PARTICLE SELECTION, INGESTION, AND ABSORPTION IN FILTER-FEEDING BIVALVES

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Abstract: Measurements were made of the clearance rate of six bivalve species each in the presence of mixed cell suspensions of the dinoflagellate Prorocentrum minimum (Pavillard) Schiller (clone Exuv), the diatom Phaeodactylum tricornutum Bohlin (clone Phaeo), and the cryptomonad flagellate Chroomonas salina (Wislouch) Butcher (clone 3C). Use of flow cytometry allowed estimation not only of the clearance rate of individual cell types, but also of their proportional occurrence in the pseudofaeces and faeces. It has been recognized that at least three mechanisms of selecting suspended particles may be present in isolation or in combination. These are: (a) preferential clearance on the ctenidia: Ostrea edulis L., for example, preferentially clears the dinoflagellate Exuv compared with similar sized cells of the diatom Phaeo and the cryptomonad flagellate 3C; (b) preingestive selection on the labial palps: the diatom Phaeo was consistently and preferentially rejected in the pseudofaeces of Ensis directus Conrad, Placopecten magellanicus (Gmelin) and Arctica islandica (L.), (c) differential absorption in the gut i.e., post-ingestive selection: of the mixed diet which was ingested, there is clear evidence of a preferential absorption of the cryptomonad flagellate 3C in the majority of the bivalves from which we obtained faecal material. The possibility of selective removal of particular components of the available food resource, especially in the case of our experiments with the cryptomonad flagellate 3C, suggests that such organisms may be quantitatively more important in the diet of bivalves than their relative abundance under natural conditions might lead us to suppose. The ability of the oyster Ostrea edulis to selectively clear the dinoflagellate Exuv from mixed cell suspensions also may have important implications in our understanding of how toxic dinoflagellates may be concentrated on the ctenidia of commercially significant bivalves.

Key words: Ensis directus; Mya arenaria; Placopecten magellanicus; Arctica islandica; Ostrea edulis; Crassostrea virginica; filtration; selective feeding; flow cytometry

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The efficiency of particle retention from algal suspensions, and its significance in the estimation of the energetics of growth has been widely studied in bivalve molluscs (for reviews, see Jørgensen, 1960, 1975a; Winter, 1978; Bayne et al., 1976a,b; Bayne & Newell, 1984). Most bivalve species retain particles \( \geq 4 \mu m \) diameter with an efficiency of 100% and retain particles of 1 \( \mu m \) diameter with a reduced efficiency of up to 50% (Haven & Morales-Alamo, 1970; Vahl, 1972a,b, 1973a,b; Jørgensen, 1975b; Møhlenberg & Riisgård, 1978; Palmer & Williams, 1980). There are, however, important and apparently adaptive differences between bivalves in their efficiency of retention of fine particles. Species, such as *Pecten opercularis* and *P. septemradiata*, have ctenidia that lack eulaterofrontal ciliary tracts, and which live on coarse deposits in open phytoplankton-dominated waters. They show a decrease in retention efficiency of particles \(< 7 \mu m \) and have a retention efficiency of only 20% at 1 \( \mu m \) (Møhlenberg & Riisgård, 1978; see also Moore, 1971; Owen & McCrae, 1976). Thus they appear to be structurally adapted to exploit the larger suspended particles, including algal cells, as a primary component of their diet.

Although the retention efficiency of large particles is clearly dependent partly on the ctenidium acting as a simple mechanical sieve, there is now a good deal of evidence that the capture of fine particles may depend not only on particle size, but on electrical charge and other factors (Rubenstein & Koehl, 1977; LaBarbera, 1978; Jørgensen, 1983; Silvester & Sleigh, 1984). These factors may allow capture of fine particles including bacterioplankton with a greater efficiency than would be predicted from the simple mechanical properties of the ctenidial filter itself. Some bivalves, especially those living near to bacteria-rich detrital sources, are able to retain even bacterioplankton with a high efficiency. Wright et al. (1982), for example, showed that the saltmarsh mussel *Geukensia demissa* was capable of the efficient clearance of natural bacterioplankton compared with *Mytilus edulis* and *Mya arenaria* which removed only the phytoplankton from mixed algal and bacterioplankton suspensions.

Berry & Schleyer (1983) have, in addition, shown that the mussel *Perna perna* is capable of removal of latex particles of 0.46 \( \mu m \) diameter from suspension. These correspond approximately to the mean diameter of the free-living coccolid bacteria which occur in the water column over a reef off Durban, South Africa, and which comprise a potentially significant food resource for the mussels. The ctenidium itself thus appears to act as a pre-ingestive site of particle selection in some bivalves in the sense that the spectrum of particles which can be retained appears to be an adaptation by the animals to exploit the local resource available in the water column, and in some bivalves, such as *Mytilus*, the proportion of particles which are trapped may vary with particle concentration in the water column (Famme & Kofoed, 1983).

A second potential site of pre-ingestive selection is the labial palps which sort material prior to ingestion. Loosanoff (1949) showed that the oyster, *Crassostrea virginica*, may be capable of selection of food material on a qualitative basis through rejection tracts
on the labial palps. More recently, Newell & Jordan (1983) have shown that when the oyster, C. virginica, was fed a mixed diet of silt of <32 μm diameter and the alga, Tetraselmis suecica, the oyster could reduce the concentration of the alga voided in the pseudofaeces by over 50% compared with levels in the food supply. They inferred that chemoselection of particles on the palps allowed preferential ingestion of algae compared with inert silt particles. Differential utilization of various algal species has also been demonstrated recently by Peirson (1983) in the scallop, Argopecten irradians. Hylleberg & Gallucci (1975) have also shown that as much as 97% of the dry weight of material removed from the surface sediments by the deposit-feeding bivalve Macoma nasuta is rejected as pseudofaeces, organic-rich material being preferentially ingested.

In contrast, Cucci et al. (in press) have shown that when specimens of Mytilus edulis were fed a mixed diet of the diatom Phaeodactylum tricornutum, the dinoflagellate Prorocentrum, and the cryptomonad flagellate Chroomonas salina, there was no differential clearance of cells from suspension. The proportion of each cell type rejected in the pseudofaeces also was similar to that in the original food supply, so that no pre-ingestive selection occurred, either at the ctenidial surface or on the labial palps, between the three different cell types. However, the cells of the cryptomonad were absent from the faeces whereas the diatom and dinoflagellate passed through the gut apparently without significant digestion. A post-ingestive selection of the cryptomonad flagellate thus occurred when the mussel was presented with a mixed diet of this type. Veligers of Mercenaria mercenaria also appear to be capable of post-ingestive and/or pre-ingestive selection of Isochrysis galbana (5.7 ± 0.6 μm) over inert latex spheres of a similar diameter (Robinson, in press).

The purpose of the present study was to determine whether selective digestion as well as selective ingestion of suspended material occurs in other bivalves and to determine the relative involvement of palps and ctenidia in this selection process. A mixed cell suspension of Phaeodactylum tricornutum, Prorocentrum sp. and Chroomonas salina was presented to six species of bivalves. We have then analyzed the relative concentration of each cell type in the filtration media, the pseudofaeces and in the faeces by means of analytical flow cytometry as in previous experiments with Mytilus edulis (Cucci et al., in press). Our data thus add to the results summarized above insofar as we have investigated selection between different cell types, rather than between a single potential food source and inert suspended particles, and have included simultaneous estimates of pre-ingestive selection mechanisms and post-ingestive selection in these bivalves.

Materials and Methods

Specimens of the following bivalve molluscs were collected at various localities in Maine: Ensis directus Conrad (reared at the Ira C. Darling Center, University of Maine), Mya arenaria Linne (Long Cove, Searsport), Placopecten magellanicus (Gmelin) (lower Damariscotta River, Arctica islandica (L.) (Barter's Island, Sheepscot River), Ostrea
edulis L. (Dodge Cove, Damariscotta River) and *Crassostrea virginica* (Gmelin) (Damariscotta River, Walpole). Animals were transported to the laboratory immediately and scrubbed to remove all epiphytes. The animals were maintained in unfiltered, running sea water from Boothbay Harbor prior to use in experiments and the animals were not fed any supplementary food.

All animals were purged in filtered sea water (0.7 μm Gelman glass fiber) for 24 h prior to use in feeding experiments and the water was changed twice. All experiments were carried out at the same time of day (early a.m.) at 12 °C (ambient temperature at time of experiments). Individual specimens were placed in bell jars containing the algal culture mixtures (see below) which were gently aerated. Control vessels were left without animals to correct for algal cell division during experiments. Experiments lasted for 1 h, samples being taken for flow cytometry analyses at 30- and 60-min intervals. Any pseudofeces produced during the experiments were collected and analyzed. At the end of 1 h, the animals were removed from the feeding media and placed in filtered sea water (same as above) for faeces collection, which 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 cytometry measurements.

Algal cultures were supplied from the Culture Center for Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences and consisted of the following: the spindle shaped diatom *Phaeodactylum tricornutum* Bohlin (clone Phaeo) which is \( \approx 2.5-3.5 \times 12-23 \mu m \) in size; the dinoflagellate *Prorocentrum minimum* (Pavillard) Schiller (clone Exuv) which is \( \approx 5-6.15 \times 8.75-12.5 \times 12.5-15 \mu m \) in size, and the cryptomonad flagellate *Chroomonas salina* (Wislouch) Butcher (clone 3 C) which is \( \approx 6.25-7.5 \times 8.75-12.5 \mu m \) in size. Cultures were grown in f/2 media at 15 °C with a 14:10 photoperiod. All three clones were mixed just prior to the experiment to obtain equal cell densities with a final cell concentration of \( 10^4 \) cells \( \cdot \) ml \(^{-1} \) in each of the test jars.

Cells were analyzed by differences in their fluorescing intensities from the photosynthesizing pigments of chlorophyll (Phaeo, Exuv, 3C) and phycoerythrin (3C). To analyze the samples, a Coulter Epics V Flow Cytometer/Sorter with a single argon ion 5-W laser was used having an excitation wavelength of 514 nm with 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 spectral region \( > 630 \) nm such as would result from chlorophyll emission, and the other receives shorter wavelengths (530–560 nm) such as would result from phyocerythrin and phycocyanin emission. The events (number of cells) registered met gate criteria based on chlorophyll fluorescence, therefore only algal cells were analyzed. (See Yentsch et al., 1983 and in press, for a complete description of flow cytometry methodology.) A total of 2000 cells were analyzed for each sample, with the total being partitioned among the three clones. Since all samples were run at a constant flow rate throughout an entire experiment, we were able to calculate the clearance rate of each individual by the differences in the
amount of time required to analyze 2000 cells (initial analysis times averaged ≈ 300 s; after 60 min, average analysis times ranged from 500 to >1000 s). All bivariate histograms are on the same scale and show number of cells analyzed plotted with increasing fluorescence approximating phycoerythrin (X-axis) and increasing fluorescence approximating chlorophyll (Y-axis).

RESULTS AND DISCUSSION

The concentrations (cells · ml⁻¹) of the three cell types, referred to as "Phaeo", "3C" and "Exuv" (see p. 80), at time zero, after 30 min, and after 60 min are summarized in Table I. Each point is the mean of six vessels, each containing one bivalve, and the standard deviation is shown in parentheses. The percentage of the total cell count represented by each of the cell types at each time interval is also shown. From these data, some estimates can be made both of the clearance rate and the relative retention of each cell type at the ctenidial surface.

CLEARANCE RATE

One difficulty with estimates of the clearance rate of particles from a fixed volume of medium is that the experimental organism is likely to be subject to a continuous decline in particle concentration with time, depending on the clearance rate and the volume of medium used.

Coughlan (1969) suggested an exponential model for the calculation of filtration rate from an observed decrease of cells in suspension. Although this method of calculation has been widely used in the past, Williams (1982) pointed out that it has serious drawbacks when applied to mixed particle spectra with differing retention efficiencies. Larger particles are likely to be removed first, leading to a progressive increase in the proportion of small particles and an apparent decline in the filtration rate. Similar objections apply to systems maintaining a constant concentration of cells (Winter, 1973) where a mixture of cells in the original proportions is added to an experimental medium which is subject to a preferential removal of the large size fraction.

The problem can be minimized if a relatively large volume of suspension medium is used, and if the system for measuring cell concentration is sufficiently sensitive to avoid the necessity of a major decrease in cell concentration with time. In the case of the measurements summarized in Table I, we were able to record a reduction in cell concentration with time, and hence to estimate clearance rate without a major decrease in residual cell concentration. Regressions, based on time required to analyze 2000 events at the beginning and end of experiments, are summarized in Table II.

From these, we can make some estimates of the cell clearance rates for each bivalve. We also can calculate minimum values for the "irrigation rate" (\(\dot{V}_w\)) by assuming 100% particle retention efficiency (Newell, 1979). These rates can be obtained both from the
### Table I

Mean values of cell concentration (cells·ml⁻¹) of "Phaeo", "3C" and "Exuv" at time zero, after 30 min and after 60 min in experimental vessels containing individual *Arctica*, *Ensis*, *Placopesten*, *Crassostrea*, *Ostrea* and *Mya*: volume in vessels indicated in parentheses under species names; each point is the mean of six vessels each containing one bivalve; the percentage of the total cell count represented by each of the cell types at each time interval is also shown; SD in parentheses.

<table>
<thead>
<tr>
<th>Bivalve</th>
<th>Time (min)</th>
<th>Phaeo</th>
<th></th>
<th>Exuv</th>
<th></th>
<th>3C</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cells·ml⁻¹</td>
<td></td>
<td>Percent of total cell count</td>
<td>Percent of total cell count</td>
<td>Cells·ml⁻¹</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>× 10⁴</td>
<td></td>
<td>± SD</td>
<td></td>
<td>× 10⁴</td>
<td></td>
</tr>
<tr>
<td><em>Arctica</em></td>
<td>0</td>
<td>7.71 (0.55)</td>
<td>36.0 (1.63)</td>
<td>7.45 (0.38)</td>
<td>34.0 (1.10)</td>
<td>6.71 (0.30)</td>
<td>30.8 (1.33)</td>
</tr>
<tr>
<td>(2000 ml)</td>
<td>30</td>
<td>7.67 (0.78)</td>
<td>37.2 (0.75)</td>
<td>6.72 (0.62)</td>
<td>32.3 (0.52)</td>
<td>6.25 (0.51)</td>
<td>30.3 (0.82)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>6.08 (2.12)</td>
<td>38.3 (1.97)</td>
<td>5.03 (2.18)</td>
<td>30.3 (4.46)</td>
<td>4.97 (1.69)</td>
<td>31.3 (3.99)</td>
</tr>
<tr>
<td><em>Ensis</em></td>
<td>0</td>
<td>6.69 (0.11)</td>
<td>33.5 (0.84)</td>
<td>6.85 (0.36)</td>
<td>34.2 (1.17)</td>
<td>6.51 (0.46)</td>
<td>32.3 (1.63)</td>
</tr>
<tr>
<td>(500 ml)</td>
<td>30</td>
<td>6.40 (0.62)</td>
<td>35.2 (1.17)</td>
<td>5.60 (0.57)</td>
<td>31.2 (0.98)</td>
<td>6.08 (0.67)</td>
<td>33.7 (1.37)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>6.06 (2.18)</td>
<td>36.2 (6.40)</td>
<td>5.10 (2.35)</td>
<td>29.5 (10.3)</td>
<td>5.78 (1.80)</td>
<td>34.5 (5.13)</td>
</tr>
<tr>
<td><em>Placopesten</em></td>
<td>0</td>
<td>7.36 (0.34)</td>
<td>37.0 (0.63)</td>
<td>7.09 (0.55)</td>
<td>35.7 (1.60)</td>
<td>5.45 (0.45)</td>
<td>27.3 (1.50)</td>
</tr>
<tr>
<td>(2000 ml)</td>
<td>20</td>
<td>2.74 (1.97)</td>
<td>40.2 (2.20)</td>
<td>2.92 (1.69)</td>
<td>30.5 (4.00)</td>
<td>2.72 (1.45)</td>
<td>29.0 (1.90)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1.52 (1.07)</td>
<td>43.0 (6.70)</td>
<td>1.24 (0.88)</td>
<td>32.0 (5.40)</td>
<td>1.01 (0.78)</td>
<td>25.0 (3.00)</td>
</tr>
<tr>
<td><em>Crassostrea</em></td>
<td>0</td>
<td>6.43 (0.41)</td>
<td>29.8 (1.70)</td>
<td>6.16 (0.32)</td>
<td>28.5 (1.40)</td>
<td>9.14 (0.33)</td>
<td>42.2 (1.50)</td>
</tr>
<tr>
<td>(2000 ml)</td>
<td>30</td>
<td>6.54 (1.30)</td>
<td>30.7 (2.10)</td>
<td>5.82 (1.21)</td>
<td>27.2 (0.75)</td>
<td>8.72 (1.67)</td>
<td>41.7 (1.07)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>5.55 (1.72)</td>
<td>31.2 (0.98)</td>
<td>4.88 (1.44)</td>
<td>27.7 (1.63)</td>
<td>7.37 (2.37)</td>
<td>41.2 (1.37)</td>
</tr>
<tr>
<td><em>Ostrea</em></td>
<td>0</td>
<td>10.14 (0.66)</td>
<td>31.2 (1.48)</td>
<td>9.70 (0.38)</td>
<td>30.0 (1.00)</td>
<td>12.57 (0.54)</td>
<td>38.8 (0.84)</td>
</tr>
<tr>
<td>(2000 ml)</td>
<td>30</td>
<td>7.88 (2.51)</td>
<td>37.3 (6.70)</td>
<td>4.10 (2.95)</td>
<td>18.5 (8.00)</td>
<td>9.74 (2.74)</td>
<td>44.3 (3.30)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>5.12 (3.81)</td>
<td>39.0 (8.10)</td>
<td>3.12 (3.59)</td>
<td>18.3 (7.80)</td>
<td>6.40 (5.10)</td>
<td>43.0 (6.90)</td>
</tr>
<tr>
<td><em>Mya</em></td>
<td>0</td>
<td>10.66 (0.17)</td>
<td>29.3 (1.60)</td>
<td>15.73 (1.18)</td>
<td>28.8 (0.98)</td>
<td>10.73 (0.21)</td>
<td>42.0 (2.00)</td>
</tr>
<tr>
<td>(1000 ml)</td>
<td>30</td>
<td>10.66 (0.41)</td>
<td>32.7 (2.40)</td>
<td>11.72 (1.30)</td>
<td>30.2 (1.30)</td>
<td>9.82 (1.47)</td>
<td>37.2 (2.00)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>7.55 (1.60)</td>
<td>33.0 (2.20)</td>
<td>9.43 (3.53)</td>
<td>29.0 (1.20)</td>
<td>6.91 (2.09)</td>
<td>38.2 (3.10)</td>
</tr>
</tbody>
</table>
Regression equations for cell concentration versus time in the presence of various bivalve species:

\[ Y = a + bX \]

where \( Y \) = cells \( \cdot \) ml\(^{-1} \times 10^4 \) and \( X \) = time in min; \( N \) in each case = 36.

### Table II

<table>
<thead>
<tr>
<th>Food source</th>
<th>Phaeo ( a + bX )</th>
<th>( r^2 )</th>
<th>Exuv ( a + bX )</th>
<th>( r^2 )</th>
<th>3C ( a + bX )</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arctica</td>
<td>7.97-0.027</td>
<td>0.768</td>
<td>7.61-0.040</td>
<td>0.950</td>
<td>6.85-0.029</td>
<td>0.931</td>
</tr>
<tr>
<td>Ensis</td>
<td>6.70-0.011</td>
<td>0.998</td>
<td>6.72-0.029</td>
<td>0.941</td>
<td>6.49-0.012</td>
<td>0.989</td>
</tr>
<tr>
<td>Placopecten</td>
<td>7.13-0.097</td>
<td>0.981</td>
<td>6.68-0.098</td>
<td>0.943</td>
<td>5.28-0.074</td>
<td>0.983</td>
</tr>
<tr>
<td>Crassostrea</td>
<td>6.61-0.015</td>
<td>0.658</td>
<td>6.26-0.021</td>
<td>0.932</td>
<td>9.30-0.030</td>
<td>0.916</td>
</tr>
<tr>
<td>Ostrea</td>
<td>10.22-0.084</td>
<td>0.997</td>
<td>8.93-0.110</td>
<td>0.859</td>
<td>12.66-0.103</td>
<td>0.998</td>
</tr>
<tr>
<td>Mya</td>
<td>11.16-0.052</td>
<td>0.764</td>
<td>15.44-0.105</td>
<td>0.976</td>
<td>11.06-0.064</td>
<td>0.916</td>
</tr>
</tbody>
</table>

The numbers of cells of each type cleared per hour for each of the six bivalve species are summarized in Table III. The total clearance \( \cdot \) h\(^{-1} \) (i.e., the yield at that ration level) and the weight-specific yield are also shown. It can be seen that, as would be anticipated from the relation between filtration rate and body size in many invertebrates including bivalves (Jørgensen, 1975b; Newell, 1979), small specimens of Ensis (0.083 g dry tissue) cleared as many as \( 1 \times 10^6 \) cells \( \cdot \) ml\(^{-1} \cdot \) h\(^{-1} \) whilst the largest of the bivalves, Arctica (3.6 g dry tissue) removed only \( 3 \times 10^4 \) cells \( \cdot \) ml\(^{-1} \) after 60 min.

Table IV shows the clearance rate (cells \( \cdot \) \( 1^{-1} \cdot \) h\(^{-1} \times 10^5 \)) and the minimum values for the irrigation rate (\( \dot{V}_w \), ml \( \cdot \) h\(^{-1} \)) assuming 100\% particle retention efficiency. Values for \( \dot{V}_w \) calculated from Equation (1) are followed in parentheses by those estimated from the linear regressions shown in Table II. The minimum weight-specific irrigation rate (\( \dot{V}_w \) \( \cdot \) g\(^{-1} \)), assuming 100\% retention efficiency, is also shown.

Except in the case of Placopecten, where a major decrease in particles occurred within 60 min in the experimental medium (Table I), there are substantial differences between the irrigation rates estimated from the linear regressions and those calculated from the exponential model of Coughlan (1969) (Table IV). Values fell generally within the range reported for other bivalves (Jørgensen, 1975a).
### TABLE III

The decline in cell counts (cells·ml⁻¹) per litre of sea water containing *Arctica, Ensis, Placopecten, Crassostrea, Ostrea* and *Mya* after 1 h: values calculated from the difference between cell counts at zero time and after 60 min (calculated from the regressions shown in Table II and corrected for sea-water volume).

<table>
<thead>
<tr>
<th>Species</th>
<th>Dry tissue (g)</th>
<th>Reduction in cell concentration after 60 min (cells·ml⁻¹ × 10⁴)</th>
<th>Total (cells·ml⁻¹ × 10⁴)</th>
<th>Reduction in cell concentration per g tissue·h⁻¹ (cells·ml⁻¹ × 10⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Arctica</em></td>
<td>3.600 ± 0.162</td>
<td>3.18, 5.21, 2.56</td>
<td>10.95</td>
<td>3.04</td>
</tr>
<tr>
<td><em>Ensis</em></td>
<td>0.083 ± 0.03</td>
<td>3.02, 2.49, 2.88</td>
<td>8.39</td>
<td>101.08</td>
</tr>
<tr>
<td><em>Placopecten</em></td>
<td>2.590 ± 0.63</td>
<td>2.62, 1.59, 1.68</td>
<td>5.89</td>
<td>2.27</td>
</tr>
<tr>
<td><em>Crassostrea</em></td>
<td>1.860 ± 0.69</td>
<td>11.43, 10.00, 4.99</td>
<td>36.42</td>
<td>19.58</td>
</tr>
<tr>
<td><em>Ostrea</em></td>
<td>1.898 ± 0.03</td>
<td>10.37, 4.66, 12.95</td>
<td>27.98</td>
<td>14.74</td>
</tr>
<tr>
<td><em>Mya</em></td>
<td>0.748 ± 0.11</td>
<td>8.04, 9.14, 7.22</td>
<td>24.40</td>
<td>32.62</td>
</tr>
</tbody>
</table>

### TABLE IV

Clearance rate and minimum values for $\dot{V}_w$ and $\dot{V}_w'$ (irrigation rate and weight·specific irrigation rates, respectively) estimated assuming 100% retention efficiency of Phaeo, 3C and Exuv: calculated from Tables I and III, where $\dot{V}_w = \frac{M}{n} \cdot \log_e \left( \frac{\text{conc}_i}{\text{conc}_0} \right)$ (see Equation 1); values in parentheses are estimates for $\dot{V}_w$ calculated from the linear regressions in Table II.

<table>
<thead>
<tr>
<th>Species</th>
<th>Dry weight (g)</th>
<th>Ration (cells·ml⁻¹ × 10⁴)</th>
<th>Clearance rate (cells·ml⁻¹·h⁻¹ × 10⁴)</th>
<th>$\dot{V}_w$ (ml·h⁻¹)</th>
<th>$\dot{V}_w'$ (ml·h⁻¹·g⁻¹)</th>
<th>Coughlan vs. linear regression* (%)</th>
<th>$\dot{V}_w'$ (ml·h⁻¹·g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Arctica</em></td>
<td>3.600</td>
<td>2.19</td>
<td>1095</td>
<td>615 (479)</td>
<td>78 (170.9)</td>
<td>479</td>
<td>170.9</td>
</tr>
<tr>
<td><em>Ensis</em></td>
<td>0.083</td>
<td>2.00</td>
<td>839</td>
<td>84.2 (53.3)</td>
<td>63 (1014.6)</td>
<td>53.3</td>
<td>1014.6</td>
</tr>
<tr>
<td><em>Placopecten</em></td>
<td>2.590</td>
<td>1.99</td>
<td>589</td>
<td>3272.3 (3157.2)</td>
<td>95 (1289.6)</td>
<td>3157.2</td>
<td>1289.6</td>
</tr>
<tr>
<td><em>Crassostrea</em></td>
<td>1.860</td>
<td>2.17</td>
<td>3642</td>
<td>396.2 (292.2)</td>
<td>74 (213.0)</td>
<td>292.2</td>
<td>213.0</td>
</tr>
<tr>
<td><em>Ostrea</em></td>
<td>1.898</td>
<td>3.24</td>
<td>2798</td>
<td>1594.3 (1002.2)</td>
<td>63 (839.1)</td>
<td>1002.2</td>
<td>839.1</td>
</tr>
<tr>
<td><em>Mya</em></td>
<td>0.748</td>
<td>3.71</td>
<td>2440</td>
<td>439.7 (364.3)</td>
<td>83 (586.3)</td>
<td>364.3</td>
<td>586.3</td>
</tr>
</tbody>
</table>

* Percentage of $\dot{V}_w$ from Coughlan's formula represented by $\dot{V}_w'$ from linear regressions.
Differential Clearance, Ingestion, and Absorption

The relative proportions of each of the three cell types Phaeo, Exuv and 3C in the experimental media at time zero, after 30 min and 60 min are shown in Table I and Fig. 1. The relative proportions in pseudofaeces and in faeces collected in filtered sea water after 2–3 h in the experimental vessels are also shown in Fig. 1.

From this result, some obvious differences in the relative proportions of the cells occur. In the case of *Ostrea edulis*, for example, there was a preferential retention of the dinoflagellate Exuv compared with Phaeo or 3C. Again, the pseudofaeces of *Arctica*, *Placopecten*, *Ensis*, and *Ostrea* all showed that a much higher proportion of Phaeo was
rejected than that of Exuv or 3C. This suggests the presence of pre-ingestive selection at the labial palps, much as described for the distinction between Tetraselmis cells and inert particles in Crassostrea virginica by Newell & Jordan (1983) and as inferred by Loosanoff (1949).

Finally, there were marked differences in the proportion of cell types emerging in the faeces. In the case of Arctica, Placopecten and Ostrea, there was no evidence of Phaeo cells in the faeces. Presumably, these had been mainly rejected prior to ingestion on the gills or the labial palps, as indicated by the high proportion of Phaeo cells in the pseudofaeces. The 3C cells appeared to be mainly or entirely absorbed during passage through the gut, only a small proportion being rejected in the faeces. Cells of Exuv were, however, dominant in the faeces.

This suggests that although all the bivalves showed a clearance of the suspended cells (Table IV), these may not have been utilized uniformly. In Arctica, Placopecten, and Ostrea, cells of Phaeo are mainly rejected at the palps prior to ingestion, Exuv is mainly undigested and is rejected in the faeces, whilst of the three potential food sources, the cryptomonad flagellate 3C is preferentially removed during passage through the gut. The results reported here clearly resemble those reported for Mytilus edulis by Cucci et al. (in press) and suggest that some cells, such as 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.

We were not able to obtain pseudofaeces from Mya arenaria at the ration level used, but from analysis of the faeces, it is clear that Phaeo was evidently ingested and that a relatively high proportion of Exuv and Phaeo was ejected in the faeces. The cryptomonad flagellate 3C was again preferentially removed during passage through the gut. Crassostrea also shows complete removal of 3C during passage through the gut with egestion of some Exuv and, above all, Phaeo cells. Since there appears to be no pre-ingestive selection between the three cell types in Crassostrea, it seems that post-ingestive preferential digestion of the 3C primarily controlled dietary intake.

From these results, there appear to be at least three different mechanisms of selection which may be present in isolation or in combination.

**Preferential clearance on the ctenidia**

Fig. 2 shows a bivariate histogram of events and log integrated green fluorescence \((X = \text{fluorescence due to phycoerythrin})\) versus log integrated red fluorescence \((Y = \text{fluorescence due to chlorophyll})\) for time zero and after 60 min in the experimental sea water 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.

This result is of interest since the ability of many commercially significant bivalves, including Ostrea edulis, to accumulate toxic dinoflagellate cells preferentially from the
SELECTIVE FEEDING IN BIVALVES

plankton is well-known, although the mechanisms by which this is accomplished are not yet clear.

Sorting on the palps, i.e. rejection in the pseudofaeces

Fig. 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 min in experimental sea water in the presence of a specimen of *Arctica islandica*. A similar histogram for the pseudofaeces resuspended in filtered sea water 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 or the cryptomonad flagellate 3C. This indicates that pre-ingestive selection between the three cell types occurs on the labial palps in *Ensis, Placopecten* and *Arctica*.

No differential clearance or sorting on the palps, but differential absorption in the gut i.e., post-ingestive sorting

Fig. 4 shows a bivariate histogram of events and log integrated green fluorescence (X) versus log integrated red fluorescence (Y) at time zero and after 60 min in the experimental sea water in the presence of a specimen of *Crassostrea virginica*. The corresponding plots for pseudofaeces and faeces resuspended in filtered sea water are also shown.
Fig. 3. Bivariate histogram plots of number of cells and $X = $ fluorescence approximating phycoerythrin vs. $Y = $ fluorescence approximating chlorophyll comparing relative cell numbers within an algal mixture of Phaeo, 3C and Exuv due to the grazing by *Arctica islandica* at time 0 min (A), 60 min (B) and within the pseudofaeces (C).

There is evidently no preferential clearance of any one cell type on the ctenidium since after 60 min of filtration the proportion of the three cell types is similar to that at time zero. The proportions of cell types in the pseudofaeces suggests 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 Fig. 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 & Gallucci, 1975;
Newell & Jordan, 1983). The ability to exploit 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 & McCrae, 1976; Möhlenberg & Riisgoârd, 1978). However, species from detritus-dominated habitats where the suspended bacterial resource is high, may show an efficient clearance of bacterioplankton compared with other bivalves (Wright et al., 1982; Berry & 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 & Bayne, 1972).
The results which we have presented, and those which we have published in an earlier paper on *Mytilus edulis* (Cucci et al., in press) suggest that particle selection in several bivalve species may occur both prior to ingestion and during passage through the gut. We are also able to confirm the early suggestion of Loosanoff (1949) that bivalves may be capable of selection of food on a qualitative basis through rejection tracts on the palps.

The cells which were used in our experiments though not identical in size, were sufficiently similar to reduce the likelihood of an obvious selection on a basis of particle size. Nevertheless, preferential clearance of cells of the dinoflagellate Exuv after 60 min of filtration by the oyster *Ostrea edulis* can be clearly seen from Fig. 2. The second and major site of pre-ingestive selection is at the labial palps where material is sorted following transfer from the ctenidia. Of the three cells supplied, the diatom Phaeo was consistently and preferentially rejected in the pseudofaeces of *Ensis directus*, *Placopesten magellanicus*, and *Arctica islandica*.

Finally, of the mixed diet which is ingested, there is clear evidence of a preferential absorption of the cryptomonad flagellate 3C in the majority of the bivalves from which we obtained faecal material. In some cases, as in *Crassostrea virginica*, post-ingestive selection of 3C appears to be the sole method of differential utilization of these three cell types, although, of course, in the presence of other types of suspended particles pre-ingestive selection may also occur.

The occurrence of several sites at which preferential ingestion of particular components of the suspended food resource can occur, and the likelihood of differential digestion and egestion of components of the ingested ration, may be of considerable importance where co-existing suspension-feeders, particularly juveniles and adults of the same species, are likely to be competing for a common suspended food resource. The possibility of selective removal of particular components of the available food resource, especially the cryptomonad 3C, also suggests that such organisms may be quantitatively more important in the diet of bivalves than their relative abundance under natural conditions would lead us to suppose.

The ability of the oyster *Ostrea edulis* to selectively clear the dinoflagellate Exuv from mixed cell suspensions may, in addition, have important implications in our understanding of how toxic dinoflagellates may be concentrated on the ctenidia of commercially significant bivalves.

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REFERENCES


