Cytometry 10:659–669 (1989)

Using Phytoplankton and Flow Cytometry to Analyze Grazing by Marine Organisms

Terry L. Cucci, Sandra E. Shumway, Wendy S. Brown, and Carter R. Newell

Department of Marine Resources (S.E.S.) and Bigelow Laboratory for Ocean Sciences (T.L.C., S.E.S.), West Boothbay Harbor, Maine 04575; Chemistry Department, Bowdoin College (W.S.B.), Brunswick, Maine 04011; Great Eastern Mussel Farms (C.R.N.), Tenants Harbor, Maine 04857

Received for publication November 2, 1988; accepted April 17, 1989

Phytoplankton can, through their autofluorescent characteristics, be thought of as tracer particles in much the same way as fluorescent microspheres when used in particle uptake experiments. Flow cytometric techniques can be used to differentiate phytoplankton from other suspended particles by the two primary autofluorescing photosynthetic pigments, chlorophyll and phycoerythrin. Based on these characteristics, phytoplankton assemblages have been used to assess grazing rates, particle selectivity, and endocytotic abilities in various marine species, from single-celled organisms to higher invertebrates.

Key terms: Endocytosis, filtration, invertebrates

Numerous studies have been devoted to secondary production in the marine environment. Studies on grazing rates of autotrophic and heterotrophic flagellates (5,16,18), micro- and macrozooplankton (6,19, 32,39), and higher invertebrates (4,25) have been instrumental in determining energy budgets for specific animal groups and energy flow within ecosystems. Filter feeding organisms in the marine environment remove particles from the water column; however, this removal does not always imply ingestion or assimilation. During the process of particle selection in bivalve molluscs, for example, particles may be removed from the water without actual ingestion, and the animals can produce pseudofeces which are then ejected from the mantle cavity (22). In other instances, particles may be ingested but not digested (36).

Historically, experiments designed to study feeding rates (grazing rates) and/or particle selection by various marine species incorporate various particle types, such as laboratory cultures of phytoplankton (11,27,30) or bacteria (3,31), encapsulated organic material (21), and inert particles (fluorescent microspheres) (13, 28,29). The type of particle used in such experiments must be recognizable to measure accurately changes in particle concentrations. In essence, these particles are used as tracer particles to observe an organism's ability to remove particles from water. For example, fluorescent microspheres (non-biological particles of uniform shape) may be presented to organisms to determine grazing and particle selection based solely on particle size. The fluorescent properties of the spheres accelerate the microscopic enumeration of these particles using fluorescence microscopy.

Only recently have researchers been able to assess grazing rates and particle selection using natural assemblages of particles (12,24). While the use of microcapsules and inert beads may provide some useful information with regard to size selectivity, phytoplankton and assemblages of naturally occurring particles provide the most realistic estimates of feeding and particle selection by filter-feeding organisms.

Whatever the material supplied to the grazers, the particles must be counted in some manner. Microscopic cell counting is both tedious and time-consuming. Particle counters provided a more rapid, although limited, means of counting particles (see below), and the recent development of flow cytometric techniques, based on the autofluorescent properties of algal cells or on fluorescent dyes, has provided the best means yet of assessing particle uptake and selection of filter feeding organisms.

AUTOFLUORESCENT PROPERTIES OF PHYTOPLANKTON

It wasn't until the early twentieth century that autofluorescence of plants was exploited as chlorophyll in chlorophasts (41). Since then, other autofluorescent pigments have been identified as photosynthetic accessory pigments. Figure 1 illustrates the fluorescence signatures of the major oceanic phytoplankton. There are

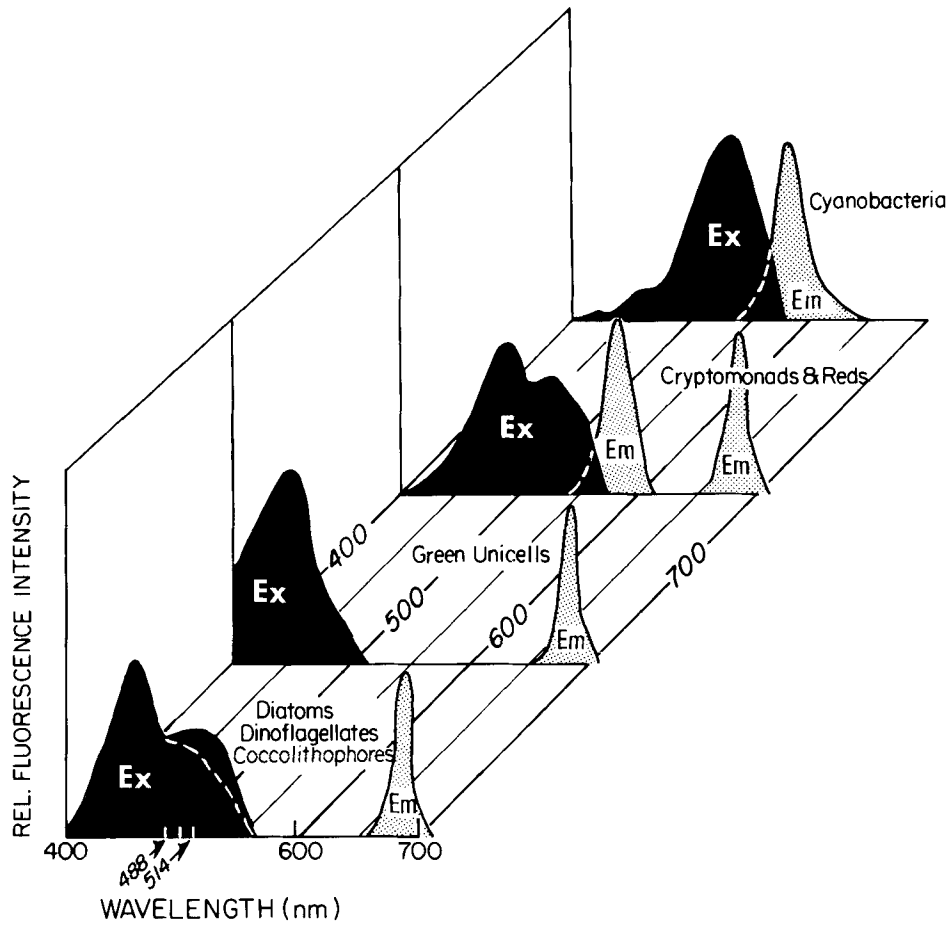


FIG. 1. Spectral fluorescence signatures of the major oceanic phytoplankton color groups. From Yentsch and Phinney (45).

two primary emission bands among phytoplankton, one at 560–590 nm, attributed to phycoerythrin (PE), and the other centered at 685 nm, which is the result of chlorophyll *a* (45). Emission signatures resulting from chlorophyll are exhibited by diatoms, dinoflagellates, coccolithophores, and chlorophytes. Phycoerythrin is the most abundant pigment of the cyanobacteria, whereas cryptomonads possess both chlorophyll and phycoerythrin. These autofluorescent properties have enabled researchers to utilize flow cytometric techniques to study grazing rates in an array of marine organisms. The first studies to utilize flow cytometry as a means of estimating grazing rates in marine organisms involved bivalve molluscs (10,36). It is now possible to discriminate between fluorescent (phytoplankton) and non-fluorescent particles (detritus, heterotrophs, bacteria) in the diet of filter feeding animals.

EARLIER METHODS TO STUDY GRAZING

Methods to assess concentration changes in suspended phytoplankton cells due to filter feeding organisms were either direct measurements of changes in optical density of the suspension or the tedious counting of cells by microscopy. These measurements were only qualitative in nature.

Subsequent studies were aided by the advent of the Coulter Counter, which, for the first time, allowed a more quantitative approach to selective feeding (34). Estimation of feeding rates and of size selective particle retention were results easily obtained by this new technology. The major limitation of the Coulter Counter is its inability to distinguish between particles having the same volume range but varying nutritive values. Consequently, it becomes impossible to determine if an organism can select particles by parameters other than size when presented with a wide variety of particles as occurs in the natural environment.

FLOW CYTOMETRY: A NEW METHOD

The introduction of flow cytometry to marine science (38,42,44) has provided us with the ability to distinguish particles of similar size which exhibit different optical properties due to photosynthetic pigments and chemical composition of the cell. As a result, differential uptake of phytoplankton from several different pig-

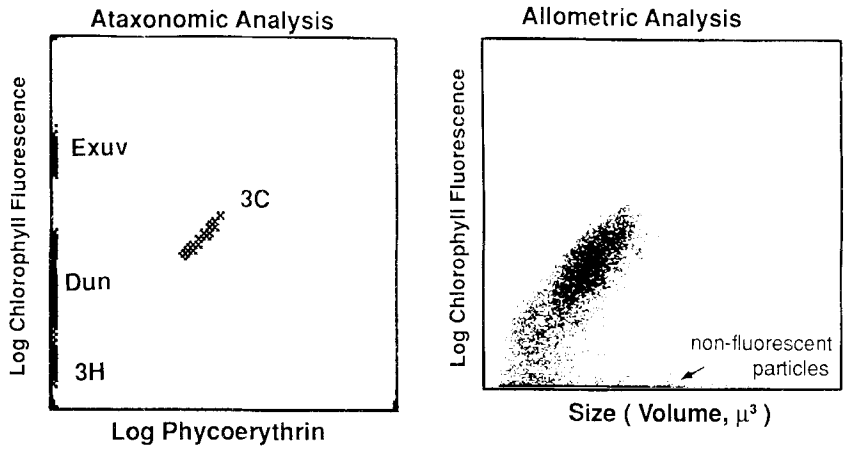


FIG. 2. Examples of ataxonomic analysis and allometric analysis plots. Color group characterization for ataxonomic analysis is based on the autofluorescing photosynthetic pigments of chlorophyll and phycoerythrin and distinguishes between chlorophyll-containing eucaryotes, phycoerythrin-containing procaryotes, and cryptomonads which contain both chlorophyll and phycoerythrin. Characterization of particles using allometric analysis is based on the size of the particle and one photosynthetic pigment. The percentage of nonfluorescing particles (detritus, heterotrophs, inorganic material) and fluorescing particles (autotrophs) can be determined.

ment groups can be simultaneously detected. In addition, the preferential uptake of similar-sized phytoplankton and nonbiogenic material can also be determined. The data are also quantitative, which allows estimation of filtration rates and retention efficiencies for all types of particles.

The principles of flow cytometry can be summarized as the simultaneous detection of the optical properties of particles moving in a flowing stream of water through a light source. Shapiro (33) describes, in excellent detail, the principles of flow cytometry and its applications. Flow cytometry is an ideal method for detecting photosynthesizing pigments in phytoplankton which autofluoresce when excited with blue or green light. To exploit flow cytometry for grazing experiments using phytoplankton, we simultaneously detect chlorophyll and phycoerythrin autofluorescence, forward and 90° light scatter, and/or volume of the cell (Coulter volume, μm^3).

Figure 2 illustrates the two types of analyses used to characterize marine particles. Ataxonomic analysis groups particles by pigment group, i.e., the two major autofluorescing pigments being chlorophyll and phycoerythrin. Size only becomes important in that, generally, large cells have more chlorophyll and/or phycoerythrin content. However, the same species of phytoplankton grown in different light intensities will contain varying amounts of chlorophyll per cell, corresponding to different fluorescent intensity per cell (43). In Figure 2, all four species of phytoplankton have chlorophyll *a*, yet clone 3C, *Chroomonas salina*, a cryptomonad, also possesses phycoerythrin. The majority of our work on bivalve grazing was carried out under laboratory conditions with mixtures of 3 or 4 phytoplankton species, easily distinguished from one another with the ataxonomic type of analysis.

Allometric analysis groups particles by size; size being measured as Coulter volume (μm^3 or equivalent spherical diameter) or based on light scattering properties which are indicators of size. In Figure 2, the allometric scattergram depicts size (volume) versus chlorophyll fluorescence, demonstrating the relationship between cell size and chlorophyll content. Of significant importance in this type of analysis is the relationship of non-fluorescent to fluorescent particles, in this case, those containing chlorophyll. In grazing experiments utilizing both phytoplankton and non-living material, the uptake of both particle types in relation to size can be determined (24).

PARTICLE SELECTION IN BIVALVE MOLLUSCS

Particulate components of seawater, whether in oligotrophic or eutrophic conditions are numerous and vary in size, shape, and chemical makeup. The composition of these particles ranges from inorganic material (silts and clays) to biogenic in origin, i.e., detritus and living organisms (plankton). The combination of these particles presents challenges to filter-feeding organisms that derive their nutritive requirements from certain components of the total (17,20,26,40). The question then arises, do these organisms selectively filter particles for consumption and utilization or do they ingest all particles and utilize a portion of the total for survival?

Experiments designed to determine if filter feeding organisms can i) select and ii) preferentially ingest, and/or preferentially digest suspended particles were the major thrust of a series of laboratory studies with bivalve molluscs (10,35,36). These studies demonstrated the ability of flow cytometry to distinguish phytoplankton cells quantitatively, even when of similar size, by detection of their autofluorescing photosynthetic pigments. The general method for all exper-

iments was to purge animals in 0.7 μm filtered seawater for 24 h prior to use in feeding experiments to clear any previously ingested material. Individual animals were placed in bell jars and incubated for 1 h in mixtures of phytoplankton, after which they were placed in fresh filtered seawater for the collection of fecal material. Samples were analyzed using an EPICS V flow cytometer (Coulter Electronics) equipped with a 5 W argon laser.

Mixtures of phytoplankton cultures consisted of three to four different species, all of which contained chlorophyll fluorescence and one containing both chlorophyll and phycoerythrin. Based on differences in total chlorophyll and phycoerythrin fluorescence, ataxonimc analyses were used to analyze the samples. Of significance was the fact that some experiments used similar sized cells, only varying in total chlorophyll per cell (~differences in fluorescence intensities) with one of the species also containing phycoerythrin. This experimental design incorporated the phytoplankton cells as the tracer particles much the same way as fluorescent microspheres are used in similar experiments. The difference between fluorescent particles (phytoplankton) at time zero and at the termination of the experiment reflects the quantitative rate of grazing by the organism.

Based on the clearance rates and the relative proportions of the phytoplankton cells in the water after 1 h incubation time, three methods of particle selection in bivalves were observed (36) (Fig. 3).

Preferential Clearance on the Ctenidia (Gills)

The preferential clearance of the dinoflagellate Exuv compared with cells of the diatom Phaeo and the cryptomonad flagellate 3C can be clearly seen in Figure 3. The apparent absence of Phaeo in the feces probably reflects pre-ingestive sorting on the gills where these cells were bound in mucus and shunted out of the animal as pseudofeces.

Pre-Ingestive Sorting on the Palps

The relative proportions of cells are similar throughout the incubation period; therefore all cell types are cleared from the water with equal efficiency. However, there is a large increase in the proportion of Phaeo compared with the dinoflagellate Exuv or the cryptomonad flagellate 3C within the pseudofeces. This indicates that pre-ingestive selection occurred on the labial palps.

Differential Absorption in the Gut

There is no evidence of preferential clearance of any one cell type on the ctenidia, since after 60 min of filtration, the proportion of the three cell types remains similar to that at time zero (see Fig. 5c). The proportions of cell types in the pseudofeces suggest some rejection of the dinoflagellate Exuv compared with the diatom Phaeo. The most obvious feature, however, is the absence of the cryptomonad flagellate 3C in the feces and the dominance of the alga Phaeo. A preferential absorption of the 3C has evidently occurred during passage through the gut.

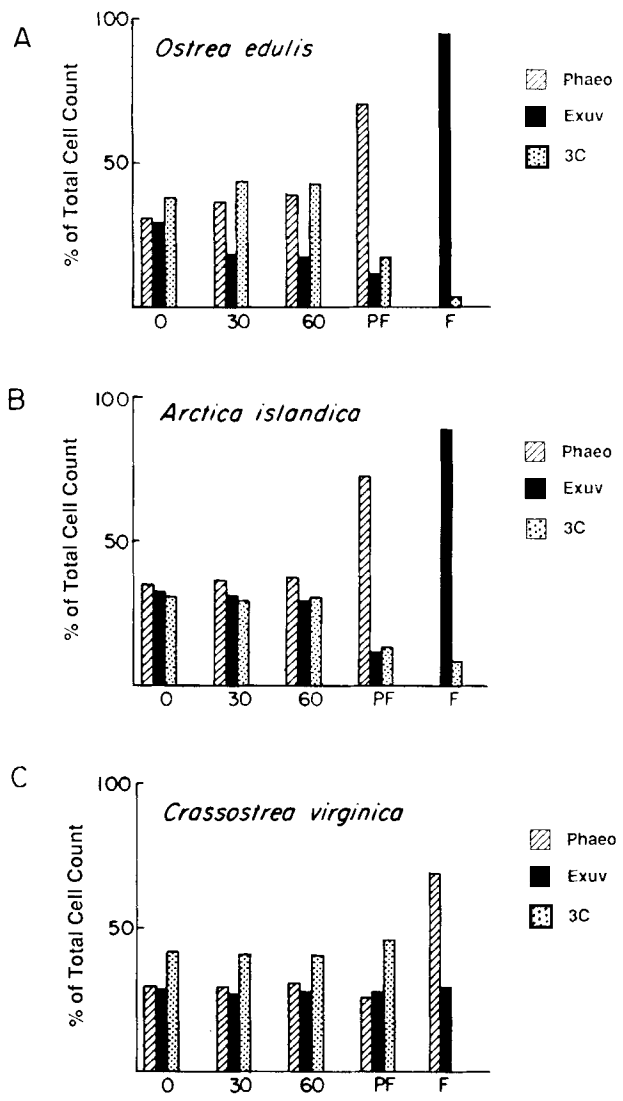


FIG. 3. Summary of the percent of the total cell count within an algal mixture of the clones Phaeo, 3C, and Exuv after 60 min and within the pseudofeces (PF) and feces (F) during the grazing experiments with (**A**) European oysters, *Ostrea edulis;* (**B**) ocean quahogs, *Arctica islandica;* and (**C**) American oysters, *Crassostrea virginica.* From Shumway et al. (36).

Figure 4 shows a typical bivariate plot of events of phycoerythrin fluorescence per cell (X) versus chlorophyll fluorescence per cell (Y) at time zero and after 60 min in the experimental seawater in the presence of a specimen of *Crassostrea virginica,* the American oyster. The corresponding plots for pseudofeces and feces resuspended in filtered seawater are also shown.

FOOD QUALITY AS A BASIS OF FOOD SELECTION BY COPEPODS

Within the natural assemblages of phytoplankton are mixtures of cells in different physiological states.

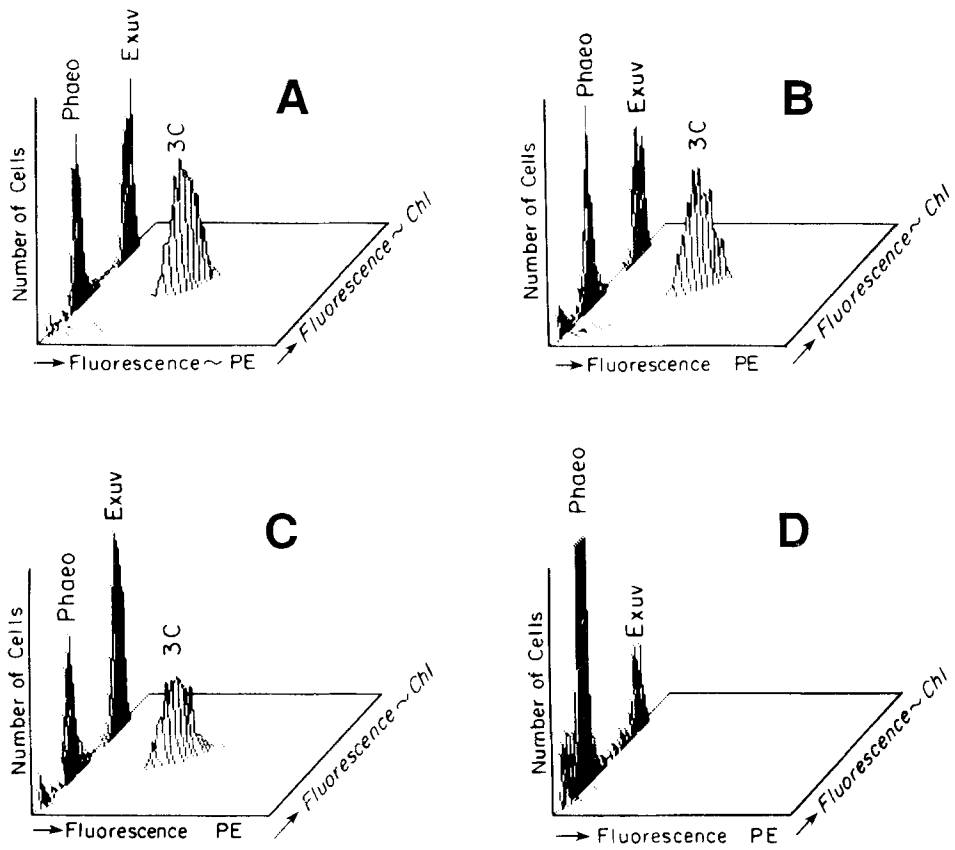


FIG. 4. An example of bivariate histogram plots of number of cells and X = phycoerythrin fluorescence per cell vs. Y = chlorophyll fluorescence per cell comparing relative cell numbers within an algal mixture of Phaeo, 3C, and Exuv due to the grazing by *Crassostrea virginica* at time 0 min (**A**), 60 min (**B**), and within the pseudofeces (**C**) and feces (**D**). Total of analyzed cells was 2,000. From Shumway et al. (36).

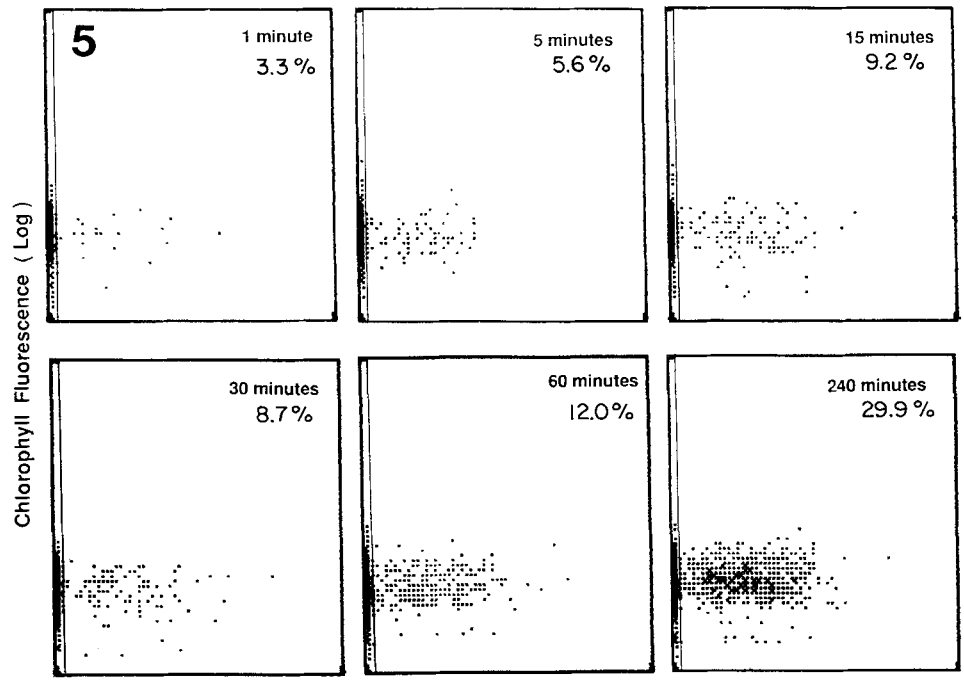
Therefore, the condition of the cell will reflect its nutritive quality. The question then arises, can zooplankton preferentially ingest cells of higher quality? A laboratory study was designed by Cowles and co-workers to investigate this topic (9).

A diatom species was grown at two different growth rates, which changed their physiological states, but kept constant their size (cell diameter). As a result, the copepods were able to graze on two different populations of the same species of phytoplankton, differing only in their nutritive quality. It was possible, using flow cytometry, to analyze the phytoplankton mixtures by differences in total chlorophyll fluorescence from both populations of the diatom. The phytoplankton cells grown in a low-growth-rate environment showed approximately a 3.5× lower chlorophyll fluorescence per cell than cells grown in a high-growth-rate environment. The need to analyze two populations of the same diatom having identical size but varying in chemical makeup and chlorophyll fluorescence supported the value of flow cytometry.

Results showed nearly a twofold higher ingestion rate by the copepods when fed the higher quality food (cells of higher growth rate). When both diatom populations were mixed together in different proportions, the copepods selectively ingested the cells of higher growth rate, suggesting that physiological differences among cells in natural assemblages may be an important factor in determining food selectivity by zooplankton.

SELECTIVE FEEDING BY HETEROTROPHIC CILIATES

The fact that planktonic ciliates are important selective grazers may impose consequences to a particular phytoplankton species in the natural environment. Therefore, it is important to assess the role of planktonic ciliates on the population dynamics of a phytoplankton community. A combined laboratory and field study was designed to investigate the seasonal distribution, food requirements, and feeding selectivity of a marine ciliate, *Balanion* sp. (37). The laboratory experimental design was similar to the bivalve experiments (10,36), whereby groups of ciliates were incubated with a mixture of two or more phytoplankton cultures. The amount of cells grazed was taken as the difference between cell concentrations at time zero and concentrations at the end of the incubation period. Again, these



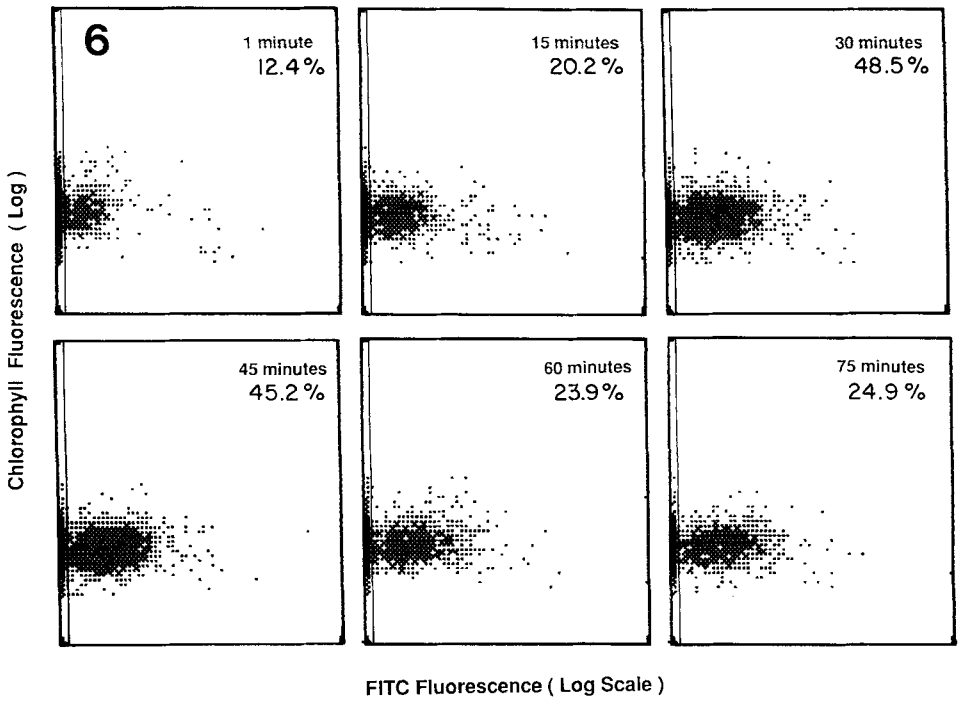


FIG. 5. Bivariate plotes of phycoerythrin fluorescence (560–590 nm) from *Synechococcus* (clone DC2) and chlorophyll fluorescence (>665 nm) from *Ochromonas* (clone IC1) representing a time series for four hours. *Ochromonas* cells that phagocytize *Synechococcus* emit both fluorescence and appear away from the X- and Y-axis. Percentages represent the percent of *ochromonas* cells that accumulated *Synechococcus*.

FIG. 6. Bivariate plots of fluorescein fluorescence (515–530 nm) from FITC-dextran and chlorophyll fluorescence (>665 nm) from *Ochromonas* (clone IIC2) representing a time series of 75 min. *ochromonas* cells that pinocytize the dextran emit both fluorescence and appear away from the X and Y axis. Percentages represent the percent of *Ochromonas* cells that took up the dextran.

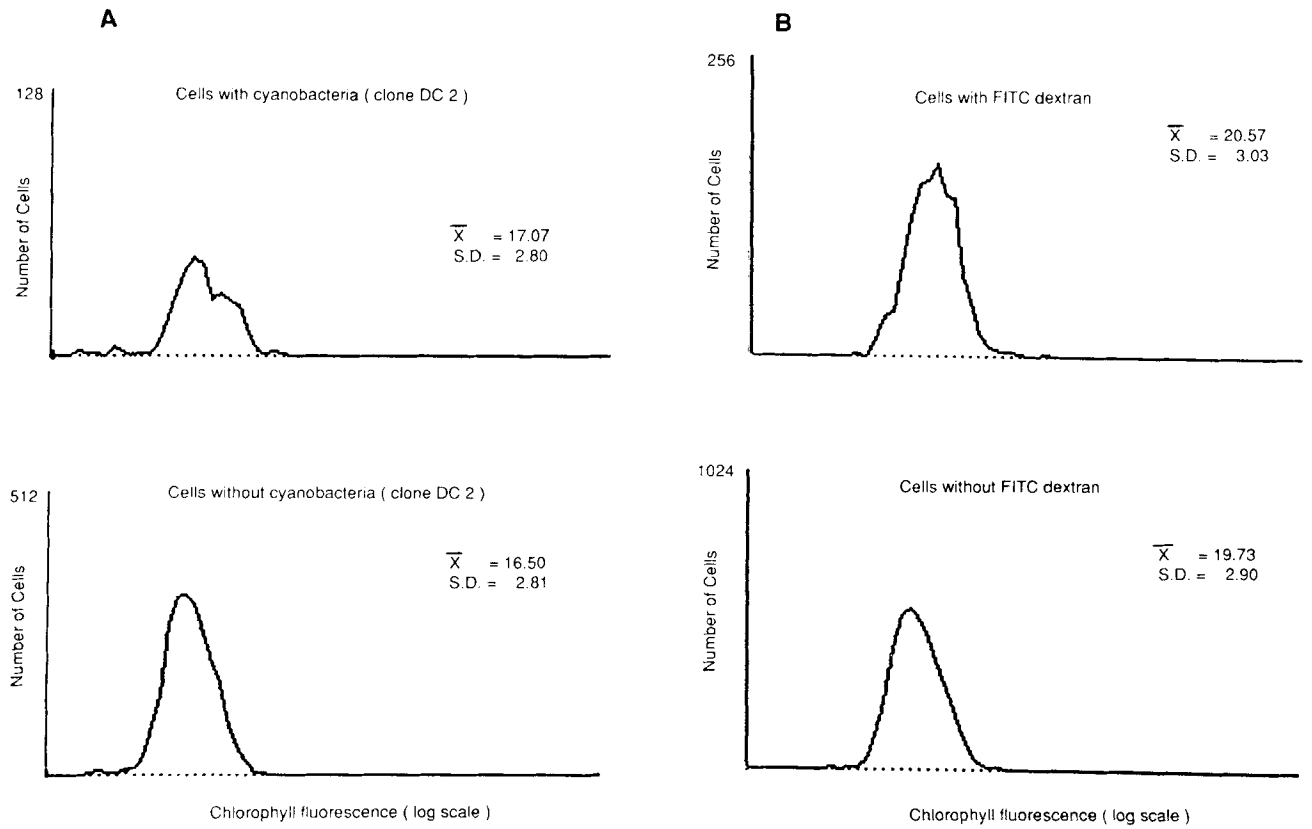


FIG. 7. Frequency histograms of chlorophyll fluorescence (>665 nm) for cells of *Ochromonas* that did or did not phagocytize cyanobacteria (clone DC2) (**A**) and pinocytize FITC-dextran (**B**). Note the difference in Y-axis scales (numbers at the top of the axis). Means represent the mean channel of the distribution on a 256 channel X-axis. These histograms depict the 60 min sample time for both experiments.

experiments made use of the phytoplankton cells as particle tracers to assess the grazing rates and food perferences of the ciliates. Species differentiation was made possible through flow cytometric analysis based on the amount of fluorescence approximating chlorophyll *a* per cell and the presence or absence of phycoerythrin.

Results of the laboratory study showed a preference by the ciliates for a particular dinoflagellate which has also been shown to support highest growth rates in the ciliates. From the field study, it was shown that the ciliate, *Balanion,* was found associated with phytoplankton assemblages consisting of dinoflagellates even if another algal species was dominant. Thus, selective feeding is a mechanism by which a ciliate can maintain maximum growth.

ENDOCYTOSIS IN PHOTOAUTOTROPHS

It has been reported that five algal groups are capable of both endocytosis and photosynthesis as energy sources (5). The two major processes of endocytosis are i) phagocytosis, the engulfing of particulate material by a cell and ii) pinocytosis, the uptake of dissolved organics in the medium outside the cell.

We investigated both the phagocytosis of a cyanobacteria and the pinocytosis of a dextran solution by a marine chrysophyte, *Ochromonas* sp. All marine and freshwater species of *Ochromonas* have been shown to be phagotrophic, ingesting bacteria, detritus, and other microflagellates (1,8,14–16) as well as photosynthetic (2,23). *Ochromonas* species are maintained in culture on organic media and are able to utilize a wide variety of soluble organic compounds as sources of carbon, nitrogen, and phosphorus for their energy requirements (2).

For studying phagocytosis in *Ochromonas,* 2 ml samples were incubated in low light (125 μEin m^{-2} s^{-1}) with a culture of a 1 μm size cyanobacteria (*Synechococcus* sp., clone DC2) whose cell density was comparable to natural concentrations of cyanobacteria (in the

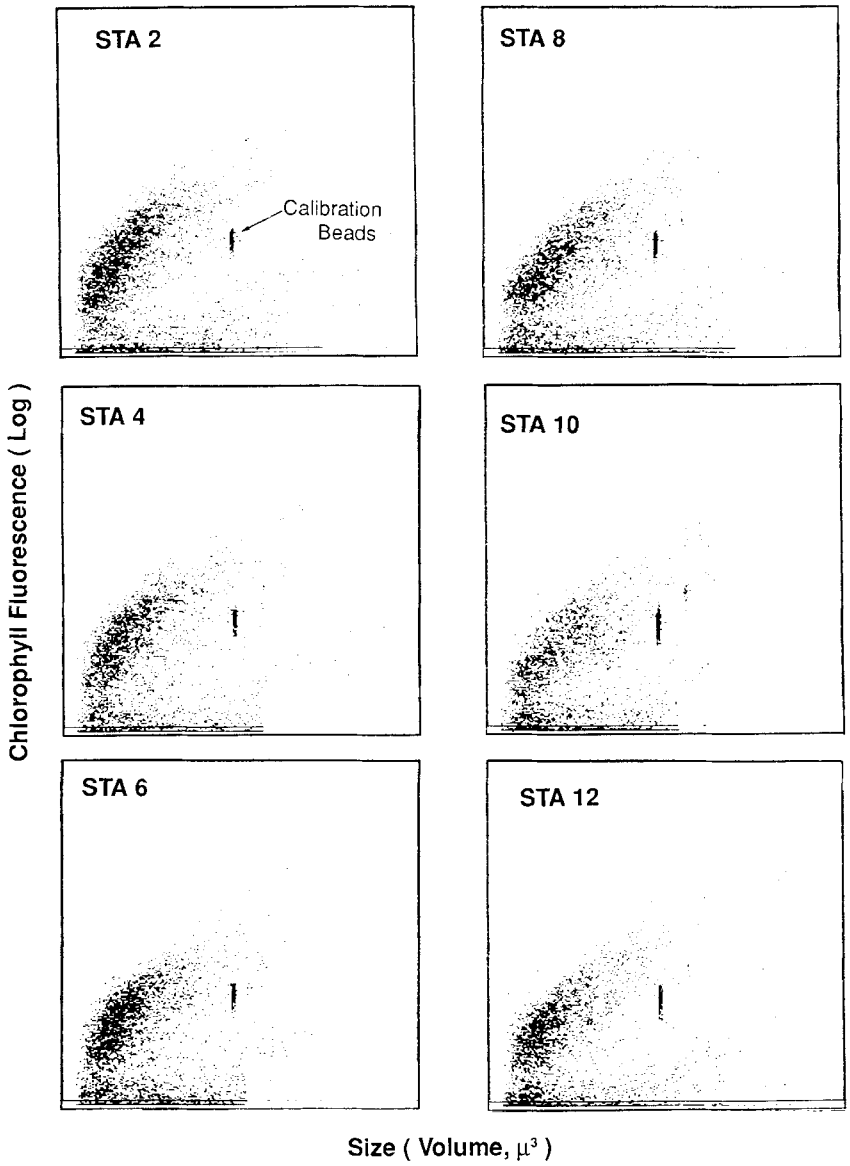


FIG. 8. Allometric analysis of particles based on size and one autofluorescing photosynthetic pigment (chlorophyll) from stations at a Maine mussel bed site. The histograms appear to look the same however, the number of cells per ml change. From Newell et al. (24).

range of 10^4–10^5 per ml). Incubations lasted for 4 h with all but one analysis taking place in the first hour. For direct observations of DC2 uptake, a Zeiss epi-fluorescence microscope of magnification 250× was used, with a 488 nm barrier filter for the excitation light source allowing the detection of the autofluorescing pigment of phycoerythrin in DC2 to be observed in the cells of *Ochromonas*.

For the pinocytosis studies, a 15 ml culture of each *Ochromonas* clone was incubated with a fluorescein-labeled polymer of glucose, known as FITC-dextran (MW of 40,000) (Sigma Chemical Co., St. Louis, MO). In our experiments, the medium used for *Ochromonas* has a final dextran concentration of 1 mg ml^{-1}. A Zeiss epifluorescence microscope with a 488 nm barrier filter was used to observe the FITC-dextran in the vacuoles of *Ochromonas*. A Coulter EPICS V flow cytometer with a 5 W argon laser was used to analyze both the phagocytotic and pinocytotic activities of *Ochromonas*.

Figure 5 illustrates the ataxonomic analysis of the chlorophyll containing *Ochromonas* and its phagocytosis of DC2. In this case, *Ochromonas* can be separated from the other non-fluorescing particles in the medium using its chlorophyll fluorescence signature. An *Ochromonas* cell that has phagocytized a DC2 cell exhibits the fluorescence signature (tracer signal) from

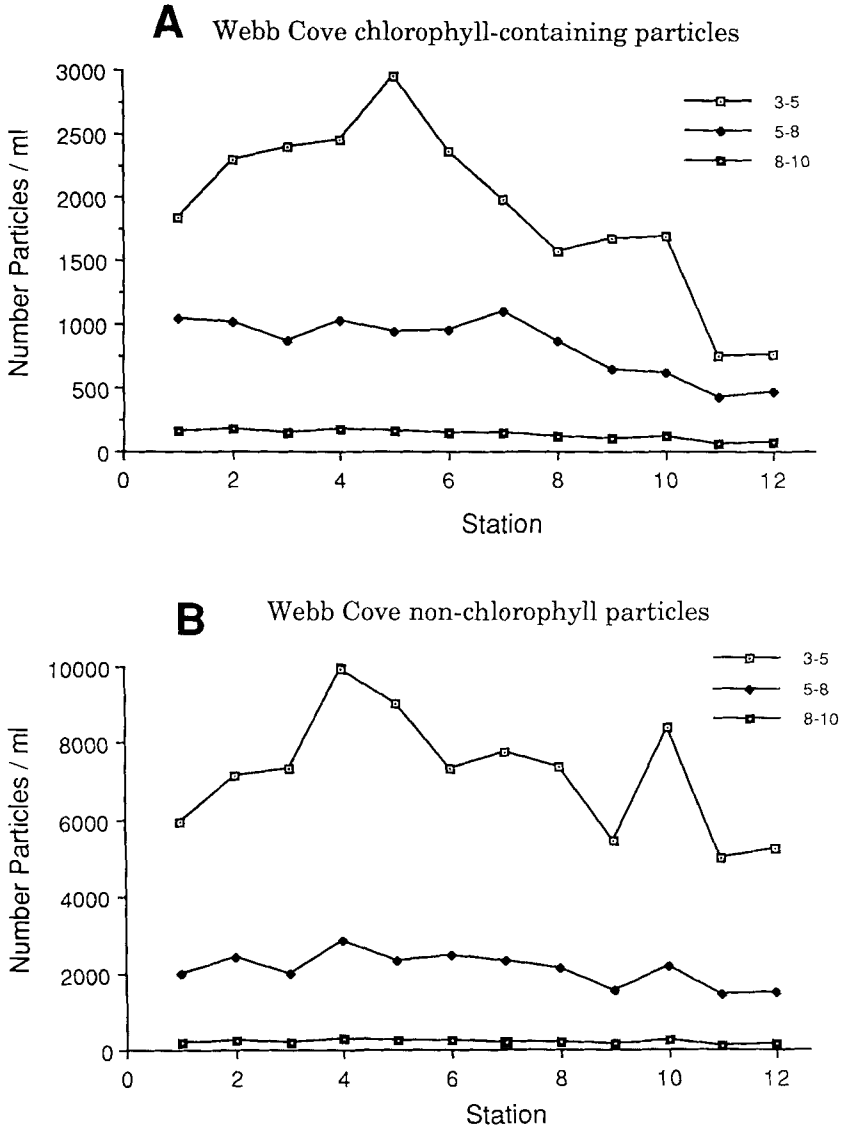


FIG. 9. Separation of chlorophyll (**A**) and non-chlorophyll (**B**) particles using flow cytometry. Samples were collected along a transect directly above a mussel bed and separated according to size and pigment content. From Newell et al. (24).

phycoerythrin. Percentages of cells that phagocytized DC2 can be obtained using chlorophyll and phycoerythrin emissions.

Figure 6 demonstrates the pinocytosis of FITC-dextran when incorporated in a food vacuole of *Ochromonas*. Upon entering the cell, the fluorescein (emission at 515–530 nm) is simultaneously detected with chlorophyll. Therefore, percentages of cells that pinocytized the soluble FITC-dextran can be quantitatively analyzed using the chlorophyll fluorescence as a biological tracer coupled with fluorescein fluorescence.

From the data, we can also determine the degree to which cells utilize endocytotic activities at different stages in the life cycle. Figure 7 represents frequency histograms of chlorophyll fluorescence for those cells that either did or did not phagocytize the cyanobacteria (DC2) (A) and pinocytize the FITC-dextran (B). It has been shown that a frequency histogram of chlorophyll represents the amount of chlorophyll fluorescence per cell in all stages of the cell cycle for a particular culture of phytoplankton (7). The left side of a distribution curve depicts chlorophyll fluorescence per cell for newly divided daughter cells, while the right side represents chlorophyll fluorescence per cell for older cells ready to divide. Figure 7A reveals that the means of the distribution for cells that phagocytized the cyanobacteria and those that did not are not significantly different, and the same applies to the means of the

distributions for cells that pinocytized the FITC-dextran and those that did not, as shown in Figure 7B. While these distributions only refer to the 60 min sample time, they are representative of the entire time course, suggesting that there is no difference in phagotrophic or pinocytotic activity during any one time in the life cycle of *Ochromonas*.

FEEDING ON NATURAL ASSEMBLAGES OF PARTICLES

The complex size overlap presented by natural particles in seawater once limited the scope and design of experiments on the differential utilization of particulate matter. As a result, laboratory experiments were designed to determine selectivity from a narrow range of particles, primarily phytoplankton cells, representative of the total found in natural seawater.

Recently, we studied the particle selectivity of mussels under natural conditions (24). The scope of the work was to assess the type and amount of particles removed by mussels and the impact that this depletion has on the growth of mussels further along a bed. The study site was an estuarine cove located along coastal Maine. Water samples were taken along a transect in the cove by pumping water, from 0.5 m above the bottom sediment, through 15 m of Tygon tubing to a boat moored above. Station 2 was before (seaward) the mussel bed; stations 4, 6, 8, and 10 were over the bed; station 12 was over a mudflat inside the mussel bed. A FACS Analyzer (Becton Dickinson) flow cytometer was used to analyze the water samples. Allometric analyses were employed for each sample, to determine both the size of particles filtered by the mussels, and the clearance of chlorophyll versus non-chlorophyll-containing particles.

In this experimental design, all particles within the water sample were of interest. However, the use of the phytoplankton cells as tracer particles enabled the differentiation of particle types (chlorophyll versus non-chlorophyll) being ingested. These analyses can be used to determine the densities of chlorophyll particles (phytoplankton) and non-chlorophyll particles (a combination of detritus, inorganic material, and heterotrophs).

Figure 8 illustrates the scatter plots of size (μm^3) and chlorophyll fluorescence from the six stations. The dynamic size range of particles was calibrated at 2.5 μm to 35 μm. Figure 9 plots the data for particle densities of chlorophyll cells and non-chlorophyll particles. There was a trend towards reduction of chlorophyll-containing cells (phytoplankton) into the cove (from station 2 to 12), particularly in the 3–5 μm size range. The non-chlorophyll particles remained at approximately the same density. The data indicate a reduction of about 30–42% of the phytoplankton cells available to the mussels in the inner vs. outer (seaward) bed. Results indicate that mussels inhabiting the inner areas of the cove experience lower food availability than those on the seaward side, leading to retarded rates of growth and reproduction.

SUMMARY

Through the use of flow cytometry, we have been able to demonstrate grazing rates/particle selection by various species of marine organisms, not only in the laboratory, but under field conditions. This technique will now allow us to carry out more complex feeding studies than were previously possible and provide a more sensitive and unified approach to the interrelated problems of particle selection, food preferences, and material flow in marine ecosystems. In the future, studies on the nutritional value of the food (phytoplankton) may be possible with the use of protein/lipid/carbohydrate-specific fluorescent stains.

ACKNOWLEDGMENTS

This work was supported by grant awards to Clarice M. Yentsch (NSF grant OCE-84-16217, ONR contract N0001481C, and a grant from Exxon Educational Foundation) and Carter Newell (NSF grant ISI-8660201). This is Bigelow Laboratory contribution No. 89002. We thank P. Colby for typing the manuscript and J. Rollins for the graphics.

LITERATURE CITED

1. Aaronson S: Particle aggregation and phagotrophy by *Ochromonas*. Arch Microbiol 92:39–44, 1973.
2. Aaronson S: Descriptive biochemistry and physiology of the chrysophyceae (with some comparisons to Prymnesiophyceae). In: Biochemistry and Physiology of Protozoa, Levandowsky M, Hutner SH (eds). Second Edition, Vol 3, Academic Press, New York, 1980, pp 117–169.
3. Amouroux JM: Comparative study of the carbon cycle in *Venus verrucosa* fed on bacteria and phytoplankton, I. Consumption of bacteria (*Lactobacillus* sp.). Mar Biol 90:237–241, 1986.
4. Bayne BL, Newell RC: Physiological energetics of marine molluscs. In: Mollusca, Saleuddin HSM, Wilbur KM (eds). Vol 4, Physiology, Part 1, Academic Press, New York, 1983, pp 407–515.
5. Boraas ME, Estep KW, Johnson PW, Sieburth JM: Phagotrophic phototrophs: The ecological significance of mixotrophy. J Protozool 35:249–252, 1988.
6. Burkill PH, Mantoura RFC, Llewellyn CA, Owens NJP: Microzooplankton grazing and selectivity of phytoplankton in coastal waters. Mar Biol 93:581–590, 1987.
7. Campbell JW, Yentsch CM: Effects of variance among individual cells on their collective (bulk) optical properties. Cytometry: this issue.
8. Cole GT, Wynne MJ: Edocytosis of *Microcystis aeruginosa* by *Ochromonas danica*. J Phycol 10:397–410, 1974.
9. Cowles TJ, Olson RJ, Chisholm SW: Food selection by copepods: Discrimination on the basis of food quality. Mar Biol 100:41–49, 1988.
10. Cucci TL, Shumway SE, Newell RC, Selvin R, Guillard RRL, Yentsch CM: Flow cytometry: A new method for characterization of differential ingestion, digestion and egestion by suspension feeders. Mar Ecol Prog Ser 24:201–204, 1985.
11. Deibel D: Laboratory-measured grazing and ingestion rates of the salp, *Thalia democratica* (Forskal, and the doliolid, *Dolioletta gegenbauri* Uljanin (Tunicata, Thaliacea). J Plank Res 4:189–201, 1982.
12. Deibel D: Clearance rates of the salp *Thalia democratica* fed naturally occurring particles. Mar Biol 86:47–54, 1985.
13. DeMott WR: Discrimination between algae and artificial particles by freshwater and marine copepods. Limnol Oceanogr 33: 397–408, 1988.

14. Doddema H, van der Veer J: *Ochromonas monicis* sp. nov., a particle feeder with bacterial endosymbionts. Cryptogamie Algolgie 4:89–97, 1983.
15. Dubowski N: Selectivity of ingestion and digestion in the chrysomonad flagellate *Ochromonas malhamensis*. J Protozool 21: 295–298, 1974.
16. Estep KW, Davis PG, Keller MD, Sieburth JM: How important are oceanic algal nanoflagellates in bacterivory? Limnol Oceanogr 31:646–650, 1986.
17. Foster-Smith RL: The effect of concentration of suspension and inert material on the assimilation of algae by three bivalves. J Mar Biol Asso UK 55:411–418, 1975.
18. Haas LW, Webb KL: Nutritional mode of several non-pigmented microflagellates from the York River estuary, Virginia. J Exp Mar Biol Ecol 39:125–134, 1979.
19. Jonsson, PR: Particle size selection, feeding rates and growth dynamics of marine planktonic oligotrichous ciliates (Ciliophora: Oligotrichina). Mar Ecol Prog Ser 33:265–277, 1986.
20. Kiørboe T, Møhlenberg F, Nøhr O: Particle selection in suspension-feeding bivalves. Mar Ecol Prog Ser 5:291–296, 1981.
21. Langdon CJ: New techniques and their application to studies of bivalve nutrition. In: Proceedings of the Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition, Pruder GD, Langdon CJ, Conklin DE (eds). Louisiana State University Division of Continuing Education, Baton Rouge, LA, 1982, pp 305–320.
22. Morton BS: Feeding and digestion in bivalvia. In: The Mollusea, Vol. 5, Physiology Part 2, Saleuddin ASM, Wilbur KM (eds). Academic Press, New York, 1983, pp 65–147.
23. Myers J, Graham JR: The role of photosynthesis in the physiology of *Ochromonas*. J Cell Comp Physiol 47:397–414, 1956.
24. Newell CR, Shumway SE, Cucci TL, Selvin R: The effects of natural seston particle size and type on feeding rates, feeding selectivity, and food resource availability for the mussel, *Mytilus edulis*, at bottom culture sites in Maine. J Shellfish Res 8:187–196, 1989.
25. Newell RC: Biology of Intertidal Animals. Marine Ecological Surveys Ltd., Faversham, Kent, 1979.
26. Newell RIE, Jordan SJ: Preferential ingestion of organic material by the American oyster *Crassostrea virginica*. Mar Ecol Prog Ser 13:47–53, 1983.
27. Nival P, Nival S: Particle retention efficiencies of an herbivorous copepod, *Acartia clausi* (adult and copepodite stages): Effects of grazing. Limnol Oceanogr 21:24–38, 1976.
28. Nygaard K, Børsheim KY, Thinstad TF: Grazing rates on bacteria by marine heterotrophic microflagellates compared to uptake rates of bacterial-sized monodisperse fluorescent latex beads. Mar Ecol Prog Ser 44:159–165, 1988.
29. Paffenhöfer GA, Van Sant KB: The feeding response of a marine planktonic copepod to quantity and quality of particles. Mar Ecol Prog Ser 27:55–65, 1985.
30. Randløv A, Riisgard HU: Efficiency of particle retention and filtration rate in four species of Ascidians. Mar Ecol Prog Ser 1: 55–59, 1979.
31. Reiswig HM: Bacteria as food for temperate-water marine sponges. Can J Zool 53:582–589, 1975.
32. Schoenberg SA, Maccubbin AE: Relative feeding rates on free and particle-bound bacteria by freshwater macrozooplankton. Limnol Oceanogr 30:1084–1090, 1985.
33. Shapiro HM: Practical Flow Cytometry, 2nd edition. Alan R. Liss, Inc., New York, 1988.
34. Sheldon RW, Parsons TR: A practical manual on the use of the Coulter Counter in marine research. Coulter Electronics Sales Co., Canada, 1967.
35. Shumway SE, Cucci TL: The effects of the toxic dinoflagellate *Protogonyaulax tamarensis* on the feeding and behavior of bivalve molluscs. Aquatic Toxicol 10:9–27, 1987.
36. Shumway SE, Cucci TL, Newell RC, Yentsch CM: Particle selection, ingestion, and absorption in filter-feeding bivalves. J Exp Mar Biol Ecol 91:77–92, 1985.
37. Stoecker DK, Cucci TL, Hulburt EM, Yentsch CM: Selective feeding by *Balanion* sp. (Ciliata:Balanionidae) on phytoplankton that best support its growth. J Exp Mar Biol Ecol 95:113–130, 1986.
38. Trask BJ, van den Engh GJ, Elgershuizen JHBW: Analysis of phytoplankton by flow cytometry. Cytometry 2:258–264, 1982.
39. Verity PG, Villareal TA: The relative food value of diatoms, dinoflagellates, flagellates, and cyanobacteria for tintinnid ciliates. Arch Protistenkd 131:71–84, 1986.
40. Widdows JP, Fieth P, Worral CM: Relationships between seston, available food and feeding activity in the common mussel *Mytilus edulis*. Mar Biol 50:195–207, 1979.
41. Yentsch CM, Yentsch CS: Emergence of optical instrumentation for measuring biological properties. Oceanogr Mar Biol Annu Rev 22:55–98, 1984.
42. Yentsch CM, Cucci TL, Phinney DA, Topinka JA: Real time characterization of marine particles at sea: Flow cytometry. In: Lecture Notes on Coastal and Estuarine Studies, Bowman M, Peterson W, Yentsch CM (eds). Vol 17, Springer-Verlag, New York, 1986, pp. 414–448.
43. Yentsch CM, Cucci TL, Phinney DA, Selvin R, Glover HE: Adaptation to low photon flux densities in *Protogonyaulax tamarensis* var. *excavata*, with reference to chloroplast photomorphogenesis. Mar Biol 89:9–20, 1985.
44. Yentsch CM, Horan PK, Muirhead K, Dortch Q, Haugen, E. Legendre L, Murphy LS, Perry MJ, Phinney DA, Pomponi SA, Spinrad RW, Wood, M, Yentsch CS, Zahuranec BJ: Flow cytometry and cell sorting: A powerful technique for analysis and sorting of aquatic particles. Limnol Oceanogr 28:1275–1280, 1983.
45. Yentsch CS, Phinney DA: Spectral fluorescence: An ataxonomic tool for studying the structure of phytoplankton populations. J Plank Res 7:617–632, 1985.