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What makes diatoms attractive for suspensivores? The organic casing and associated organic molecules of *Coscinodiscus perforatus* are quality cues for the bivalve *Pecten maximus*

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A major question in the study of seston particle dynamics is the properties of particles which induce selection in suspension feeders. In order to determine whether the diatom organic casing and associated organic molecules (herein termed 'perifrustular envelope') influences selection by the Great Scallop Pecten maximus L., a mixed culture containing intact and naturally dead (empty frustules) Coscinodiscus perforatus was presented to normally feeding scallops. The presence of the envelope on all cells of the culture was verified using scanning electron microscopy. Proportions of intact and dead cells were determined via endoscopy-assisted sampling at strategic points in the particle processing chain. The use of C. perforatus as a food item was verified by microscopic examination of faeces at the end of the experiment. Selection indices were calculated to quantify eventual enrichment of empty, dead cell proportions at the rejection sites, in relation to the previous steps in the particle-processing chain. No significant difference was observed in the proportions of intact and dead cells at any of the sites, nor in the selection indices, and the large P-values suggest that both cell types were treated in the same manner by these actively feeding scallops ($P = 0.760$ and 0.839 for proportions and selection indices, respectively). Together with previous studies which have shown that empty, cleaned (perifrustular envelope absent) diatom frustules provoke rejection; these results strongly support the hypothesis that the presence of the external organic components is a major selection cue. This has implications for phytoplankton dynamics, notably with respect to the potential fates of species captured by this suspension feeder.

INTRODUCTION

Suspension-feeding bivalves may exert considerable influence on the seston dynamics of coastal and freshwater ecosystems (Officer *et al.*, 1982; Gili and Coma, 1998; Davenport *et al.*, 2000; Newell, 2004). Two aspects of bivalve suspension feeding will obviously be critical determinants of their interaction with the seston and its plankton populations: the overall rate of feeding (clearance rate) and the degree of particle selection for ingestion or rejection. There is a very large literature concerning the clearance rate, usually based upon laboratory studies using easily cultured phytoplankton species which are often not representative of the *in situ* populations (Bayne, 1998; Kraeuter and Castagna, 2001; Gosling, 2003). Similarly, a considerable number of studies relate to selection, and the majority of these focus on the types of easily cultured plankton or other seston particles which are preferentially retained by various bivalve suspensivores (Kiørboe and Møhlenberg, 1981; Fritz *et al.*, 1984; Shumway *et al.*, 1990; Prins *et al.*, 1991; Bougrier *et al.*, 1997; Defossez and Hawkins, 1997; Levinton *et al.*, 2002). However, despite the progress made recently in the understanding of suspension-feeding mechanisms in bivalves, as reviewed by Riisgård and Larsen (Riisgård and Larsen 2000, 2001)

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and Gosling (Gosling, 2003), and in the understanding of the transport processes involved in rejection (Beninger and St-Jean, 1997a,b; Beninger *et al.*, 1997a,b; Beninger and Veniot, 1999; Beninger *et al.*, 1999), the mechanisms of qualitative selection remain relatively obscure.

Characteristics such as particle size (Hughes, 1975; Shumway *et al.*, 1985; Defossez and Hawkins, 1997) and presence of ectocrines (Ward and Targett, 1989) have been shown to affect selection, and indirect evidence of qualitative selection based on organic content has been reported for several species (Newell and Jordan, 1983; Bayne *et al.*, 1993; Bacon *et al.*, 1998). Bivalve feeding organs may be exposed to thousands of particles per second, and it is difficult to imagine how so many selection decisions may be made so quickly; indeed, the individual quality detectors and effectors are as yet unknown. At present, the elucidation of indisputable general features of qualitative particle selection would constitute progress in this field.

Recently, evidence of the importance of particle composition in the selection process has been advanced. Ward *et al.*, (1998) demonstrated preferential retention of algal particles over *Spartina* sp. detritus in oysters; however, it may be argued that particle shapes were also different in this experiment, and that the oyster gill system does not give an unambiguous response (since particles sent to the ventral gill tract are not necessarily rejected). Stronger evidence for the importance of particle composition in qualitative selection has been provided by Cognie *et al.* (2003) in oysters and Beninger *et al.* (2004) in scallops, via endoscope-directed sampling in the pallial cavity, combined with the use of diatom particles which differed only in organic content: intact versus empty, cleaned frustules, and the inclusion of the labial palps as final decisional sites. In these bivalves, qualitative selection was shown to occur both at the heterorhabdic gills and at the labial palps. However, the selection choice was quite radical: intact cells versus empty, cleaned frustules. While being useful for determining the sites of selection, these results do not provide insights into the eventual diatom quality cues which determine selection.

Diatoms are a major dietary component of bivalves (Buley, 1936; Davis and Marshall, 1961; Shumway *et al.*, 1987), and among the obvious candidates for such selection cues is the organic casing and associated organic molecules (hereafter termed the perifrustular envelope) of these unicellular algae. Here, we demonstrate that the diatom perifrustular envelope alone is capable of preventing the negative selection that has been observed in *Pecten maximus* with empty, cleaned diatoms (Beninger *et al.*, 2004).

METHOD

Test algae culture and characteristics

With the exception of Cognie *et al.*, (2003) and Beninger *et al.*, (2004), previous studies on selection in bivalves have relied upon stock algal cultures which are relatively easy to culture in the laboratory but not necessarily representative of the types of algae present or dominant in the natural habitats of the various bivalves tested. For the present study, the naturally occurring *Coscinodiscus perforatus* was cultured (as in Beninger *et al.*, 2004) in order to test the effects of the presence or absence of the perifrustular envelope on qualitative selection in scallops. *C. perforatus* was isolated from a net tow in a natural population off Le Croisic, France (2°30′W and 48°17′N) in June 2000. The diatom was cultured in Guillard F/2 medium (Guillard, 1982); however, it was not possible to achieve large-volume culture or high densities under laboratory conditions.

Empty frustules

All diatom cultures contain some dead cells (empty frustules), which accumulate over the life of the culture. Hence, a senescent-phase culture of *C. perforatus* was used as a source of both empty and intact frustules. The culture contained a mixture of homogeneously sized intact (28%) and dead (empty, uncleaned frustules: 72%) cells. Dead cells were defined as those which were empty under light microscopic examination (Fig. 1). Cell dimensions were 96 ± 2 μm diameter and 74 ± 3 μm per valvar axis (light microscope measurements of 30 cells). There was negligible cell size decrease over the course of the experiments.

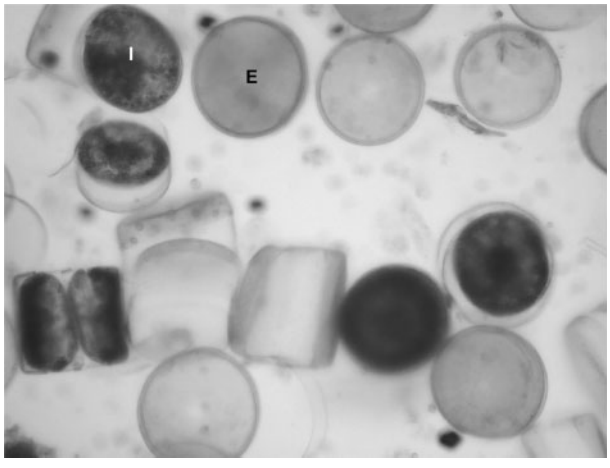

Fig. 1. Intact (I) and empty, dead (E) *Coscinodiscus perforatus* cells, as viewed under light microscope.

Unambiguous verification of the presence of the perifrustular envelope on the two cell types was only possible using scanning electron microscopy, since the envelope was not visible under light microscopy and not totally distinguishable from associated/adsorbed organic molecules using histochemical techniques. Sedimented samples of the culture were fixed in a solution of 2.5% glutaraldehyde in a hyperosmotic 0.1 M cacodylate buffer, rinsed five times in distilled water, freeze dried for 12 h, sputter coated with gold–palladium and observed using a JEOL 6400 scanning electron microscope (SEM).

Scallop sampling and maintenance

The five *P. maximus* (mean shell length 11.5 cm) used in the present study were collected by divers in the Baie de St-Brieuc, France (2°49′W and 48°38′N) in March 2002. The shells were cleaned of epibionts and maintained in a 400 L recirculating seawater tank, at a mean temperature and salinity close to that recorded at the sampling habitat. The specimens were fed 2–5 times weekly with a culture of *Skeletonema costatum* (Grev.) Cleve for a 4-week stabilization period prior to experimentation.

Experimental conditions

Within the 17 L experimental chamber, each scallop was fixed via a Velcro patch attached to an inclined platform and to a removable restraining collar on the scallop. The collar prevented both valve closure and further valve opening when the endoscope optical insertion tube (OIT) was inserted. The valve opening thus obtained was similar to that observed in normally feeding specimens. The design of the experimental chamber has been explained previously (Beninger *et al.*, 2004); briefly, it was provided with refrigerated, flow-through filtered (0.2 μm) seawater and contained two longitudinal separations, in order to decrease turbulence.

The large cell size of *C. perforatus* rendered numerical comparisons of experimental cell concentration with the majority of previous bivalve feeding studies impractical, as the latter use much smaller test species in spite of the frequent prevalence of large diatoms in the field. Hence, cell concentrations were determined on the basis of comparable organic matter concentrations. A calibration of cell numbers and organic matter (loss on ignition: 48 h at 60°C and 4 h at 450°C) was established using the *C. perforatus* culture. Particle concentrations were verified at six points in the experimental chamber in order to ensure homogeneous distribution.

Endoscopic observation and particle sampling

Individuals were fasted for 24 h and were allowed to acclimate to the presence of the OIT for 1 h prior to beginning observations. A suspension of 5–50 mg L^{-1} organic matter of the algal mixture, similar to the natural concentrations from the sampling site in the wild (Arzul *et al.*, 1990), was presented to normally feeding scallops in the experimental chamber. Endoscopy-directed sampling (Beninger *et al.*, 1992; Cognie *et al.*, 2003; Beninger *et al.*, 2004) was performed every 15 min throughout the 2 h experimental period in the chamber water column, the gill dorsal (acceptance) tract and the gill ventral (rejection) tract (Beninger *et al.*, 1992; Beninger *et al.*, 2004). Palp pseudofaeces were collected from the ventro-posterior extremity of the labial palps, where they accumulated over the course of the experimental period (valve clapping being impeded by the restraining collar). All samples were fixed in Lugol's solution for subsequent counts using light microscopy, according to the method described by Utermöhl (Utermöhl, 1958), allowing the ready distinction of intact (live) versus dead (empty, uncleaned) diatoms. The proportions of each cell type were determined for a minimum of 300 cells of the total volume sampled over the 2 h course of the experiment for each scallop.

Video sequences were digitized and stored directly on a computer using a miroVIDEO DC 1000 video card (Pinnacle Systems) and Adobe Premiere 5.0 Image Software (Adobe Systems).

The use of *C. perforatus* cells as food items by these specimens of *P. maximus* was confirmed by microscopic examination of stomach contents and faeces. Similarly, the structural integrity of the gill of each scallop specimen was verified histologically (5 μm paraffin sections, Masson's trichrome stain).

Data analysis

The null hypothesis was that no difference in particle proportions (intact versus empty, uncleaned) would be observed at any of the sampling sites (in which case the presence of the perifrustular envelope in the dead, uncleaned cells sufficed to have them treated as intact cells). The experimental hypothesis was that the dead, uncleaned frustules would be preferentially rejected (in which case the lack of cytoplasmic organic content would be a determining characteristic of negative qualitative selection). A selection index was calculated for successive pallial cavity sampling sites, in order to monitor potential enrichment in empty, uncleaned cells:

$$\mathrm{SI} = \left(\frac{S_n}{S_{n-1}} - 1\right) \times 100 \qquad (1)$$

where S_n is the proportion of intact cells sampled at a given site, and S_{n-1} is the percent of intact cells sampled at the previous step in the particle processing sequence (water, dorsal and ventral tracts, and palp pseudofaeces).

Uncleaned cells were chosen as the selection criterion because if the experimental hypothesis is true, this cell type should be found in increasing proportions at the sites concerned with rejection. Arcsine transformations of the proportions were performed (Sokal and Rohlf, 1995) prior to a one-way parametric ANOVA on the normally distributed, homoscedastic data. The selection index data were treated in the same manner.

RESULTS

All scallops demonstrated active, normal feeding behaviour (tentacles and velum normally deployed, particle entrainment in inhalant current visible under endoscopy).

The perisfrustular envelope was present in all cells from the *C. perforatus* culture observed under SEM (Fig. 2). The extent of coverage of the envelope varied greatly between cells, with abundant evidence of breakage and exfoliation, presumably due to unavoidable mechanical disturbance during sample preparation (Fig. 2A and B). The dehydrated envelope measured ~0.6 μm in thickness, and in specimens which did not suffer extensive breakage, it appeared as a uniform sheet covering the frustules (Fig. 2C and D). It was not possible to identify associated mucopolysaccharides visually in these micrographs.

The results of the ANOVAs, both for the proportions of cells and for the selection indices at the various experimental sampling sites, showed no significant differences (Figs 3 and 4). Indeed, the *P*-values were extremely large ($P = 0.760$ and 0.839 for proportions and selection indices, respectively), confirming the essential equality of all values. No selection between intact and empty, uncleaned cells was thus apparent at any of the particle-processing sites in this scallop.

Examination of stomach contents showed both types of cells (intact and empty, uncleaned) were ingested by the experimental scallops, and examination of faeces confirmed that *C. perforatus* was defecated. The histological sections showed that all gills were structurally intact, confirming the behavioural observations of normal feeding.

DISCUSSION

Previous experimental studies on the particle cues which influence selection by bivalves have focussed on size or on particles of very heterogeneous natures, such as algal particles and *Spartina* sp. detritus (Ward *et al.*, 1998). Although larger seston particles (>20 μm) have been shown to contain a lower organic fraction than smaller particles and to be preferentially rejected by *Mytilus edulis, Tapes (Ruditapes) philippinarum*, *Tapes decussatus* and *Abra spp.* (Hughes, 1975; Defossez and Hawkins, 1997), it should be remembered that these bivalve species all possess homorhabdic gills which function quite differently from the heterorhabdic gills of scallops and oysters. The principal filament openings of *P. maximus* are quite large (~200 μm) and are extensible (Beninger and

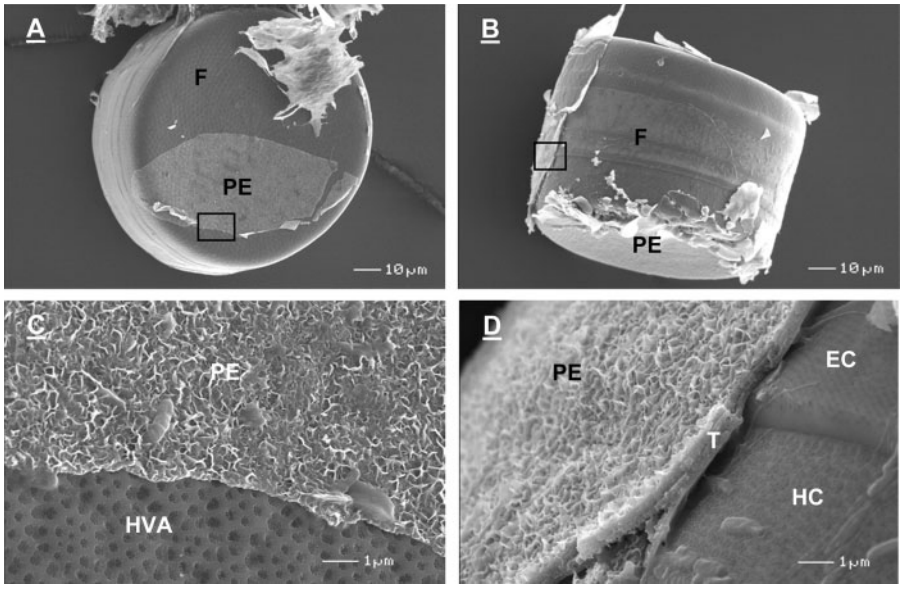

Fig. 2. Scanning electron micrographs of *Coscinodiscus perforatus* cells. (**A**) Valve view and (**B**) girdle view of frustules (F) partially covered by the perifrustular envelope (PE). The black rectangle indicates location of detailed enlargements in **C** and **D**. (C) Details of the external, dehydrated surface of the envelope. (D) Transverse view of the envelope, showing thickness (T). EC, epicingulum; HC, hypocingulum; HVA, hypovalve areolation.

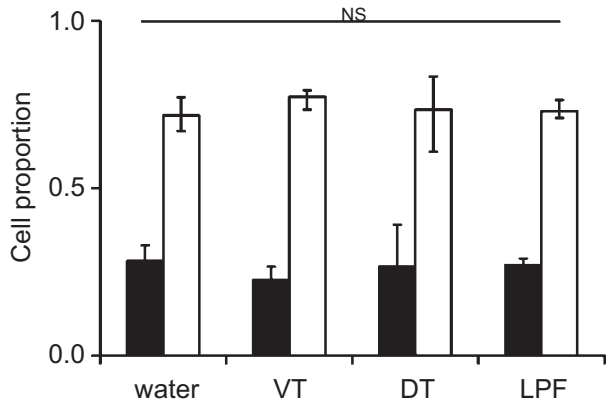

Fig. 3. *Coscinodiscus perforatus* intact and naturally dead, empty cells. Mean percentages of intact (black bars) and naturally dead, empty cells (white bars) from the different sampling sites: water, dorsal tracts (DT), ventral tracts (VT) and labial palp pseudofeces (LPF). Error bars represent the range of values (n = 3). NS: not significant ($P \gg 0.05$).

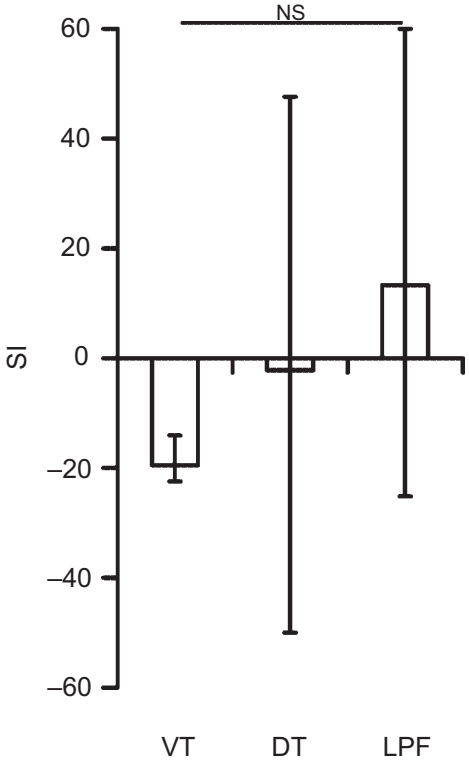

Fig. 4. Mean selection index (SI) at the different sites: dorsal tracts (DT), ventral tracts (VT) and labial palp pseudofaeces (LPF). Error bars represent the range of values (n = 3). NS: not significant ($P \gg 0.05$).

Le Pennec, 1991; Beninger *et al.*, 2004), allowing access to large particle sizes, such as the *Coscinodiscus* species which may be quite abundant in littoral waters (Levinton, 1982; Lakshminarayana, 1983; Barnes and Hughes, 1988; Rincé, 1993; Beninger *et al.*, 2004); moreover, indirect techniques suggest that size does not affect selection in the scallop *Placopecten magellanicus* (MacDonald and Ward, 1994). The results of Beninger *et al.* (2004) and the present study show that *C. perforatus* is ingested by *P. maximus*, despite its large size.

The data of the present study lead to the acceptance of the null hypothesis: the actively-feeding scallops made no distinction between intact and empty, uncleaned diatom cells. This is in contrast to their treatment of empty, cleaned *C. perforatus* frustules whose addition to the natural culture provokes an increase in the proportion of empty cells at the rejection sites: the gill ventral bend and the ventro-posterior extremity of the labial palps (Beninger *et al.*, 2004). The external organic molecules of the diatom thus constitute an unconditional quality cue for the scallop feeding structures, independent of cell organic content. Given the organically rich and diverse composition of the organic casing and associated exopolymers (Volcani, 1981), it may be that it is adaptively advantageous for scallops to treat both cell types as food items. Such a strategy would procure the additional advantage of simplifying somewhat the extremely complex task of individually sorting very large numbers of particles at an extremely high speed. To date, no data are available from any study to indicate the fine-scale locations and characteristics of the sensory receptors responsible for selection at the selection sites: gill and labial palps.

The acceptance of both intact and empty diatoms as food items may be critical to maintaining the Type 1 feeding response (positive linear consumption response to increasing particle concentrations, with a well-defined plateau at the maximum rate) which is unique to filter feeders and common among bivalves. This is the only feeding response type in the animal kingdom which achieves maximum consumption and efficiency, due to a high capture success rate and minimal handling time (Jeschke *et al.*, 2004).

The persistence of the perifrustular envelope in dead cells, when the cellular content has been entirely degraded, is intriguing. However, the presence of sulphated polysaccharides in both the casing and associated exopolymers (Duke and Reimann, 1977; Volcani, 1981; Bhosle *et al.*, 1995) no doubt stabilizes the envelope, and this same class of molecules is known to possess bacteriostatic properties (Sasikala and Subramoniam, 1987; Subramoniam, 1991). Our own unpublished histochemical observations of the perifrustular envelope of naturally occurring diatoms stained *in toto*, support the observations of the previous studies. It is thus likely that dead cells surrounded by their perifrustular envelope accumulate in the pelagic littoral environment, as diatom populations increase, peak and decline. Although such dead cells do not represent as nutritious a particle type as the corresponding intact cells,

they do represent a potentially abundant source of organic matter and energy. In addition, it must be remembered that these external organic compounds are more readily available for digestion, compared to the cytoplasm within the frustules.

Although these results do not provide insight on the selection effect of removing only the external organic components, while maintaining cytoplasmic content, such a procedure might be envisaged in future work using enzymatic degradation. This is likely to be rather difficult to achieve, however, given the extremely heterogeneous and complex assortment of organic molecules which compose the envelope (Duke and Reimann, 1977; Volcani, 1981).

The present study clearly demonstrates that the presence of the perifrustular envelope allows even empty diatom cells to be treated as intact cells by *P. maximus*, and hence the importance of these substances as quality cues in particle selection by scallops. The envelope thus increases the ingestion probability for both intact and dead cells. Since the exact composition of the envelope varies between diatom species (Volcani, 1981), this may constitute a basis for qualitative discrimination of mixed diatom populations. While most ingested diatoms will be at least partially digested, and hence removed from the diatom population dynamics, some cells may survive passage through the digestive system and exit in a viable state within highly mucoid faeces (Barillé and Cognie, 2000). Similarly, rejected material is ejected from the pallial cavity in a viscous mucus (Beninger and Veniot, 1999; Beninger *et al.*, 1999). The ultimate importance and fate of both of these categories of live diatoms is a subject for potentially fruitful, if complex, future research.

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