

NOTE

Ingestion of a bacterivorous ciliate by the oyster *Crassostrea gigas*: protozoa as a trophic link between picoplankton and benthic suspension-feeders

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ABSTRACT: The linked concepts of 'microbial loop' and 'protozoan trophic link' have been very well documented in filter-feeding microzooplankton such as copepods, but have not been applied to energy transfer to benthic suspension-feeding macrofauna, with the exception of the recent demonstration of heterotrophic flagellate assimilation by mussels. The oyster *Crassostrea gigas* obtains energy resources by filtering microalgae (~5 to 100 µm). However, in turbid estuaries, light-limited phytoplanktonic production cannot entirely account for oyster energy requirements. Conversely, picoplankters (<2 µm), which are main effectors of coastal energy flow and matter cycling, are not efficiently retained by oyster filtration. Ciliate protozoa, as both micro-sized cells (~5 to 100 µm) and bacteria grazers, may represent a major intermediary in trophic transfer between picoplankton and metazoa. The ciliate *Uronema* was intensely cultured and labelled, using the cyanobacteria *Synechococcus* as an auto-fluorescent biomarker. The labelled ciliates were offered as potential prey to oysters. We report here the first experimental evidence of a significant retention and ingestion of ciliates by oysters, supporting the role of protozoa as a realistic trophic link between picoplankters and filter-feeding bivalves and thus enhancing their potential importance in estuarine microbial food webs.

KEY WORDS: Oyster · Food sources · Picoplankton · Protozoa · Trophic link

Oysters obtain energy resources by filtering particles from sea water, and their growth and fecundity depends upon the nutritive value of the retained seston (Berg & Newell 1986). In turbid estuaries such as the oyster-rearing Marennes-Oléron Bay (France), the light-limited amounts of phytoplankton cannot entirely account for the energy requirements of the benthic suspension-feeding oyster *Crassostrea gigas* (Héral 1987).

Conversely, microbial consortia play a key role in marine food webs (Pomeroy 1974). In coastal ecosystems, heterotrophic bacterioplankters significantly contribute to the microbial biomass (Fuhrman & Azam 1980) and up to 50 to 60% of total carbon flow passes through bacterioplankters (Newell & Linley 1984). An important part of production and respiration in marine ecosystems is processed through picoplankters less than 2 µm in size, such as heterotrophic bacteria (Ferguson & Rublee 1976, Fuhrman 1992), autotrophic cyanobacteria (Johnson & Sieburth 1979, Waterbury et al. 1979, Glover et al. 1986) and small eukaryotic phototrophic plankters (Johnson & Sieburth 1982, Courties 1994). With rapid growth rates and high growth efficiencies, heterotrophic bacterioplankters constitute a significant pathway of C/N flow by recycling dissolved organic matter (DOM) into particles potentially available to upper trophic levels (Pomeroy 1974, Williams 1981, Azam et al. 1983, Sherr & Sherr 1988).

However, micrometre-sized bacteria or cyanobacteria are not efficiently retained by bivalve filtration, particularly not by oysters (Shumway et al. 1985, Héral 1987, Riisgård 1988, Barillé et al. 1993). Ciliate protozoa, as both bacteria grazers (Hamilton & Preslan 1969, Berk et al. 1976, Sherr & Sherr 1987, Kemp 1988, Bernard & Rassoulzadegan 1990, Caron et al. 1991) and copepod prey (Berk et al. 1977, Sheldon et al. 1986), have been suggested as a major trophic link between bacteria and micro- or macrozooplankton (Porter et al. 1979, Sherr et al. 1986b, Sime-Ngando et al. 1995).

In the same way, do picoplankters unretained by filter-feeding bivalves contribute indirectly to their diet via ingestion of protists supplementing the phytoplankton (Fig. 1)? Some data support this assumption:

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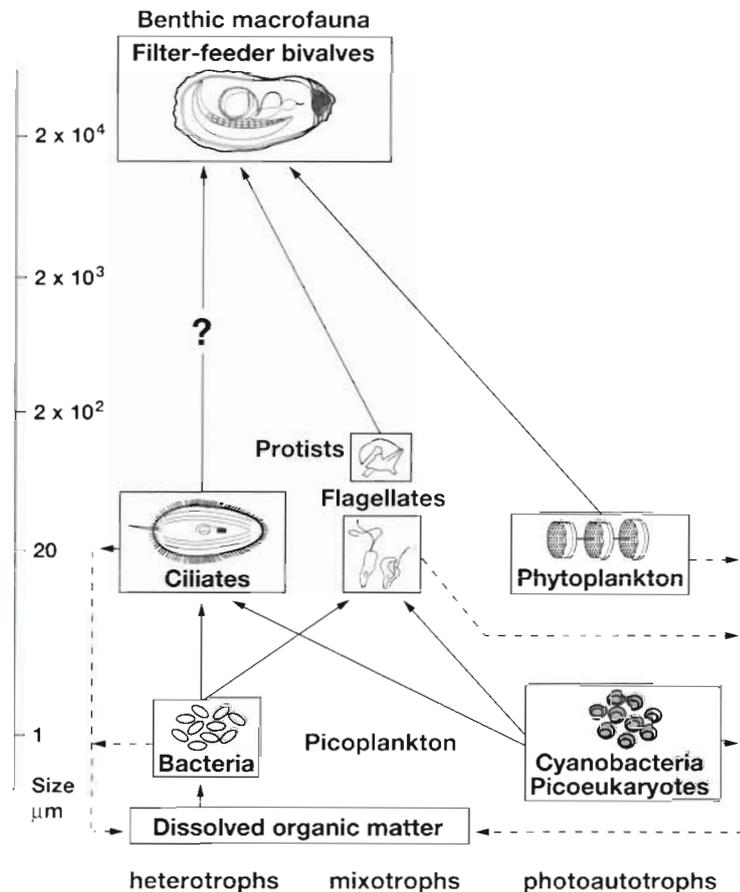


Fig. 1. Hypothetical diagram of microbial food webs in a coastal shellfish ecosystem

presence of tintinnids was observed in the stomach contents of oysters (Paulmier 1972) and mussels added into experimental marine enclosures reduced by more than 50% the biomass of microzooplankton, mainly tintinnids and oligotrich ciliates (Riemann et al. 1990). Moreover, protists are retained by filter-feeding mollusks, 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 clear selectively and digest the dinoflagellates (Shumway et al. 1985). Recently, ingestion and assimilation of bacterial carbon via heterotrophic flagellates was clearly demonstrated in mussels (Kreeger & Newell 1996).

Experimental evidence of oysters grazing on ciliate protozoa is presented here via a model system simulating a simplified food web: bacteria \Rightarrow ciliate \Rightarrow oyster, using *Uronema*, a bacterivorous ciliate, in the size range of phytoplanktonic cells that oysters usually feed upon.

Methods and results. Intensive culture of the bacterivorous ciliate *Uronema*: *Uronema* were caught in the field and cultured (Hamilton & Preslan 1969) for

48 h on suspension of natural bacterial consortia at a concentration of 1×10^6 bacteria ml^{-1} . Ciliate abundances were estimated from alkaline lugol-fixed samples (Sherr et al. 1989) in Nageotte counting cells or Utermöhl settling columns, according to the expected concentration. The cultured ciliates in early stationary phase (90×10^3 cells ml^{-1}) were separated from their bacterial prey by differential centrifugation (Ohman & Snyder 1991), washed twice in phosphate buffer saline (PBS) and resuspended in autoclaved $0.2 \mu\text{m}$ filtered sea water (FSW) at experimental concentrations of 5×10^3 cells ml^{-1} and 8.8×10^3 cells l^{-1} . Collected cells were motile and able to develop intensely in culture. Carbon and nitrogen analysis of *Uronema* was performed on an ERBA elemental auto analyser. The determined C:N ratio was 6.2 ± 0.5 ($n = 3$), a value closely related to C:N estimates in phytoplanktonic cells.

Grazing of *Crassostrea gigas* on ciliates:

The *Uronema* were offered as potential prey to oysters at a concentration regulated in the range of the natural abundance of ciliates: at the time of the experiment, 12×10^3 ciliates l^{-1} were enumerated in the oyster pond. One year old *Crassostrea gigas* (5 cm long and 1 g dry weight flesh) were kept overnight in FSW. The possible influence of oyster filtration was studied by comparing the evolution of ciliate abundances in triplicate

400 ml suspensions, with or without an oyster. In 3 isolated ciliate suspensions, gently homogenized to prevent sedimentation, the abundance of *Uronema* (8.8×10^3 cells l^{-1}) remained relatively constant for 20 min (Fig. 2). The introduction of *Uronema* in 3 experimental oyster trays induced the set-up of an active pumping behaviour of the mollusks. The oyster filtration triggered in ciliate suspensions an 88% decrease in *Uronema* abundance within 20 min (Fig. 2).

Moreover, careful withdrawal of seawater aliquots from the oysters' exhalant siphons with a bent Pasteur pipet (Le Gall & Béchemin 1994) allowed a comparison between the protozoan concentrations in the inhalant versus exhalant siphon: after a single passage through the oyster gills, the decrease in *Uronema* abundance was $85 \pm 9\%$ ($n = 3$) at the natural 8.8×10^3 cells l^{-1} concentration. In a more concentrated suspension (5×10^3 cells ml^{-1}), only $44 \pm 13\%$ ($n = 5$) of *Uronema* were retained on the gills: unnaturally high concentration of particulate food is likely to restrict retention efficiency in oysters (Barillé et al. 1993). Even in the range of natural ciliate abundances, the retention of *Uronema* (85%) is lower than the retention of algal particles

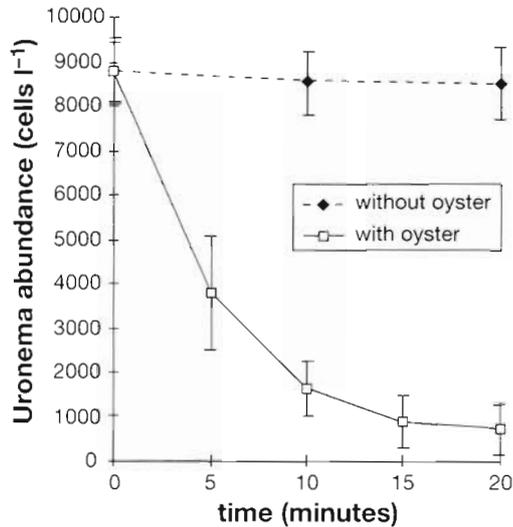


Fig. 2. Retention of *Uronema* by oyster *Crassostrea gigas*. Data were collected from 6 separate experiments performed in 400 ml ciliate suspensions without an oyster (◆) or with an oyster (□). Ciliate abundances (mean \pm SD) were estimated from alkaline lugol-fixed samples

(100% for microalgae $>5 \mu\text{m}$) (Shumway et al. 1985, Riisgård 1988); the active swimming behaviour of *Uronema* might allow some of them to escape the strong inhalant current of the bivalve.

Ingestion of the ciliates by *Crassostrea gigas*: The disappearance of *Uronema* during the passage of the suspension through the branchial chamber might have resulted from the destruction of those fragile organisms upon contact with the mollusk filtration apparatus. The fate of the ciliates in the *Crassostrea gigas* gut was studied through fluorescent labelling, using autofluorescent cyanobacterial prey as the biotracer for ciliates.

Uronema which had been starved overnight were fed a *Synechococcus* ROSO4 culture at a natural (6×10^5 cells ml^{-1}) concentration (Glover et al. 1986). Cyanobacteria had been sonicated (75 W, 2 min) to avoid cell clumping. Two hours later, ciliates observed by epifluorescence microscopy under blue light excitation appeared intensely labelled by their yellow-gold autofluorescent cyanobacterial prey, which had been both caught on cilia and ingested in the internal vacuoles of the protozoa (Fig. 3A).

Oysters were offered 3 different experimental suspensions: (1) FSW without any particles, (2) *Synechococcus* suspensions (6×10^5 cells ml^{-1}) or (3) *Uronema* suspensions (5×10^3 cells ml^{-1}) labelled with previously ingested *Synechococcus*. Non-filtrating oysters were discarded. Stomach and intestine contents of oysters were dissected 30 min later, gently pipetted, filtered on black $0.2 \mu\text{m}$ Nuclepore and observed by epifluorescence microscopy to detect the labelled ciliates possibly ingested by the mollusk.

In 3 oysters filtering FSW, the stomachs and intestines were flat and empty, as could easily have been predicted (Fig. 3B). The 3 filtering oysters offered the *Synechococcus* suspensions also had flat guts. Epifluorescent examination of filtered stomach contents revealed phytoplanktonic residues, characterized by chlorophyll red autofluorescence and rare cyanobacterial cells (Fig. 3C). Conversely, oysters offered the biolabelled *Uronema* suspensions exhibited expanded guts filled with a pinkish material. Epifluorescent examination revealed large amounts of autofluorescent cyanobacteria clumped on residual membranes of labelled ciliates in the stomach (Fig. 3D) and intestine (Fig. 3E) contents of oysters.

Discussion. The oyster *Crassostrea gigas* intensely retains and ingests the $20 \mu\text{m}$ bacterivorous ciliate prey: at a concentration close to natural abundances of ciliates in the field, about 85% of the suspended *Uronema* were retained on the oyster gills. The C:N value in *Uronema* (~ 6), similar to the ratio usually found in phytoplankton, makes them potentially valuable food sources. Moreover, picocyanobacterial cells at a natural concentration, which were inefficiently filtered by oysters, were indirectly ingested via our experimental protozoan trophic link. Questions always remain concerning the extrapolation to nature of *in vitro* data: the ciliate *Uronema* we used here may not realistically represent the natural planktonic consortium, but it can be considered as a model for picoplanktivorous protozoa, sized in a range of oyster retention.

Oysters have been shown to preferentially ingest organic particles (Newell & Jordan 1983). They are able to selectively clear dinoflagellate protists from diatom/dinoflagellate mixtures (Shumway et al. 1985), although toxic species reduce molluskan filtration (Newell & Shumway 1993) and feeding (Bardouil et al. 1996). Moreover, heterotrophic flagellates contribute to the C requirements of 2 mussel species, with assimilation efficiencies in the same range as for phytoplankton (Kreeger & Newell 1996). Tintinnid protozoa were observed to be abundant in the stomachs of wild oysters (Paulmier 1972). Such ability of oysters to ingest and assimilate protozoan cells as well as phytoplankton supports the idea that they have wide trophic adaptability (Kreeger & Newell 1996, Riera & Richard 1996).

Picoplankters may be responsible for 25 to 80% of the primary production in marine coastal ecosystems and are main effectors of energy flow and nutrient cycling. In Atlantic oyster ponds, bacteria contribute up to 56% of the particulate organic C biomass (Frikha et al. 1987). In the marine Thau lagoon, a major shellfish coastal zone in France, the photosynthetic picoeukaryote *Ostreococcus tauri* is the main component of the phytoplankton (Courties et al. 1994). Similarly, cyanobacteria and picoeukaryotes represent more

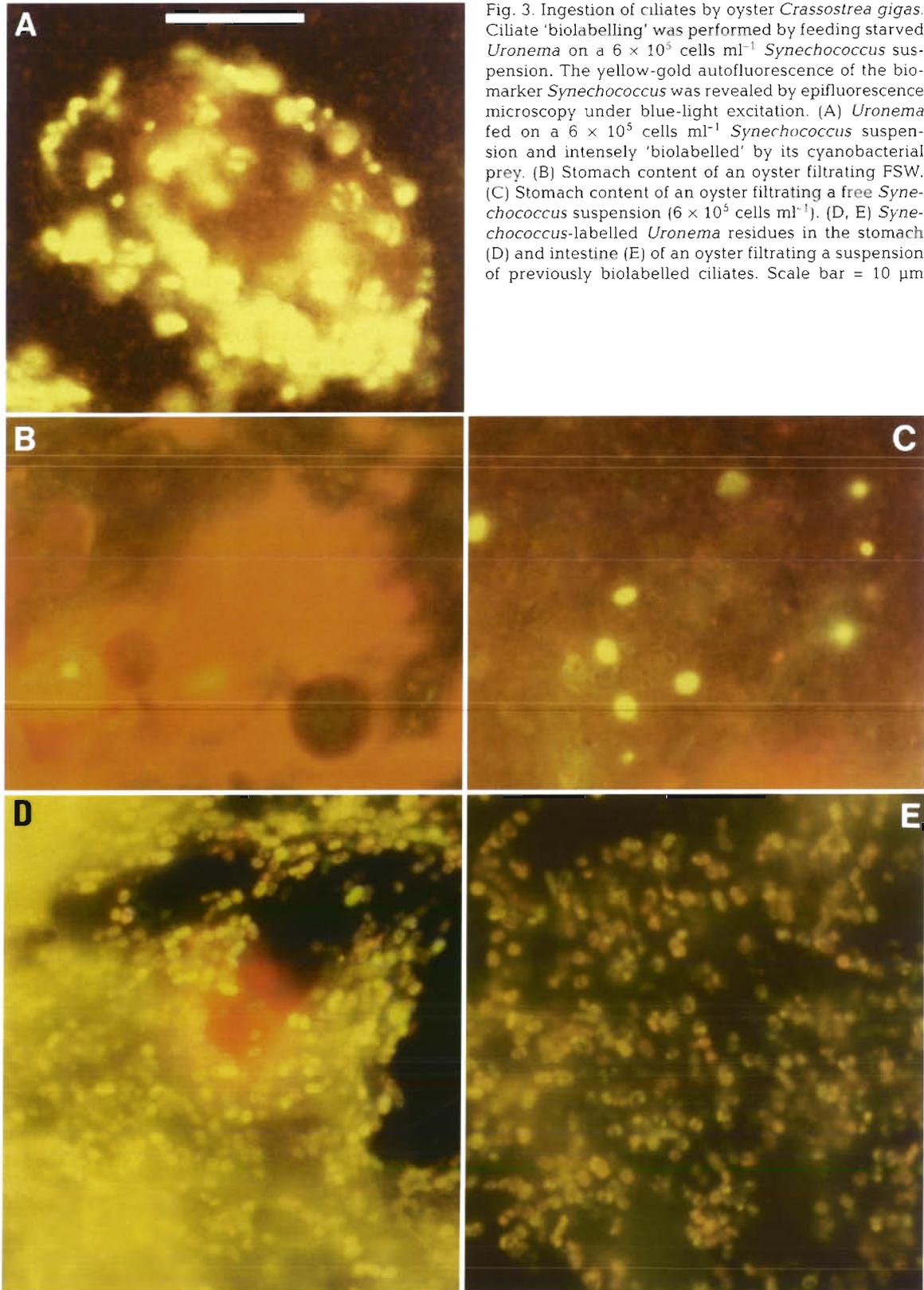


Fig. 3. Ingestion of ciliates by oyster *Crassostrea gigas*. Ciliate 'biolabelling' was performed by feeding starved *Uronema* on a 6×10^5 cells ml^{-1} *Synechococcus* suspension. The yellow-gold autofluorescence of the biomarker *Synechococcus* was revealed by epifluorescence microscopy under blue-light excitation. (A) *Uronema* fed on a 6×10^5 cells ml^{-1} *Synechococcus* suspension and intensely 'biolabelled' by its cyanobacterial prey. (B) Stomach content of an oyster filtering FSW. (C) Stomach content of an oyster filtering a free *Synechococcus* suspension (6×10^5 cells ml^{-1}). (D, E) *Synechococcus*-labelled *Uronema* residues in the stomach (D) and intestine (E) of an oyster filtering a suspension of previously biolabelled ciliates. Scale bar = 10 μm

than 60% of phytoplanktonic biomass and production in the pearl oyster culture grounds of the Polynesian lagoons (Charpy & Blanchot 1996). Bacteria are considered as a major food resource in marine Atlantic ponds (Linley 1986) and in coral reef lagoons (Linley & Koop 1986). These tiny cells are absorbed by deposit-feeding bivalves (Newell & Shumway 1993) and some mussels (Kreeger & Newell 1996), but they are not efficiently retained by most filter-feeders, particularly oysters (Shumway et al. 1985, Riisgård 1988). Conversely, the bulk of bacterial production is rapidly consumed by protozoa (Frikha & Linley 1989, Van Wambeke et al. 1996), these constituting a potential trophic link between picoplankton and oysters.

The transfer of the picoplanktonic biomass through the heterotrophic food webs and the significance of its link or sink value (Pomeroy 1974, Ducklow et al. 1986, Sherr et al. 1987) is still a controversial issue. Moreover, direct use of primary decomposers (bacteria) rather than protozoa would allow conservation of more energy for macrometazoa, because of losses occurring along the food chain (Linley & Newell 1981). However, in coastal waters, such as nutrient-rich estuaries, ciliate protozoa with abundances of 10^3 to 10^5 cells l^{-1} are a major component of zooplankton (Sherr et al. 1986a). They are considered efficient grazers of bacteria and cyanobacteria, at least temporally and locally (Rassoulzadegan 1993). Our experimental evidence of an important retention and ingestion of a ciliate by *Crassostrea gigas* is consistent with the observation of ciliates in the stomach contents of wild oysters (Paulmier 1972). Protozoa may thus represent a realistic trophic link through which DOM and picoplankton contribute to the food resources of large benthic metazoan suspension feeders, such as oysters.

Acknowledgements. We thank Martine Bréret (CNRS) for technical assistance in cyanobacteria and ciliate culture, Lucette Joassard (IFREMER) for C and N analysis and Daniel Vaultot (Roscoff) for the gift of the *Synechococcus* ROSO4 cell line.

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This note was submitted to the editor

Manuscript first received: December 2, 1996

Revised version accepted: April 7, 1997