haptorida (8900 ± 4400 cells $h^{-1}\ g^{-1}$) or Oligotrichida (19600 ±

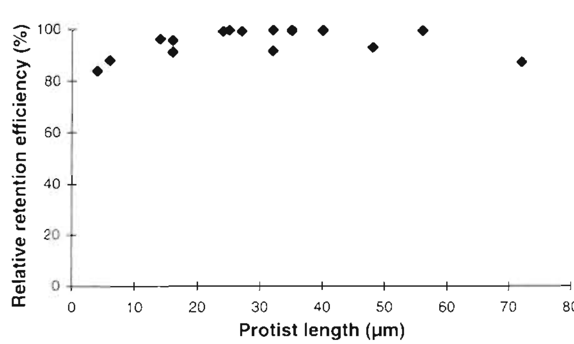

Fig. 3. Relative retention efficiencies of protists related to their size class

Table 2. Cell abundances in experimental suspensions (cells l^{-1} at t_0) and standardized clearance rates by *Crassostrea gigas* ($l\ h^{-1}\ g^{-1}$) for the different ciliate and flagellate taxa (mean ± SD, n = 3). When species were unidentifiable, taxa were typified by their size (length and width in µm)

Taxon (length/width in µm)	Cell abundances (l^{-1}) at t_0 in experimental suspension with an actively filtering oyster		Standardized clearance rate ($l\ h^{-1}\ g^{-1}$)	
	Mean	SD	Mean	SD
Haptorida				
Mesodiniuim sp. (16/16)	1178	237	3.6	0.7
Mesodiniidae (16/16)	1748	776	7.9	4.3
Mesodiniuim pulex (14/10)	2736	3178	4.1	4.5
Askenasia sp. (24/16)	76	132	2.5	4.4
Haptorida average	**5738**	**4192**	**5.5**	**2.3**
Oligotrichida				
Strombidinum sp. (25/22.5)	1026	1777	3.7	6.5
Strombidinum sp. (32/24)	3648	3288	4.9	6.4
Strombidinum sp. (35/25.6)	2318	4015	4.1	7.1
Strombidinum sp. (40/28.5)	114	197	2.7	4.7
Strombidinum conicum (72/32)	1064	628	4.9	3.5
Halteria sp. (27/19)	76	132	0.0	0.0
Oligotrichida average	**8284**	**2028**	**7.2**	**3.5**
Tintinnina				
Tintinnopsis sp. (35/24)	152	174	4.9	4.3
Tintinnopsis sp. (40/24)	1102	1425	8.7	2.2
Tintinnopsis sp. (48/24)	9082	3933	6.5	5.8
Tintinnopsis sp. (48/40)	760	1316	3.6	6.2
Tintinnopsis sp. (56/24)	114	114	4.7	4.1
Tintinnina average	**11210**	**2146**	**7.8**	**1.5**
Flagellates				
Autotrophic flagellate	1.38×10^6	1.07×10^6	4.9	3.0
Heterotrophic flagellate	3.55×10^6	5.00×10^4	3.1	1.8
Flagellate average	**4.93×10^6**	**2.47×10^6**	**4.0**	**1.3**

10200 cells $h^{-1}\ g^{-1}$). By multiplying filtered particles (cells $h^{-1}\ g^{-1}$) by the carbon content per cell for each taxon, we obtained the quantity of ciliate carbon retained per hour per gram oyster dry weight (µg C $h^{-1}\ g^{-1}$), which averaged 126 µg C $h^{-1}\ g^{-1}$ (Table 3).

DISCUSSION

Marine planktonic protists (ciliates and flagellates) have recently been shown to be abundant in Atlantic coastal ponds: our estimations of protist abundances in our coastal pond at the time of the grazing experiment were respectively 23700 ± 3600 ciliates l^{-1} and $4.5 \times 10^6 \pm 1.12 \times 10^6$ flagellates l^{-1}. These protist abundances fell within the range estimated for the same pond by O. Robin (pers. comm.), 10000 to 30000 cells l^{-1} for ciliates and 53×10^4 to 2.2×10^6 flagellates l^{-1}.

In the absence of published data on ciliate abundances in the Atlantic coastal ecosystem near the coastal pond, we compared our data to results from distant estuaries and bays. In other temperate estuaries, ciliate abundances were in the same range, from 200 to 19000 cells l^{-1} (St. Lawrence estuary, Sime-Ngando et al. 1995) and from 220 to 56000 cells l^{-1} (northern Adriatic, River Po estuary, Revelante & Gilmartin 1983). However, in the Gulf of Maine, ciliate abundances were higher: 350000 to 6000000 cells l^{-1} (Montagnes et al. 1988).

In our study, the ciliate community was dominated by the order Choreotrichida with *Tintinnopsis* spp. (10000 to 11200 cells l^{-1}) and by the order Oligotrichida with *Strombidium* spp. (5700 to 8500 cells l^{-1}). O. Robin (pers. comm.) observed up to 300000 Tintinnina l^{-1} in June 1996 in the same coastal pond of L'Houmeau. Tintinnina are also abundant in the Mediterranean Sea: 10000 ciliates l^{-1} in Villefranche-sur-mer (Rassoulzadegan & Gostan 1976) and 8000 cells l^{-1} in the southeastern Mediterranean (Alger Bay, Vitiello 1964). On the other hand, in a northern Mediterranean coastal lagoon (Etang de Thau), Tintinnina abundance was only 75 cells l^{-1} (Lam-hoai et al. 1997), a value much lower than ours. Oligotrichida abundances (5700 to 8500 cells l^{-1}) were in the range of values collected by O. Robin (pers. comm.) during the spring of 1996 (4300 to 11500 cells l^{-1}) but lower than abundances (90000 cells l^{-1}) during the summer in Mediterranean Sea (Rassoulzadegan 1977).

Table 3. Retention of various ciliate taxa by *Crassostrea gigas* expressed as filtered particles per unit time and unit oyster dry weight (cells h^{-1} g^{-1} or ng C h^{-1} g^{-1}). When species were unidentifiable, taxa were typified by their size (length and width in µm)

Taxon (length/width in µm)	Filtered particles (cells h^{-1} g^{-1}) Mean	SD	Carbon per cell (pg $cell^{-1}$)	Filtered particles (ng h^{-1} g^{-1}) Mean	SD
Haptorida					
Mesodiniuim sp. (16/16)	2707	823	365	988	301
Mesodiniidae (16/16)	4441	2933	365	1621	1070
Mesodiniuim pulex (14/10)	1488	1394	125	186	174
Askenasia sp. (24/16)	227	393	547	124	215
Haptorida sum	**8863**	**4390**		**2919**	**1583**
Oligotrichida					
Strombidinum sp. (25/22.5)	3063	5305	732	2242	3883
Strombidinum sp. (32/24)	7811	7739	948	7405	7337
Strombidinum sp. (35/25.6)	6919	11985	1167	8075	13986
Strombidinum sp. (40/28.5)	276	479	1627	450	779
Strombidinum conicum (72/32)	2154	1126	3509	7560	3953
Halteria sp. (27/19)	158	274	514	81	141
Oligotrichida sum	**19643**	**10285**		**25812**	**8643**
Tintinnina					
Tintinnopsis sp. (35/24)	432	528	2379	1029	1256
Tintinnopsis sp. (40/24)	2382	2899	2717	6472	7876
Tintinnopsis sp. (48/24)	22770	14798	3261	74254	48257
Tintinnopsis sp. (48/40)	1583	2742	9058	14342	24841
Tintinnopsis sp. (56/24)	318	342	3804	1210	1303
Tintinnina sum	**27487**	**11584**		**97307**	**32081**
Mean sum for all ciliates	**55993**			**126038**	

Our values for flagellate abundances were close to those obtained in the St. Lawrence estuary, 1.9×10^6 to 6×10^6 cells l^{-1} (Lovejoy et al. 1993), and in the marine shallow-water Limfjorden in Denmark, 2×10^6 cells l^{-1} (Andersen & Sørensen 1986).

The wide range of these data shows the natural variability of protist abundances in the field. Moreover, since the coastal ponds are periodically closed systems in which the plankton community undergoes rapid fluctuations, it remains difficult to establish valid criteria for comparisons with open coastal systems. Nevertheless, in terms of potential carbon resources available to the oysters, the amounts calculated for ciliates (63.5 µg C l^{-1}) were at the high level found for protozoa in coastal waters (St. Lawrence Estuary: 0.23 to 51.6 µg C l^{-1}, Sime-Ngando et al. 1995).

When a coastal pond planktonic community was provided as potential food, clearance rates of oysters for protists (4.0 ± 1.3 l h^{-1} g^{-1} for flagellates and 7.2 ± 3.5 l h^{-1} g^{-1} for Oligotrichida ciliates) were in a range similar to values measured for phytoplankton by Gerdes (1983): 4.8 l h^{-1} g^{-1}, Deslous-Paoli et al. (1987): 4.7 l h^{-1} g^{-1}, Riisgård (1988): 6.8 l h^{-1} g^{-1} and Soletchnik et al. (1991): 3 to 4 l h^{-1} g^{-1}. However, in our experimental closed system, the concentration of particles rapidly declines during the experiment (Fig. 2); the standard time for our clearance experiment (15 min), selected to avoid drawbacks related to the irregular establishment of oyster filtration during the first 5 min, is too long to allow an accurate evaluation of clearance rates. Nevertheless, the possible negative effects of our suboptimal laboratory conditions on bivalve filtering efficiency (Jørgensen 1996) would only have resulted in the underestimation of our experimental values; field clearance rates of oysters for protists might be even higher.

The relative retention efficiency was 94% for the ciliates and 86% for the flagellates within 15 min from 400 ml suspensions. This finding supports the results of Le Gall et al. (1997), who demonstrated that the oyster *Crassostrea gigas* retained *Uronema* sp., a cultured ciliate isolated from the oyster pond, with a 85% relative retention efficiency when present at a concentration close to field ciliate abundances. It also corroborates the observations by Paulmier (1972), who reported tintinnids to be abundant in the stomachs of wild oysters from the Atlantic coast. Likewise, Kreeger & Newell (1996) estimated that 58% and 44% respectively of cultured heterotrophic nanoflagellates were retained by *Geukensia demissa* and *Mytilus edulis*, compared to values of 66% and 77%, respectively, for the autotroph *Isochrysis galbana*. Ciliates and flagellates thus represent a potentially valuable food source and might be a significant component in the natural diet of suspension-feeding bivalves, provided their relative abundance is sufficiently high in the available seston.

To investigate the possible influence of particle size on oyster retention, we followed the abundance for

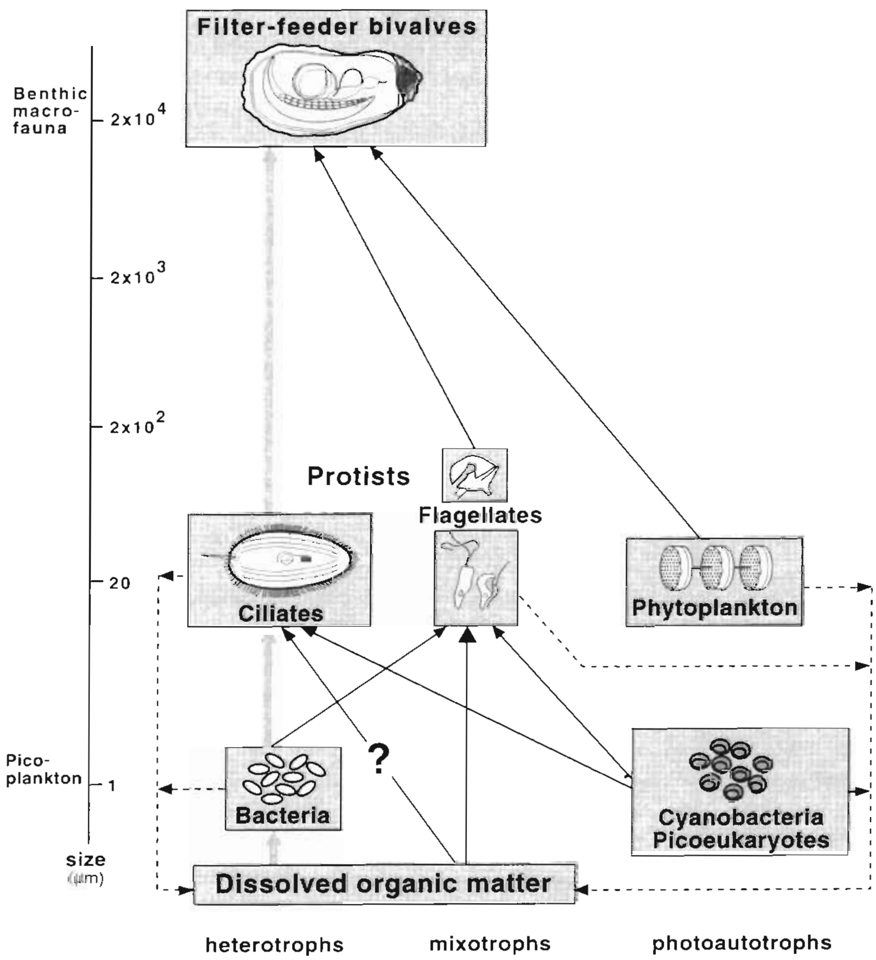

Fig. 4. Hypothetical microbial food web in an oyster growing area (modified from Le Gall et al. 1997)

each separate protist taxon in the experimental suspensions. In our experiments, ciliates and flagellates in a size range from 4 to 72 µm were retained by the oyster, but the smallest heterotrophic flagellates (4 µm) and the largest ciliates (*Strombidium conicum*, 72 µm by 32 µm) displayed a slightly lower relative retention efficiency than the ciliates with sizes between 20 and 40 µm. Indeed, the flagellate sizes in our suspensions were at the lower end of the particle size spectrum known to be retained by *Crassotrea gigas*. Barillé et al. (1993) showed that this oyster has a limited capacity to retain small particles: 4 µm particles (equivalent spherical diameter, ESD) were retained with 100% retention efficiency when sestonic load was low, but the limit increased to 12 µm for higher sestonic loads; for particles below these thresholds, retention efficiency quickly decreased. Similarly Deslous-Paoli et al. (1987) demonstrated that the oyster is not able to retain small particles. Bougrier et al. (1997) reported that the selection of algae by the oyster *C. gigas* was independent of the size, volume or carbon content of each species (size between 3.65 and 9 µm ESD). They observed, nevertheless, that some algae were preferentially filtered or rejected, due to cell shape and flexibility.

Conversely, mussels are able to retain even picoplankton-size particles (Kemp et al. 1990, Kreeger & Newell 1996): in particular, in high-quality food suspensions (expressed as the percentage of particles with chlorophyll fluorescence) prey retention and selection in *Mytilus edulis* is not dependent on the prey size (Newell et al. 1989). Reduction in food quality induced a drop in the ability to select living cells from silt particles, independent of size. As for oysters, however, these investigations demonstrated a selectivity based on cell shape (Newell et al. 1989). In contrast to the mussel, *Crassostrea gigas* cannot retain picoplankton-size particles at natural concentrations; therefore, the picoplankton-protozoa trophic pathway (Le Gall et al. 1997) may represent a significant energy source for the oyster (Fig. 4).

Ciliates are more nutritious prey than phytoplankton cells. They are relatively rich in nitrogen (C:N ratio near 4, Putt & Stoecker 1989, Ohman & Snyder 1991; as compared to >5 for phytoplankton, Heinbokel et al. 1978, Burkhardt & Riebesell 1997), and contain more carbon per cell than phytoplankton: our estimations of cell carbon contents, which are comparable to values previously reported in the literature (3100 pg C cell^{-1} for *Strombidium* sp. [43 µm by 42 µm], Stoecker & Egloff 1987; 1100 pg C cell^{-1} for *Strombidium* sp. [43 µm by 30 µm], Jonsson & Tiselius 1990) were much higher than phytoplankton carbon content per cell from 10 to 21 pg C cell^{-1} for *Skeletonema costatum* (Strathmann 1967, Burkhardt & Riebesell 1997, Bougrier et al. 1997), 1.61 pg C cell^{-1} for *Phaedactylum tricornatum* (Fiala-Medioni et al. 1983) and 10.3 pg C cell^{-1} for *Navicula filata* (Bougrier et al. 1997). In our experiment, on average, oysters retained 126 µg ciliate C h^{-1} g^{-1} for a ciliate concentration of 25000 ± 3900 cells l^{-1}. Fiala-Medioni et al. (1983) estimated that oyster filtering *Phaedactylum tricornatum* retained 27.5 µg C h^{-1} g^{-1} for a phytoplankton concentration of 1×10^6 cells l^{-1}. Ciliates may thus contribute to the carbon requirements of *Crassostrea gigas* in the same way as do heterotrophic flagellates for the mussels *Geukensia demissa* and *Mytilus edulis* (Kreeger & Newell 1996).

Most studies that have examined the nutritional importance of protists as a 'trophic link' have focused on pelagic consumers, such as zooplankton (Berk et al. 1977, Porter et al. 1979, Sherr et al. 1986b, Jonsson & Tiselius 1990, Gifford & Dagg 1991, Hartmann et al. 1993). However, only few studies have done the same for benthic consumers (Kreeger & Newell 1996, Le Gall et al. 1997). Trophic coupling between pelagic protists and benthic suspension-feeders is poorly documented in aquatic food models (e.g. see Legendre & Le Fèvre 1995).

In open water oyster beds, primary producers, in particular phytoplankton and resuspended microphytobenthos, can be considered important food sources for bivalve suspension feeders (Blanchard et al. 1997). In coastal ponds, on the other hand, even though microphytobenthic biomass may attain up to 25 times the higher levels of water column phytoplankton (Zanette 1980, Robert 1983), it is unlikely that the microphytobenthos is an important direct resource because its resuspension is low, due to a lack of turbulence. However, the DOM released by these autotrophs contributes to the important bacterial biomass that develops in coastal ponds: bacterioplankton constitutes 50% of the planktonic C biomass in oyster ponds of the Charente (Frikha et al. 1987). The bacteria, in turn, are a primary food source of heterotrophic/mixotrophic ciliates and flagellates which develop biomasses comparable to phytoplankton: in our coastal pond, the protist biomass was similar to the phytoplankton biomass of coastal oyster ponds from Bourgneuf Bay (Robert 1983). Since bacterivorous ciliates have a gross growth efficiency of about 40 (Johnson et al. 1982, Ohman & Snyder 1991), relatively large amounts of bacterial C must be recovered by oysters via the protist trophic link.

In coastal pond habitats, bivalve molluscs are abundant and may be the dominant consumers of seston. Oysters are most likely opportunist omnivores, balancing their C (and N) requirements by utilizing a wide variety of living and dead material (Riera & Richard 1996), including protists. In addition to phytoplankton which cannot entirely account for the energy requirements of *Crassostrea gigas* (Héral 1987), oysters may derive nutrients from microzooplankton, in particular from protists. Our experiment presents the first data on oyster nutrition through grazing on a field community of protists. These results clearly show that suspension-feeding bivalves feed on ciliates and flagellates. Such a trophic relationship could be of primary importance for the transfer of C, and probably N, from the microbial food web to higher trophic levels in the benthos.

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