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PARTICLE SELECTION IN FILTER-FEEDING BIVALVE MOLLUSCS: A NEW TECHNIQUE ON AN OLD THEME*

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ABSTRACT

The efficiency of particle retention from algal suspensions, and its significance in the estimation of energetics of growth has been widely studied in bivalve molluscs. Whether filter-feeding organisms can (1) select; (2) preferentially ingest, and/or (3) preferentially digest suspended particles, is of major importance to our understanding of material flow through marine systems. The complex size overlap presented by natural populations of phytoplankton in the sea which once limited the scope and design of experiments on the differential utilization of particulate matter have been at least partially overcome through the use of flow cytometry. Until recently, investigations have been limited by the lack of techniques that can distinguish quantitatively between different particles of the same size. The methods are now available and by taking advantage of fluorescence and light scatter characteristics of particles, we have been able to examine differential use by consumer organisms of food resources, even when those resources are comprised of groups having similar sized cells. Using this technique, we have been able to estimate not only the clearance rate of individual cell types, but also their proportional occurrence in the pseudofaeces and faeces. In addition, bacteria can now be used experimentally, provided they have been stained with fluorescent materials prior to use.

Through the use of flow cytometry, we have been able to demonstrate three different mechanisms of selection which may be present in isolation or in combination in filter-feeding bivalve molluscs: (a) preferential clearance on the ctenidia; (b) preingestive selection on the labial palps and (c) differential absorption in the gut.

Flow cytometry thus presents a potentially manageable, sensitive and unified approach

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to the study of interrelated problems of particle selection, food preferences and material flow in marine organisms previously not approachable. This technique, coupled with other newly devised methods, allows investigators to carry out more complex feeding studies using a number of particles simultaneously, and will allow us to elucidate at least some of the mechanisms involved in particle selection in filter-feeding animals.

INTRODUCTION

The possibility that bivalve molluscs may be able to select nutritive components of their diet from the relatively large quantities of inert material suspended in the water column has attracted attention for many years. Research into bivalve filter feeding, and especially the hitherto unresolved question of the extent to which bivalves select the more nutritious components of suspended particulates, has been profoundly influenced by the technologies available to investigators. Four distinct periods may be recognized as we now enter the fifth with the advent of flow cytometry.

Early in the era of descriptive morphology, Lotsy (1895) and Grave (1916) presented evidence that oysters ingested organic rather than inorganic particles. Kellogg (1915) and Yonge (1926), however, maintained that lamellibranchs distinguished between heavier, larger particles in contrast to smaller, lighter ones. Pioneer studies by Yonge (1923, 1926a, b) and Loosanoff (1949) suggested that qualitative selection could be achieved by differential sorting mechanisms on the gills and labial palps.

The second phase followed the introduction of techniques of monoculture combined with simple methods of assessing concentration changes in the suspended algal cells, such as changes in optical density or the tedious counting of cells on the haemocytometer.

The third phase was made possible through the invention of the Coulter Counter and its subsequent introduction to the field of marine science which made counts of cell concentration a routine and accurate procedure, including the possibility of separating particles of different size, and meant that the grazing of bivalve molluscs could be put on a quantitative basis. However, little advance was made on the problem of species discrimination. To the contrary, the mariculture industry sought to use highly artificial monocultures for hatchery purposes and for 'growing on' spat (Sheldon and Parsons, 1967). In the fourth phase of this study interest became deflected towards productivity problems of larval, rather than adult, bivalves, e.g., Holland and Gabbott (1971) and Holland and Spencer (1973), and to the relative nutritional value of such monocultures.

Most subsequent studies on bivalve feeding rates were concerned with quantifying the rates of uptake of standard cultures of algal cells which were distinguished on the basis of size alone (for reviews on feeding and physiological energetics of the Bivalvia, see Morton (1983) and Bayne and Newell (1983)). Because of the limitations of the Coulter Counter, which cannot be used to distinguish identical-sized particles of differing nutritional quality, little further attention was given to whether or not molluscs are able to distinguish between different types of food resources when presented with a mixed spectrum of particles, such as occurs under natural conditions. The question is an important one since it may given some insight into the means by which co-existing and potentially competing organisms could partition the available food resource (Stuart and Klumpp, 1984) and may also suggest mechanisms by which toxic dinoflagellates can be detected and actively included/excluded from the diet (Shumway and Cucci, 1986).

This paper illustrates the potential of the fifth technological advance with the advent of flow cytometry. The introduction of the flow cytometer to marine science (Yentsch *et al.*, 1983, 1986) has provided us with an opportunity to distinguish particles of a similar size but different optical properties from one another. In addition to size, two further properties of cells can now be routinely assessed and the cells sorted into categories. These are: first, the property of fluorescence by the naturally occurring pigments or, equally, the absence of fluorescence in inert or detrital particles and second, the particle shape that influences forward dispersion of the light beam. Differential uptake of algae from several different pigment groups can be detected simultaneously and we have been able to quantify differential ingestion, digestion and egestion by several molluscs in the presence of mixed food resources of a similar cell size. Though the mix of three alga we have used is simpler than the complex assemblage of particles in the sea, it gives the lie to the defeatist view of Lasker (1966) that 'you may never get an experiment which (sic) will be comparable to the sea. . .'. The study by Lucas *et al.* (1986) on the different-sized particles taken up by *Mytilus* took the Coulter Counter as far as its limitations allowed, but the smaller bacteria required an independent method. Flow cytometry has no such minimum size restriction and so could be used to investigate how filter feeders utilize and partition the smallest suspended food particles available in the sea. Moreover, when cells are selectively removed from a known volume of suspension, the calculation of filtration rate from total cell counts becomes mathematically compromised (Williams, 1982). However, if each cell type is independently filtered and can be counted, the logarithmic model, usually attributed to Coughlan (1969) gives valid filtration rates which may differ for each species. This new technique comes at an opportune time since the importance of these small flagellates, suspected many years ago by Knight-Jones (1951) in his paper on *Chromulina pusilla* and by Walne (1963), has now been extended to small heterotrophic and cyanobacteria which many consider to constitute a large part of algal production in the sea.

The results confirm and amplify those of earlier workers and pose exciting questions on the relative importance of different cell types in the diet of suspension-feeding molluscs.

MATERIALS AND METHODS

Specimens of the following bivalve molluscs were collected at various localities in Maine, USA: *Crassostrea virginica* (Gmelin), *Mya arenaria* Linne, *Mytilus edulis* L., *Placopecten magellanicus* (Gmelin) and *Spisula solidissima* Dillwyn. Animals were scrubbed to remove all epiphytes and maintained in running seawater from Boothbay Harbor at 12°C prior to use in experiments.

Before each experiment, all animals were purged in filtered seawater (0.7 μm Gelman glass fibre) for 24 h and the water changed at least once. All experiments were carried out at the same time of day (early a.m. at 12°C). Individuals were placed in bell jars containing the algal mixture (see below) and gently aerated. Control vessels were left without animals to correct for algal cell division during experiments. Experiments lasted for one hour and samples were taken for flow cytometric analyses at 30 and 60 minute intervals. Any pseudofaeces produced were collected and analyzed. After 1 h, the animals were removed from the feeding media and placed in filtered seawater (as above) for faeces collection which usually occurred after 4 h. Faeces and pseudofaeces were collected with glass Pasteur pipettes and all samples were observed under a fluorescence microscope prior to flow cytometric measurements.

Algal cultures were supplied by the Provasolli-Guillard Culture Center for Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences and consisted of the following: the dinoflagellate *Prorocentrum minimum* (Pavillard) Schiller (clone Exuv) (5–6.15 x 8.75–12.5 x 12.5–15 μm), the cryptomonad flagellate *Chroomonas salina* (Wislouch) Butcher (clone 3C) (6.25–7.5 x 8.75–12.5 μm), *Thalassiosira pseudonana* (Hustedt) Hasle et. Heimdal (clone 3H) (3–4 μm). Cultures were grown in f/2 media at 15°C with a 14:10 photoperiod. The three algal clones, 3C, 3H and Exuv were mixed just prior to the experiment to obtain equal cell densities with a final cell concentration of 10^5 cells.m^{-1} in each of the test jars.

Cells were analysed on a Coulter Epics V Flow Cytometer/Sorter by utilizing differences in their fluorescing intensities from the photosynthesizing pigments of chlorophyll (Exuv. 3C, GT429 and 3H) and phycoerythrin (3C). The instrument has a single argon ion 5-W laser with an excitation wavelength of 514 nm and a power of 1000 mW fluorescence derived from each particle is split by a 590 nm dichroic mirror and is received by two photomultiplier tubes located at 90° to the intersection of the laser beam and sample stream. One receives a wavelength (630 nm) such as would result from chlorophyll emission and the other receives shorter wavelengths (540–560 nm) such as would result from phycoerythrin emission. The events (number of cells) registered met gate criteria on chlorophyll fluorescence, therefore only algal cells were analysed. (See Yentsch *et al.*, 1983, 1986 for a complete description of flow cytometry methodology.) A total of 2000 cells was analysed for each sample, with the total being partitioned among the 3 clones (4 when GT429 was added to the mixture). Since all samples were run at a constant flow rate throughout the entire experiment, we were able to calculate the clearance rate of each individual by the differences in the amount of time required to analyse 2000 cells (initial analysis times average ~300 s; after 60 min, average analysis times ranged from 500 to >1000 s). A comparison of three methods (Coulter Counter, Model ZM; uniform bead counting on flow cytometer, and the time method utilized here) used to determine actual cell density of the algal clone 3C have been shown to yield similar results (Shumway and Cucci, 1986). Actual cell concentrations were calculated according to the following formula:

$$\frac{\text{counts/sec } @T_n}{\text{counts/sec } @T_o} \text{ x initial cell concentration}$$

where T_n and T_o are times (in sec) taken to count 2000 events after experimental grazing and in control vessels, respectively.

Typical bivariate histogram plots are shown in Figures 1, 2 and 3 where the number of cells analysed is plotted with increasing fluorescence approximating phycoerythrin (X-axis) and increasing fluorescence approximating chlorophyll (Y-axis).

RESULTS

Data on clearance and irrigation rates and particle selection for the various species are given in Shumway *et al.* (1985) and Shumway and Cucci (1986) and will be discussed here only as they apply to particle selection capabilities. Three representative species of molluscs will be discussed, each representing a different site of particle selection: *Ostrea edulis* (preferential clearance on the ctenidia), *Arctica islandica* (sorting on the palps; rejection in the pseudofaeces) and *Crassostrea virginica* (no differential clearance or sorting on the palps, but differential absorption in the gut, i.e., post-ingestive sorting.

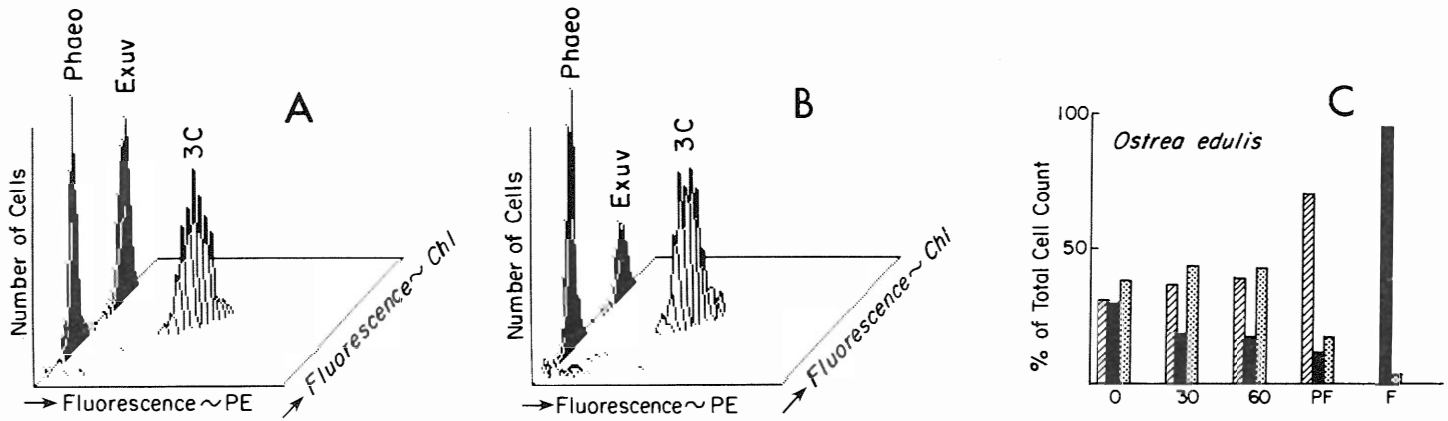

Fig. 1. Bivariate histogram plots of number of cells and X = fluorescence approximating phycoerythrin vs. Y = fluorescence approximating chlorophyll showing relative changes in cell numbers within an algal mixture of Phaeo, 3C and Exuv due to the grazing by *Ostrea edulis* at A, time 0 min; B, 60 min and C, a summary of the percent of the total cell count within the same algal mixture C. Histogram plots A and B are actual instrument printouts. (After Shumway *et al.*, 1985).

Figure 1 shows a bivariate histogram of events and log integrated green fluorescence (X = fluorescence due to phycoerythrin) versus log integrated red fluorescence (Y = fluorescence due to chlorophyll) for time zero and after 60 minutes in the experimental seawater in the presence of a specimen of *Ostrea edulis*. The preferential clearance of the dinoflagellate Exuv compared with similar-sized cells of the diatom Phaeo and the cryptomonad flagellate 3C can be seen clearly.

The result is of interest since the ability of many commercially significant bivalves, including *Ostrea edulis*, to accumulate toxic dinoflagellate cells preferentially from the plankton is well-known, although the mechanism(s) by which this is accomplished are not yet clear.

Figure 2 shows a bivariate histogram of events and log integrated green fluorescence (X) versus log integrated red fluorescence (Y) at the time zero and after 60 minutes in experimental seawater in the presence of a specimen of *Arctica islandica*. A similar histogram for the pseudofaeces resuspended in filtered seawater is also shown.

From this it is clear that the relative proportions of cell types are similar in the experimental medium after 60 min of filtration. The cells are thus cleared from suspension with equal efficiency by *Arctica*. The pseudofaeces show, however, an increase in the proportion of Phaeo compared with dinoflagellate Exuv and the cryptomonad flagellate 3C. Similar ingestive selection between the three cell types on the labial palps has been demonstrated in *Ensis directus* and *Placopecten magellanicus* (Shumway *et al.*, 1985).

Figure 3 shows a bivariate histogram of events and log integrated green fluorescence (X) versus log integrated red fluorescence (Y) at time zero and after 60 minutaes in the experimental seawater in the presence of a specimen of *Crassostrea virginica*. The corresponding plots for pseudofaeces and faeces resuspended in filtered seawater are also shown.

There is evidently no preferential clearance of any one cell type on the ctenidium since after 60 minutes of filtration the proportion of the three cell types is similar to that at time zero. The proportions of cell types in the pseudofaeces 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 faeces and the dominance of the alga Phaeo. A preferential absorption of the 3C has evidently occurred during passage through the gut. As shown in Figure 1, this is commonly combined with pre- ingestive sorting on the palps in the other bivalves.

Our review of the literature on the clearance of suspended particles by bivalves suggested that, in many instances, potentially nutritive particles may be selected relative to inert particles by pre-ingestive sorting on the labial palps (Hylleberg and Gallucci, 1975; Newell and Jordan, 1983). The ability to exploit a size range of particles appropriate to the resource available in the local environment also appears to involve a widespread pre-ingestive selective mechanism. Thus, species which characteristically inhabit phytoplankton-dominated waters appear to be structurally adapted to exploit larger suspended particles including algal cells as a primary component of their diet (Moore, 1971; Owen and McCrae, 1976; Møhlenberg and Riisgard, 1978). However, species from detritus-dominated habitats where the suspended bacterial resources is high, may show an efficient clearance of bacterioplankton compared with other bivalves (Wright *et al.* 1982; Berry and Schleyer, 1983). Finally, there is evidence which suggests that, of the cells ingested, a variable quantity may be egested as viable cells depending on ration and other factors (Thompson and Bayne, 1972).

Our results suggest that, although all of the bivalves showed clearance of suspended

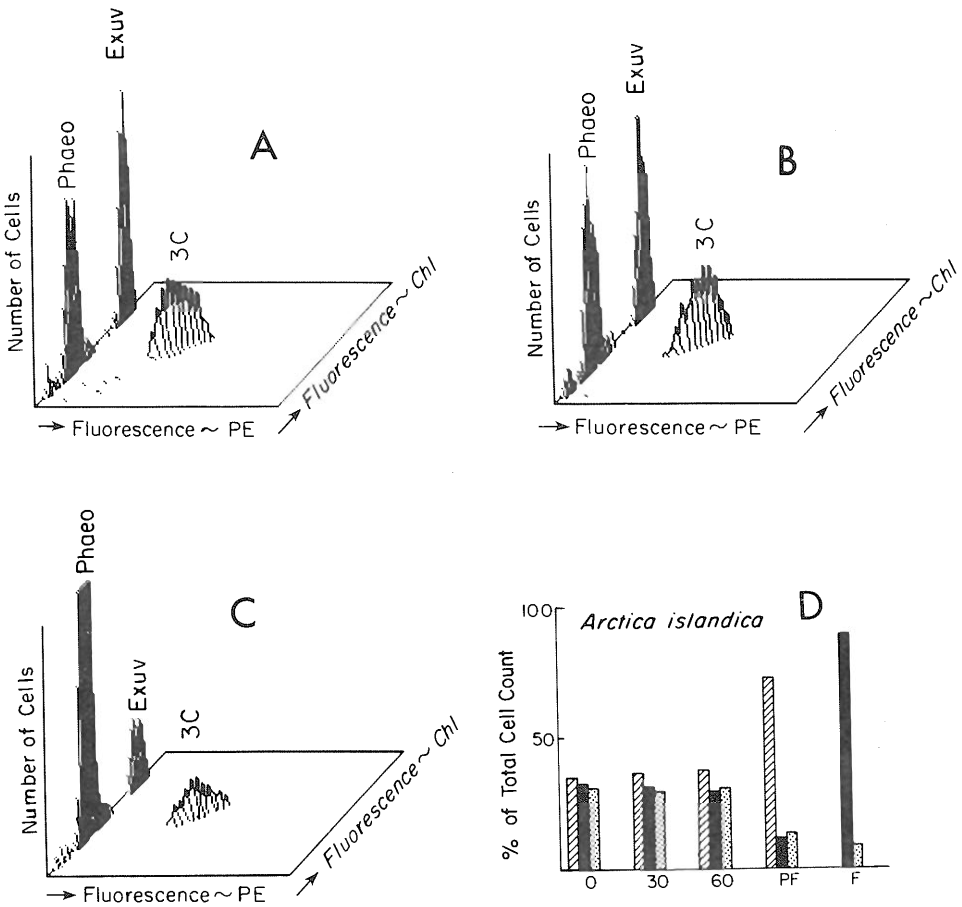

Fig. 2. Bivariate histogram plots comparing relative cell numbers due to grazing by *Arctica islandica* at A, time 0 min; B, 60 min and C, within the pseudofaeces. Also shown is the percent of the total cell count within the sample algal mixture D. See Figure 1 for description. (After Shumway *et al.*, 1985).

cells, these may not have been utilized uniformly. We do not know, however, whether these differences in utilization are the result of variable digestibility or active postingestive sorting. It is clear that some cells, such as the cryptomonad flagellates, may be of more significance in the diet of suspension feeders under natural conditions than their relative concentration in the water column would indicate.

DISCUSSION

Particle selection is an obvious advantage to filter-feeding organisms, not only in sorting organic from inorganic particles, but also in 'choosing' particles of easier digestibility (more nutritive value?) over those more difficult or impossible to digest. Alder and Hancock (1851)

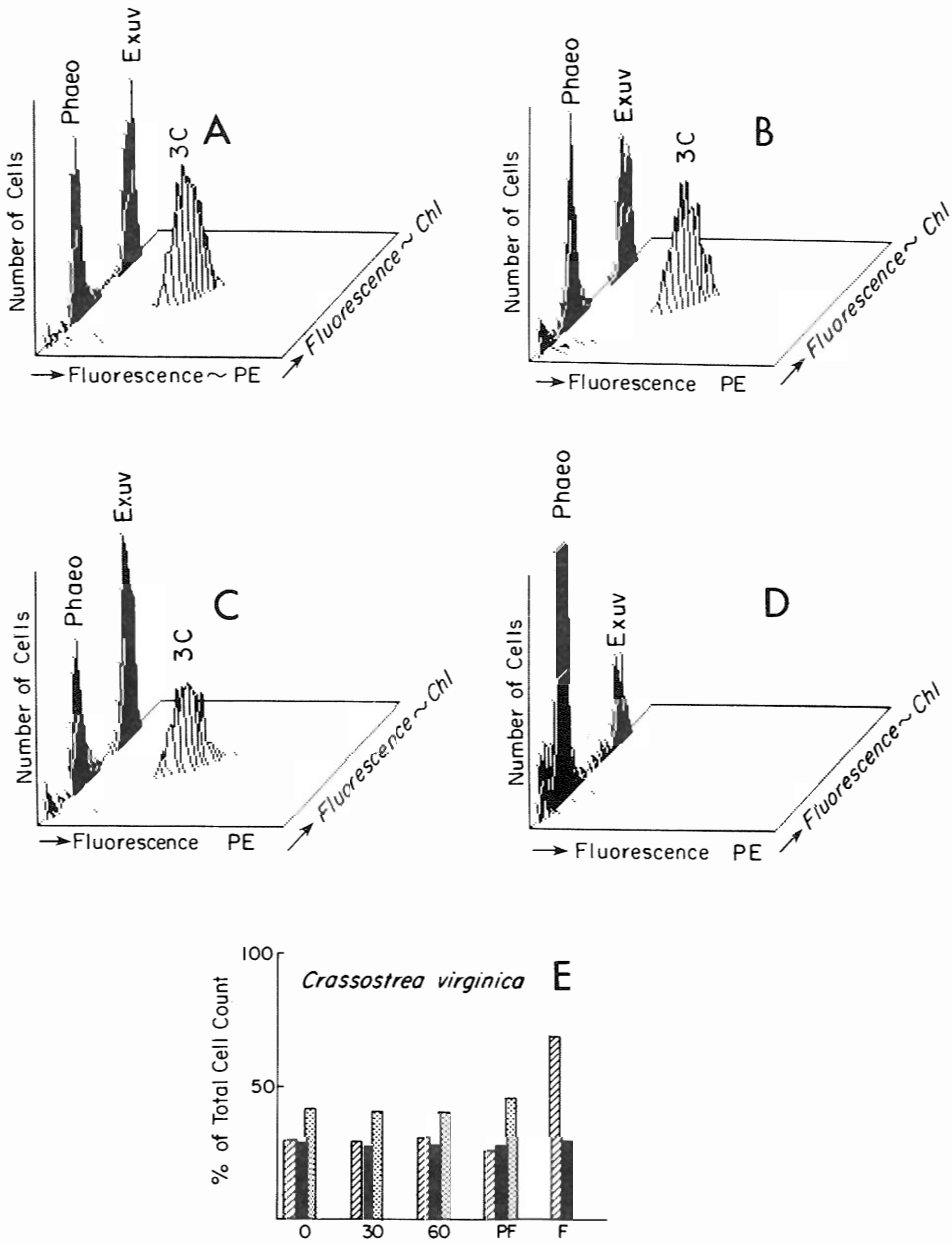

Fig. 3. Bivariate histogram plots comparing relative cell numbers due to grazing by *Crassostrea virginica* at A, time 0 min; B, 60 min; C, within the pseudofaeces and D, faeces. Also shown (E) is the relative percent of the total cell count within the same algal mixture. See Figure 1 for description. (After Shumway *et al.*, 1985).

first described the theory of particle filtration in lamellibranch molluscs and there have been a vast number of studies since. The majority of these studies have been concerned with the uptake of particles from suspension and in some cases with the sorting of organic from inorganic particles (for reviews, see Jørgensen, 1966; Bayne and Newell, 1983; Newell and Jordan, 1983). As yet, no mechanisms have been clearly elucidated, although many have been proposed. Clearly, any proposed mechanisms must take into account not only gill structure but also: nervous innervation; muscular control of the gill filaments; particle size structure; chemosensory capabilities; gut residence time; digestive enzyme suites, mucous secretions. Feeding mechanisms in bivalve molluscs are complicated and, likely, a number of these factors may be acting in consort and will be species-specific.

In a typical bivalve mollusc, a slow current of water maintained by ciliary action flows into the mantle cavity at the inhalant aperture, passes through ostia in the ctenidia into the suprabranchial chamber and passes through the exhalant siphon back into the surrounding seawater. Except for occasional reverse pulses of water which serve to clear obstructions, all water entering the coarse fringe that protects the inhalant entrance from heavy or large particles passes out at the exhalant opening. There are thus three 'gates' that control the fate of suspended particles entering the mantle cavity: (1), the ctenidia, ostia and the cilia around them; (2), the ciliated grooves of the labial palps and, in some, the ventral marginal grooves of, for example, the arcacean ctenidia which are also rejectory where pseudofaeces are rejected, and (3), the ciliated tracts of the gut wall which separate assimilated particles from faeces. Clearly, the flow of water into the mantle cavity must be identical for all genuinely suspended particulates, hence the initial concentrations reaching the surface of the ctenidia must be those of the seawater just outside the inhalant area, which if the water is sufficiently well stirred, will be that of the whole volume in which the experimental animal is immersed (Hildreth and Crisp, 1976).

The gill has been long known to have a sieve-like action (Alder and Hancock, 1851), and was thought, therefore, to allow only the finest particles to pass through the ostia and back into the sea. On such an assumption, a mixture of equal sized microalgae, large enough not to pass the ostia, would be wholly filtered off and not returned to the experimental vessel, and the clearance rate or pumping rate, as measured by the logarithmic formula, would be equal for all such microalgae. However, the results, especially those for *Ensis directus* and *Ostrea edulis*, indicate that this is not so. The change in the concentration in the medium, with time, is greater for the dinoflagellate 'Exuv' than for the other two algae, and this is significant for the bivalves used as a whole. The ordination of filtration rates between the three alga are so similar for the different bivalves that the selection observed may well be attributed to some property of the particular microalgae themselves rather than to preferences on the part of each bivalve. This is not so, however, in regard to pseudofaecal content. Separation at the labial palps and at the stomach is highly specific as our results demonstrate, whereas differences in filtration rate are less marked. Indeed, for *Mytilus edulis*, Cucci *et al.* (1985) found that the same three microalgae were equally cleared by the ctenidia but *Chromomonas* (3C) was absorbed while *Phaeodactylum* (Phaeo) and *Prorocentrum* (Exuv) were ejected.

If the bivalve alimentary system behaved exactly as stated above, the only possible explanation for differences in clearance rates would be that 'Phaeo' and '3C' were less efficiently cleared than 'Exuv', the ostia allowing more of them to pass through and so allowing them to return to the surrounding medium. However, other explanations are possible if the alimentary system has leakages. First, when short valve contractions occur, microalgae not yet fully entrained in ingestive tracts of cilia, might be preferentially expelled

from the mantle space. The 'shunt' posterior to anterior water flow described by Famme and Kofoed (1983) may also be involved. Second, some microalgae might be resuspended from expelled faeces or pseudofaeces (Hildreth, 1980). Last, as Thompson and Bayne (1972) found, some microalgae may pass in a viable condition through the gut and thus appear not to have been cleared. These potential leakages appear to us as a more probable explanation than differential passage through the ostia, though Elsey (1935) and Dral (1967, 1968) postulated control of ostial openings by changes in blood pressure or muscle action, respectively. Whatever the explanation, effective clearance is not equal for all algae and the clearance rate $dF_{x,y,z}/dt$ can be measured correctly by the logarithm formula if each cell type is distinctly recognizable. We shall call $F_{x,y,z}$ the amount of x, y or z removed from solution after a standard length of time.

At the second gate, the labial palps sort particles passed on to them by ciliated tracts in the gills. These organs are highly mobile (Foster-Smith, 1978) and operate in conjunction with the ctenidia to reject excessive or unwanted filtered material. This is bound into mucous-covered strings, the pseudofaeces, which fall into the lower mantle groove and are rejected into the sea. Their quantity and composition are probably not constant, but depend on the type and concentration of suspended matter removed by the ctenidia. the quantity of each microalga, x, rejected as pseudofaeces after a standard period will be called $P_{x,y,z}$ etc. The remainder is ingested.

The third gate, constituted by the gut, further sorts ingested particles into those which are assimilated either intracellularly in the gut diverticulae or treated with the large array of enzymes (van Weel, 1961) produced by the midgut. The remainder are expelled as faeces, the amount for each microalga being designated as $E_{x,y,z}$. The stomach, with its rotating crystalline style is the primary site for selection. The elaborate ciliated areas were first described for *Mya* by Yonge (1923) and their variation between species by Purchon (1956, 1957, 1958, 1960). As with pseudofaecal production, the quantity of algal ingestion that is voided rather than assimilated is a function of ration. Variation in gut residence time, as demonstrated by Bayne and Newell (1985) will give the enzymes more or less opportunity to work and alter assimilation efficiency (Epifanio, 1979). If we assume that particles containing specific pigments present in the faeces are cells that have not been assimilated and that cells which have lost pigment have been wholly assimilated, both assumptions probably being only partially true, we may write for each cell type

$$F_x = P_x + E_x + A_x \qquad (1)$$

where A_x is the amount of cell clearance; F_x that which has been assimilated.

Unfortunately, although F_x for each cell type can be obtained from Coughlan's equation and, by resuspending pseudofaeces and faeces, the ratios of each cell type present in each can be measured by flow cytometry (vis $P_x/P_x + P_y + P_z$, etc. and $E_x/E_x + E_y + E_z$, etc.), these data are insufficient to solve eq. 1, for A_x, A_x, A_x, etc. For a solution we also require total pseudofaecal or faecal quantity and hence, by difference, the total amount $A_x + A_y + A_z$, etc. assimilated. Alternatively, A_x might be found using radio-labelled algae. A more detailed study is needed to determine the mechanism by which selective filtration, pseudofaecal production and assimilation rates come about, and eventually a full inventory of the constituents of pseudofaeces, faeces and quantities absorbed for each of a mixture of algal species.

Mechanisms of selection

There can be no doubt that ability to select richer food particles from inorganic material must be of great value, particularly to bivalves living in muddy environments and removing deposits from the mud surface. Since some bivalves are harmed by toxic microalgae (Shumway *et al.*, 1986) qualitative selection between algae in favour of the least toxic and most nutritious or readily assimilated would also be advantageous. Hitherto, however, most workers have concentrated on mechanisms for the selection of organically rich particulates, and on commercially important species notably mussels and oysters. Some authors, including Yonge (1923, 1926a, b) and Ansell (1961), believed that particle size, and not chemical nature, was a major criterion for particle selection in *Mya*, *Ostrea* and *Venerupis*, respectively. Indeed, almost all authors agree that selection between particles takes place through ciliary action, especially the eulatero-frontal cilia (Moore, 1971; Vahl, 1973; Jørgensen, 1975; Møhlenberg and Riisgard, 1978). Particles are wafted into, or excluded from various tracts in the gills and palps, which co-operate in rejecting larger or heavier particles as pseudofaeces. Details of the hydromechemical mechanisms are not as yet argued, but there is little doubt of the sieving properties of the ostia. Thus, Lucus *et al.* (1986) found greatly reduced filtration efficiency in the uptake of 0.5 μm bacterioplankton by *M. edulis* compared with larger algae. The role of mucus in particle uptake and selection has been debated. Newell and Jordon (1983) believed that mucus was thixotropic and that its use was reduced by ciliary action within the grooves of the palps, but increased again at the free edge where particles were being rejected. Rubenstein and Koehl (1977) emphasized adhesive mucus trapping, but Jørgensen (1981), showed that in *Mytilus* at least, all ingested particles are in free suspension. Some authors, e.g., MacGinitie (1941), Korringa (1952), Jørgensen (1949, 1951, 1960), Chipman and Hawkins (1954) and Smith (1958), have even suggested that a mucous 'net' covers the ctenidia.

While there seems little doubt that inorganic particles are selectively rejected, e.g., Kiørboe *et al.* (1980, 1981), Kiørboe and Møhlenberg (1981), Newell and Jordon (1983), and that bivalves have the capacity to regulate local retention efficiency and water flow (Bayne, 1976; Winter, 1977; Hildreth and Mallet, 1980), there is less strong evidence (until now) that qualitative selection was possible. Hence, little attention has been given to the mechanisms involved, although it is known that adhesion or particle change might be important (Rubenstein and Koehl, 1977; LaBarbara, 1978; Silvester and Sleigh, 1984; Wright *et al.*, 1982). Dwivedy (1972) claims to have demonstrated chemoreceptors on the palps of *Crassostrea* which could differentiate between various chemicals. It should be remembered that on the scale of microalgal suspensions the viscosity of water becomes more important and convective processes less so. Each microalga will therefore carry an envelope of effusate which could be specifically recognized. Thus, Strickland (1972) demonstrated this effect in the context of copepod feeding, so that chemical recognition and response should not be excluded, whether on the gill surface, the palps, or in the ciliary tracts of the stomach.

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