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Localization of qualitative particle selection sites in the heterorhabdic filibranch *Pecten maximus* (Bivalvia: Pectinidae)

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ABSTRACT: Particle-processing, and specifically qualitative particle selection, was studied in *Pecten maximus* using intact and empty versions of the naturally occurring diatom *Coscinodiscus perforatus*. Endoscope-directed sampling allowed comparison of particle proportions or numbers in the experimental medium, dorsal and ventral gill tracts, and labial palp pseudofeces. The general processing of particles was closely similar to that previously described for the more primitive scallop *Placopecten magellanicus*, despite the large phylogenetic distance between these 2 species. The gill and the labial palps of *Pecten maximus* each effected particle selection (rejection of empty, cleaned frustules), both in the presence of intact *C. perforatus* particles, and when only empty, cleaned frustules were presented. Particle selection operated even when small proportions (approx. 10%) of empty, cleaned *C. perforatus* frustules were present in the particle mixture. This represents the first direct experimental demonstration of the sites of qualitative particle selection in a heterorhabdic filibranch bivalve, and shows the process to be located in both the gills and the labial palps. The qualitative selection capacity of the heterorhabdic gill could be the result of either early evolution in turbid environments, or an adaptation which avoids overloading qualitative selection of the palps in this gill type.

KEY WORDS: Bivalves · Pectinidae · Feeding · Selection · *Coscinodiscus* · Endoscopy

INTRODUCTION

In coastal areas, where suspensivorous bivalves are abundant in the benthic habitat, they can be important determinants of seston dynamics, both in natural (Officer et al. 1982, Gili & Coma 1998, Davenport et al. 2000, Lehane & Davenport 2002) and in domesticated (Soto & Mena 1999, Lefebvre et al. 2000) ecosystems. In coastal waters, suspensivorous bivalves can be major couplers between the benthic and pelagic ecosystems, contributing to the regulation of phytoplankton biomass (Cloern 1982, Hily 1991, Asmus & Asmus 1993). Considerable progress has been made in understanding the underlying mechanisms of particle-processing in these organisms (see Beninger & St-Jean 1997, Beninger & Veniot 1999, Riisgård & Larsen 2000, 2001, Gosling 2003 for reviews and references), although a certain amount of debate persists concerning particle capture (Ward et al. 1998a, Silverman et al. 1999).

Bivalve feeding has been abundantly and quantitatively studied from the standpoint of the results of this process: clearance rates, retention efficiencies and formation of biodeposits (see Jørgensen 1990, Riisgård 2001 for reviews). Such studies employ various means of collection of inhalant and exhalent water and/or biodeposits (pseudofeces and feces), both in the laboratory (see review by Riisgård 2001) and, much less frequently, in the field (Cranford & Hill 1999, Yahel et al. 2003).

Studies of qualitative particle selection in bivalves have similarly focused on the results of this process, making inferences about the sites and mechanisms (Kiørboe & Møhlenberg 1981, Newell & Jordan 1983, Shumway et al. 1985). It has been assumed that, in homorhabdic bivalves (those whose gill is composed

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of a single filament type), the gill is generally not equipped to perform particle selection in addition to capture and transport, whereas the heterorhabdic gill (composed of 2 different filament types) provides an anatomical basis for qualitative selection (Atkins 1937, Beninger & St-Jean 1997). This assumption was recently supported by Ward et al. (1998b) using endoscopic-directed sampling at various sites in the pallial cavity of the oysters *Crassostrea virginica* Gmelin and *C. gigas* Thunberg (heterorhabdic gill type), and the mussel *Mytilus edulis* L. (homorhabdic gill type). As expected, the mussel gill was incapable of effecting particle selection (although the labial palps may effect such selection), while the oyster gill demonstrated a separation of detritus and live algal particles to 2 different particle grooves (dorsal and ventral). The demonstration was not free of confounding variables, however, since this study used very different-shaped particle types (unicellular alga vs *Spartina* sp. detritus). In addition, the oyster pseudolamellibranch gill is a very particular heterorhabdic gill type, in that it possesses a ventral particle groove and thus the possibility of a secondary positive or negative selection on the labial palps of material initially directed toward this tract. The oyster is therefore not the most unambiguous system in which to search for the sites and mechanisms of selection. Furthermore, it has been shown recently that particle size alone, irrespective of particle quality, is an important determinant of selection site in oysters (gills vs labial palps: Cognie et al. 2003).

The scallop family Pectinidae possesses a heterorhabdic gill with no ventral particle groove; all particles directed ventrally are rejected directly from the gill via the pallial current and valve-clapping (Beninger et al. 1992). In addition, the principal filament openings are much larger (approx. 200 µm) than those of the oyster (approx. 70 µm), and the combination of a low degree of tissue fusion and lateral wall musculature allows these openings to be easily expanded (Beninger & Le Pennec 1991, Beninger et al. 1992). The principal filaments of the larger scallop species can thus accommodate particles covering most of the sestonic size range, in contrast to the principal filaments of the oyster, which afford unimpeded entry only to particles whose axis in all dimensions is smaller than the rigid opening of their comparatively smaller principal filaments (Cognie et al. 2003). This extension of the upper particle size limit capable of being sorted on the gill, together with the comparable or even superior retention of small particles in scallops compared to oysters (Møhlenberg & Riisgård 1978, Riisgård 1988) and the lack of ambiguity about the significance of particle presence on the ventral gill tract, all make the scallop gill a much clearer system in which to study the sites of selection in heterorhabdic bivalves.

In a detailed account of particle processing in the scallop *Placopecten magellanicus* Gmelin (Beninger et al. 1992), no attempt was made to determine the sites and mechanisms of particle selection. In the present work, we used endoscope-directed sampling and the technique of live versus empty natural diatom particles described by Cognie et al. (2003) to study the following poorly known aspects of particle-processing in the scallop *Pecten maximus*: general features, location of selection sites, and the effect (if any) of the presence/absence of cellular contents on selection in naturally occurring particles that are identical in other respects. As test particle we used the locally abundant species *Coscinodiscus perforatus* Ehrenberg. Members of the coastal diatom genus *Coscinodiscus* are often abundant (Levinton 1982, Lakshminaryana 1983, Barnes & Hughes 1988, Rincé 1993), and are commonly found in the stomachs of bivalves, including scallops (Buley 1936, Davis & Marshall 1961, Shumway et al. 1987).

Pecten maximus is a relatively recent member of the Pectinidae, belonging to a group separated by 65 million yr from the more primitive group comprising *Placopecten magellanicus* (Waller 1991), whose particle-processing mechanisms have previously been studied (Beninger et al. 1992, Ward et al. 1993). A secondary goal of this study was therefore to determine whether particle-processing mechanisms, and associated anatomical organization of the gill, have remained similar throughout pectinid evolution.

MATERIALS AND METHODS

Specimen sampling and maintenance. The 3 *Pecten maximus* (mean shell length 11.5 cm) used in the present study were collected by divers from the Baie de St-Brieuc (2° 49′ W, 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 in the sampling habitat. The specimens were fed 2 to 5 times weekly with a culture of *Skeletonema costatum* (Grev.) Cleve (equivalent to a concentration of 5 mg l^{-1} organic matter individual^{-1} feed^{-1}) for a 4 wk stabilization period prior to experimentation.

Test algae: culture and characteristics. Naturally occurring *Coscinodiscus perforatus* was cultured to test the effects of natural algal quality on selection sites and mechanisms. *C. perforatus* was isolated from a net tow in a natural population off Le Croisic (2° 30′ W, 48° 17′ N) in June 2000. The diatom was cultured on Guillard (1982) F/2 medium; 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 during the life of the culture. Hence, senescent-phase cultures of *Coscinodiscus perforatus* were used as a source of empty frustules. The cells were recovered by centrifugation and placed in a saturated solution of hydrogen peroxide in an 80°C water bath, to produce cleaned, empty frustules of size and shape identical to those of intact cells. Verification under inverted microscope showed that frustule valves very rarely separated as a result of this treatment. These empty frustules were added to younger cultures, which themselves contained accumulations of empty cells (72% empty cells in the younger-phase cultures). The final proportions of empty and intact cells in the experimental mixture were 82% empty (of which 10% were empty, cleaned frustules), 18% intact. Cell dimensions of the mixture were 96 ± 2 µm diameter and 74 ± 3 µm pervalvar axis (light microscope measurements of 30 cells). Both types of culture originated from the same source culture, and there was negligible cell size reduction during the course of the experiments.

Gill morphology and anatomy. The following general features of gill organization were observed *in vivo* using endoscopy: filament types, plication, relative lengths of descending and ascending lamellae, ciliated spurs, concertina movements and other contractions. Gill filament internal anatomy and ciliation were observed via transverse histological sections, processed and stained as described in Beninger et al. (1995).

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 experimental chamber was provided with refrigerated, flow-through filtered (0.2 µm) seawater, and contained 2 longitudinal baffles to reduce turbulence (Fig. 1). The flow in the experimental chamber was fixed at approximately 20 l h^{-1}, for a turnover time of 1 h, thus avoiding a particle depletion of >30% (Barillé et al. 1993, based on a filtration rate of approx. 8 l h^{-1}). Over the course of the 1 mo stabilization period, the water temperature was progressively raised to 17°C, at which feeding activity was frequent. Salinity was maintained at 34, identical to that of the field sampling site. Particle concentrations were verified at 6 points in the chamber in order to ensure homogeneous distribution.

Endoscopic observation and particle sampling. Individuals were starved for 24 h, and were allowed to acclimate to the presence of the OIT for 1 h prior to beginning observations. Only individuals displaying normal feeding configuration (relaxed velum, deployed tentacles, particles drawn into pallial cavity) were used for observations. The general organization of the experimental apparatus was similar to that described by Beninger et al. (1992). A 4 mm diameter rigid OIT was used, since only straight dorso–ventral movements were required within the voluminous mantle cavity of this species. 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).

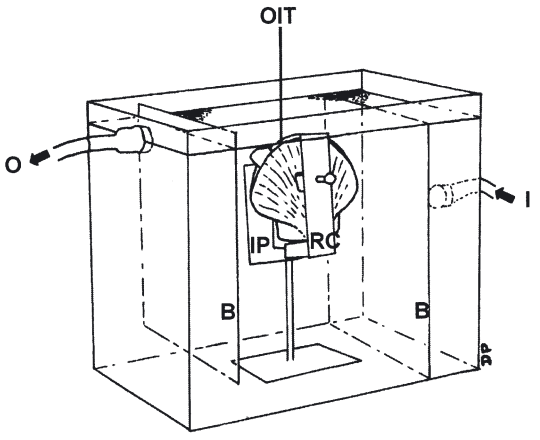

Fig. 1. Diagram of experimental chamber, showing *Pecten maximus* specimen with optical insertion tube (OIT), inclined platform (IP) and restraining collar (RC). B: baffles; I: inflow; O: outflow

The general treatment of particles was studied using cultures of *Coscinodiscus perforatus*, as well as added reflective particles (Afflair® 120 Lustre Satin Pearl Lustre Pigments, EM Industries) to better visualize particle behavior, mucus cords and suspensions.

We investigated 2 selection conditions: in Condition 1, a mixture of the exponential-phase *Coscinodiscus perforatus* culture and the empty, cleaned frustules was presented to determine whether and where selection takes place when nutritive and non-nutritive particles are present. In Condition 2, only empty, cleaned frustules were presented to the scallops to determine whether such particles are rejected if there is no other choice. A summary of the null and experimental hypotheses tested for each condition is presented in Table 1.

Endoscope-directed sampling of the gill arch (dorsal tract), ventral bend (ventral tract), and labial palps (Fig. 2) was performed with glass micropipettes (Fig. 3a) every 15 min throughout the 2 h experimental period. The water column was also sampled every 15 min using a volumetric pipette. Palp pseudofeces were collected from the ventro-posterior extremity of the labial palps, where they accumulated during the course of the experimental period (valve-clapping being impeded by the restraining collar). All samples

Table 1. *Pecten maximus*. Selection effects tested and corresponding experimental comparison, and hypotheses

Effect tested	Sites compared	Particle types presented and underlying hypotheses
Natural versus empty, cleaned *Coscinodiscus perforatus* frustules (Condition 1)		
Algal quality: poor versus high quality	Water and gill frustule proportions	H_0 (no selection at gill): cell proportions not significantly different in water and at dorsal and ventral gill tracts H_1 (selection at gill): dorsal tract enriched in intact frustules, ventral tract enriched in empty frustules; gill capable of quality selection from mixed suspensions
	Palp pseudofeces	H_0 (no further selection): frustule proportions of palp pseudofeces not significantly different from dorsal gill tract H_2 (further selection): palp pseudofeces enriched in empty frustules; palps capable of particle selection
Empty, cleaned *Coscinodiscus perforatus* frustules (Condition 2)		
Algal quality: poor quality only choice	Water and gill frustule concentrations	H_0 (no rejection at gill): frustule numbers in 2 ml sample significantly lower than those of empty cells in Condition 1 at gill ventral tract and in labial palp pseudofeces H_3 (rejection at gill): frustule numbers in 2 ml sample as great or greater than those of empty cells in Condition 1 at gill ventral tract; gill capable of identifying and rejecting poor-quality particles even when there is no other choice
	Palp pseudofeces	H_0 (no further rejection): frustule numbers in 2 ml sample significantly lower than those of empty cells in Condition 1 in labial palp pseudofeces as great or greater than those of empty cells H_4 (further rejection): frustule numbers in 2 ml sample as great or greater than those of empty cells in Condition 1 in the labial palp pseudofeces; palps capable of identifying and rejecting poor-quality particles even when there is no other choice

were fixed in Lugol's solution for subsequent counts under the inverted microscope according to the method described by Utermöhl (1958), which allowed the distinction of intact and empty frustules.

The large cell size of *Coscinodiscus perforatus* rendered numerical comparisons with the majority of previous studies impractical, as the latter used much smaller test species. Hence, cell concentrations were determined on the basis of comparable organic matter concentrations. A calibration of cell numbers and organic matter (loss on ignition) was established using the *C. perforatus* culture, and periodic samplings and cell counts were performed throughout the observations.

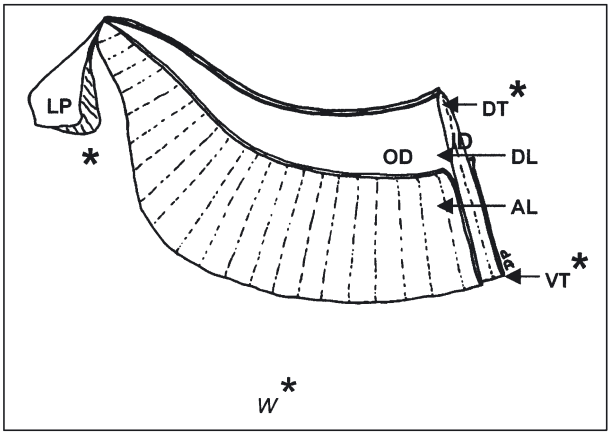

Fig. 2. *Pecten maximus*. Diagram of anterior portion of left gill and left labial palps (viewed from left side), showing location of sampling sites (*). AL: ascending lamella; DL: descending lamella; DT: dorsal tract; ID: inner demibranch; LP: labial palps; OD: outer demibranch; VT: ventral tract; *W*: water in experimental chamber

Size and speed calibrations were performed in order to determine particle speeds in the gill arch and on the ventral bend. We used 2 size calibration techniques: direct measurement of living gill plicae (30) immediately following experimentation, and measurement of plicae width on-screen using the known size *Coscinodiscus perforatus* on the gill epithelium as a standard. Particle speed was determined from the number of frames required to cross a plica of known width, at a recording speed of 25 frames s^{-1}.

To verify that *Coscinodiscus perforatus* was indeed processed as a food item, the stomach contents and feces of the 3 experimental *Pecten maximus* were examined under the microscope after feeding for 12 h with the exponential-phase culture of *C. perforatus*. Similarly, to ensure that the gills did not present regions of desquamation which would compromise their functioning under observation (see MacDonald et al. 1995, Potter et al. 1997), the whole gills were histologically processed, serially sectioned transversally, stained as indicated above, and examined for the presence of bare regions on the filaments.

Data analysis. Samples from the different organism sampling sites and ambient water represent different quantities. Although water samples are straightforward concentrations, slurry samples from the dorsal tract, mucus cord samples from the ventral tract, and

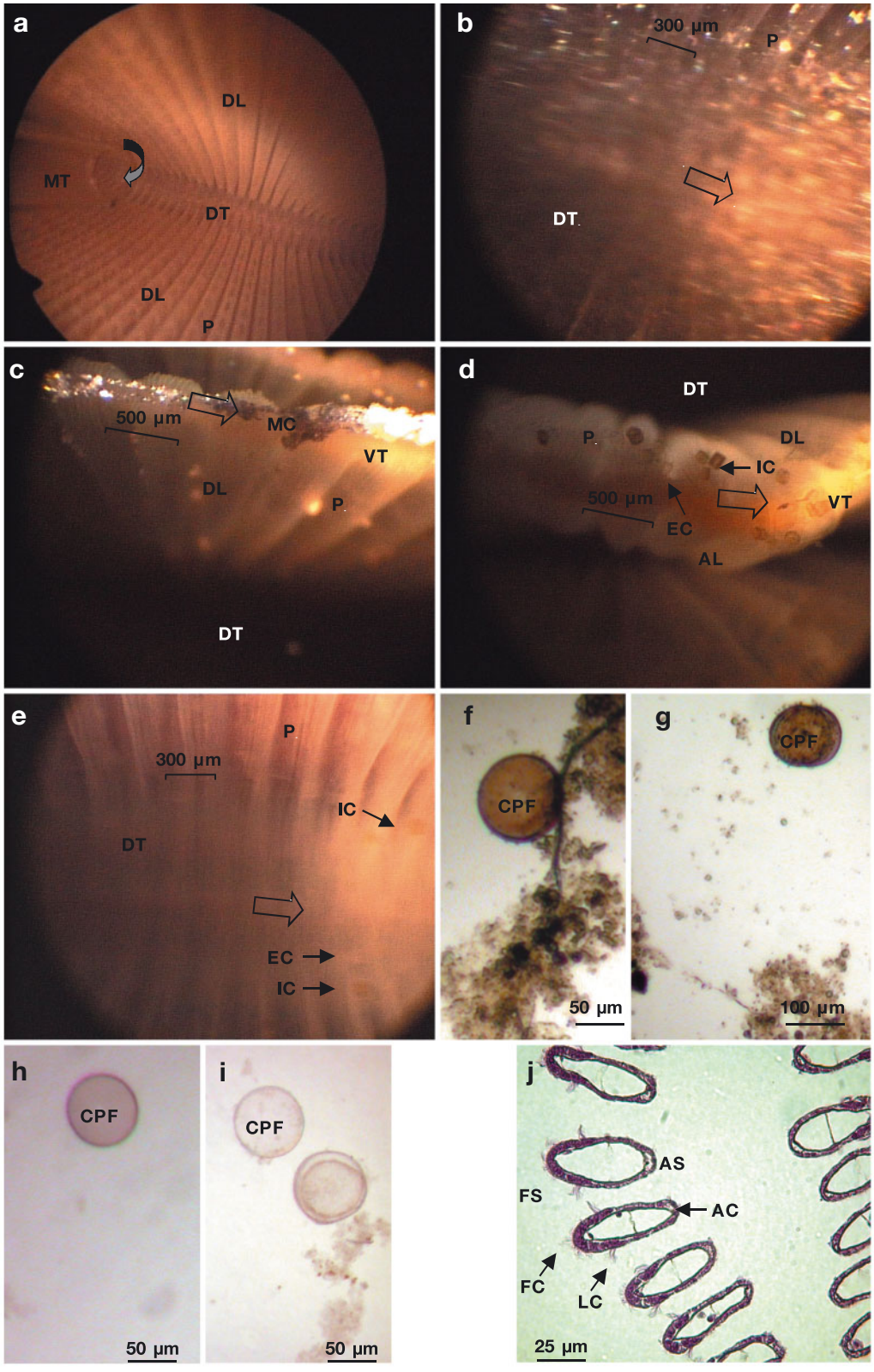

Fig. 3. *Pecten maximus*. (a)–(e): Videoendoscopic images. (a) Endoscope-directed sampling in dorsal tract (DT); DL: descending lamella; MT: glass micropipette tip; P: gill plica. (b) Dorsal transparent suspension and (c) ventral mucus-particle cord (MC) revealed by reflective particles; VT: ventral tract; direction of movement indicated by open arrow. (d) Ventral mucus cord and (e) dorsal mucus suspension observed in Condition 1 (mixture of intact and empty *Coscinodiscus perforatus*); AL: ascending lamella; EC: empty *C. perforatus*; IC: intact *C. perforatus*. (f–h): Photomicrographs of (f) stomach content and (g) feces of experimental specimen fed with *C. perforatus* culture; CPF: *C. perforatus* frustule. (h) Tranverse sections of ordinary gill filaments. AC: abfrontal cilia; AS: abfrontal surface; FC: frontal cilia; FS: frontal surface; LC, lateral cilia

mucus-particle masses from the palp pseudofeces all represent differing degrees of accumulation between sites. Therefore, valid inter-site comparisons can only be made using proportions from the mixed-cell condition (Condition 1), and intra-site comparisons can only be made within each site in the empty cell-only condition (Condition 2).

In the intact and empty cell mixture, the proportions of each cell type were determined for a minimum of 300 cells of the total volume sampled during the 2 h course of each experiment. As explained above, for the empty cell-only condition, it was not possible to present proportional data. Hence, the total number of empty cells 2 ml^{-1} was determined for each sampling site and compared to the same sites in the mixed condition to determine whether the degree of rejection of poor-quality particles was the same when there was no other choice of particles available.

Since we wished to detect eventual enrichment at successive sampling sites (water, gill arch, ventral bend, palp pseudofeces) in the mixed-cell condition, a selection index (SI), based on those of Ward et al. (1998b) and Cognie et al. (2003), was calculated. In contrast to the index of Ward et al. (1998b) and Cognie et al. (2003), who compared particle concentrations of pallial cavity sites to that of the ambient water only, our index compares the concentration at each site to that of the previous site (gill arch and ventral bend compared to water, palp pseudofeces compared to gill arch):

$$SI = [(S_n / S_{n-1}) - 1] \times 100$$

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, palp pseudofeces).

Statistical analysis. For the mixed-cell condition (Condition 1), the data were normally distributed and variances homogeneous, but since proportions were compared, an arcsine transformation was performed (Sokal & Rohlf 1995) prior to a 1-way parametric ANOVA. Student-Newman-Keuls (SNK) tests of significance were subsequently performed to test the corresponding hypotheses in Table 1. The non-normal selection index data was normalized by the arcsine transformation, and treated as above. SigmaStat 2.0 (Jandel Scientific) software was used for all statistical analyses.

In the empty cell-only condition (Condition 2), the between-site heterogeneity of accumulation times precluded statistical comparison; instead, the data were compared to those of the mixed-cell condition for the same sites. As these data were not based on proportions, but normally distributed and characterized by homogeneous variances, Student *t*-tests were performed for each sampling site (Condition 1 vs Condition 2).

RESULTS

Particle concentrations and organic matter content

Depending on the particle concentration and volume of the added culture medium, particle concentrations during endoscopic observation and sampling ranged from 30 to 250 cells ml^{-1} in the intact + empty mixture (Condition 1) and 20 to 50 cells ml^{-1} in the empty condition (Condition 2). Particulate organic matter content ranged from 5 to 50 mg l^{-1} in the mixed suspension (compared to 1 to 30 mg l^{-1} at the Baie de St-Brieuc sampling site: Arzul et al. 1990), whereas no organic matter was present in the empty condition.

Gill anatomy and organization

The topology, organization, and internal anatomy of the *Pecten maximus* gill conformed generally to that previously described by Dakin (1909) for preserved specimens of *P. maximus*, but more closely to that described for *Placopecten magellanicus* (Beninger et al. 1988, 1992, Le Pennec et al. 1988).

General particle-processing mechanisms

The incorporation of reflective particles allowed improved observation of particle behavior on the gill frontal surface, with respect to the characteristics of the medium in which they were transported. Particle movement on the observed surfaces (descending lamellae) was similar in all respects to that previously presented for *Placopecten magellanicus* (Beninger et al. 1992): particles transported dorsally in the principal filaments emerged at the gill arches (dorsal tracts) in a transparent suspension which, based on the behavior of reflective particles (Fig. 3b), appeared more viscous than the pallial fluid. Particles transported ventrally on the ordinary filaments were enveloped in visible, apparently highly viscous mucus masses at the ventral bends (ventral tracts: Fig. 3c). Particles in the dorsal tracts moved 5 times faster (2 mm s^{-1}) than those at the ventral tracts (0.4 mm s^{-1}).

Particle-selection sites and mechanisms

Endoscope-directed sampling allowed the particle composition of the dorsal and ventral tracts to be quantitatively compared. In Condition 1 (mixed suspension), a significantly greater proportion of intact cells was observed in the dorsal versus the ventral particle tracts (Figs. 3d,e & 4a); particles arrived in

the dorsal tracts via the principal filaments. The ventral particle tract was enriched in empty particles compared to both the dorsal tract ($p = 0.010$) and the ambient water ($p = 0.013$) particle concentrations; particles arrived in this tract via the ordinary filaments. The null hypothesis (no selection at the gill) may therefore be rejected and experimental hypothesis H_1 accepted, i.e. qualitative particle selection occurs on the gill of this species. Similarly, a significantly greater proportion of empty cells was observed in the palp pseudofeces than the dorsal tract ($p = 0.003$), indicating that additional enrichment in empty cells occurred at this level. The selection indices clearly reflected these differences: the ventral tract and palp pseudofeces were significantly ($p < 0.001$) more depleted in intact cells than the dorsal tract (Fig. 4b), indicating selection both at the gill and at the labial palps. The null hypothesis of no further selection may therefore be rejected and experimental hypothesis H_2 accepted, i.e. the labial palps are also capable of particle selection, and may do so after initial selection on the gills.

In Condition 2 (empty, cleaned cells only), the ventral tract contained a similar number of cells in 2 ml of sample (greatly overlapping ranges, $p > 0.05$, no significant difference), as was observed for empty cells in Condition 1 (Fig. 5), indicating that rejection in the empty, cleaned cells-only condition was equivalent to that in the mixed condition (i.e. rejection of empty cells by the gill was similar even when there was no other particle choice). Particles arrived in the dorsal tracts via the principal filaments, and in the ventral tracts via the ordinary filaments. Conversely, significantly greater numbers of empty cells in 2 ml of sample were observed in the dorsal tract ($p = 0.048$) in Condition 1 (mixed suspension). It should be noted that while all of the empty cells in Condition 2 had been cleaned of their organic casing, Condition 1 contained a large proportion of naturally dead, uncleaned cells. Finally, there was a significantly greater number of empty cells

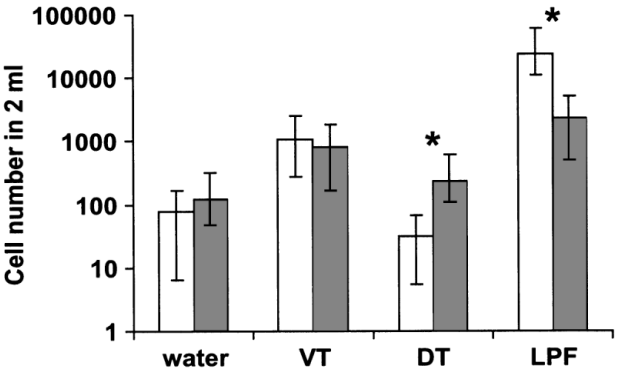

Fig. 5. *Coscinodiscus perforatus*. Mean numbers of empty cells 2 ml^{-1} sample from different sampling sites in Condition 2 (empty, cleaned frustules only: white bars) compared to those of Condition 1 (mixed suspension: shaded bars) at the same sites: water, ventral tracts (VT), dorsal tracts (DT) and labial palp pseudofeces (LPF). Note logarithmic scale of ordinate. Error bars represent range of values; asterisk indicates significant differences in empty cell numbers between Conditions 1 and 2 within the relevant site ($p < 0.05$)

in the palp pseudofeces in Condition 2 ($p = 0.045$), compared to Condition 1 (mixed suspension). The corresponding null hypotheses of no rejection by either the gills or the labial palps may thus be rejected, in favor of the experimental hypotheses H_3 and H_4, i.e. both the gills and the labial palps can detect and reject poor-quality particles, even when there is no other choice; the amplitude of this rejection is as great or greater than when the scallops are exposed to a mixed suspension.

Post-experiment examination of the individuals fed with intact suspensions of *Coscinodiscus perforatus* revealed intact cells and empty frustules of this alga in both stomach contents and feces (Fig. 3f,g), indicating that it is ingested as a food item. Histological observation of the gills of all 3 individuals showed that they were anatomically intact, with no signs of desquamation, infection, or parasitic infestation (Fig. 3h).

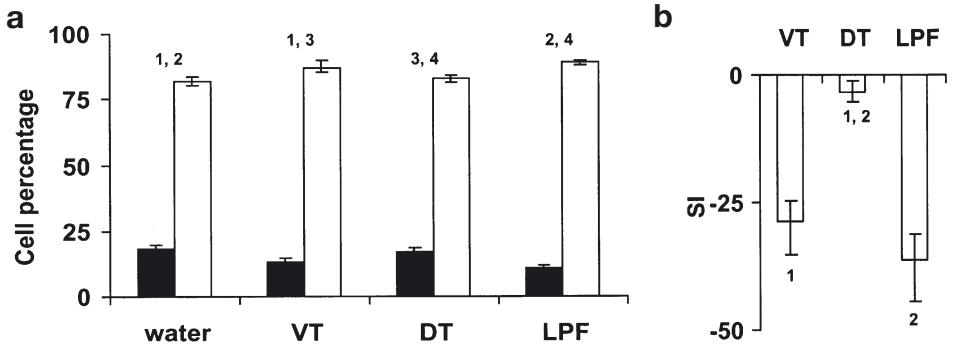

Fig. 4. *Coscinodiscus perforatus*, Condition 1 (mixed suspension). (a) Mean percentages of intact (black bars) and empty (white bars) cells for the different sampling sites: ambient water, ventral tracts (VT), dorsal tracts (DT) and labial palp pseudofeces (LPF); (b) Mean selection index (SI) at the different sites. Error bars represent range of values (n = 3). Bars with same number are significantly different from each other ($p < 0.05$)

DISCUSSION

General features of gill- and particle-processing

The great degree of similarity in particle-processing mechanisms on the gill of *Pecten maximus* to those previously described for *Placopecten magellanicus* (Beninger et al. 1992) is in line with the essentially identical anatomy of this structure in the 2 species. As the evolutionary appearances of these 2 species are separated by approximately 65 million yr (Waller 1991), we may thus conclude that not only the anatomy, but also the functioning of this organ appear to be highly conservative within the framework of a 'pectinid-type' heterorhabdic gill. In a previous study of the evolution of the bivalve gill, Beninger & Dufour (2000) argued that this organ is much more susceptible to evolutionary modification than other organs such as the heart, since it is subject to much more intense selection pressure. The present study has shown that while the gill may have demonstrated such plasticity during its diversification from the primitive protobranch condition, the present-day pectinid-type configuration may itself be quite stable due to the limited possibilities for functioning in any other way with such a specific anatomy. The high degree of structural and functional conservatism of this gill type contrasts with the differences in less vital details of features such as the shell, which serve to indicate evolutionary distance. It would be interesting to explore this line of research for the 3 other major autobranch gill types (homorhabdic filibranch, eulamellibranch, and pseudolamellibranch).

The particle speeds recorded in *Pecten maximus'* dorsal tract are considerably slower than those reported for *Placopecten magellanicus* (Beninger et al. 1992): 2 mm s^{-1} vs 5.7 to 10 mm s^{-1}. The greater speeds in the *P. magellanicus* dorsal tract were observed at all particle concentrations tested, and are probably not artefacts of temperature differences since, had this been the case, the greater water temperature for *P. maximus* should have resulted in faster speeds for this species. Similarly, the faster speeds in *P. magellanicus* cannot be imputed to differences in body size, since the individuals in both studies were of comparable size (10 to 11.5 cm, P.B. unpubl. observations, and present study). However, it should be noted that *Coscinodiscus perforatus* used in the present study is over 20 times larger than the test alga *Chaetoceros muelleri* Lemmermann used by Beninger et al. (1992) for *P. magellanicus*, and was therefore subject to considerably more frictional resistance (which is proportional to surface area) in the essentially hydrodynamic environment of the dorsal tract mucus–particle slurry. The velocity values for the transport of particles on the ventral tract (mean = 0.4 mm s^{-1}) are in the same range as those reported for *P. magellanicus* (means = 0.185 and 0.380 mm s^{-1}). Since transport on the ventral bend is mucociliary (both large and small particles enveloped in mucus masses: Beninger et al. 1992, Ward et al. 1993) and therefore depends and on the speed of effective cilia strokes, it is not surprising that the values for this mode of transport are comparable in the 2 species.

Particle-selection sites and macroscopic effectors

The results of the present study clearly demonstrate that the heterorhabdic filibranch system is capable of selection at the gill. While the observed selection is statistically significant, it is intriguing that the dorsal tract may still contain a large proportion of empty cells (approx. 82%, Fig. 4a). A possible explanation for this is that only the empty, cleaned frustules were rejected in the mixed-cell suspension (Condition 1), effectively accounting for only 5% of rejection on the gill (50% of the empty, cleaned cells presented), with a further 6% of rejection at the labial palps (the remaining 50% of empty, cleaned frustules presented). If this is the case, then it implies that the empty, natural frustules tend to be treated as intact frustules by the gill. Further support of this interpretation is found in the extremely low numbers of empty, cleaned frustules in the dorsal tract in Condition 2 (empty, cleaned frustules only). We are currently pursuing further research in order to elucidate the treatment of empty, natural frustules in *Pecten maximus*.

Potential effectors of selection may be divided into 2 broad categories: macroscopic and microscopic. Macroscopic effectors comprise the gill architecture and filament types, whereas microscopic effectors presumably involve sensory detection, and decisional behavior by individual cilia or groups of cilia. At this point in time, no data is available concerning possible mechanisms of microscopic effectors for any bivalve species. However, the present study substantially advances our knowledge of the roles of macroscopic effectors. Ingestion volume control ('cleaning') has previously been observed *in vivo* in the heterorhabdic filibranch gill, and attributed to differentiation of the 2 gill filament types (Beninger et al. 1992). The present finding of particle selection at the heterorhabdic filibranch gill of *Pecten maximus*, as has previously been reported for the heterorhabdic pseudolamellibranch oyster gill (Ward et al. 1998b), suggests that the heterorhabdic condition also allows qualitative particle selection on this organ, an idea which originated from Atkins (1937). This mechanism was later extended to include the presence of a ventral particle groove (Beninger & St-Jean 1997). The potential role of the labial palps in qualitative selection in heterorhabdic bivalves has

been somewhat less clearly established. Ward et al. (1998b) did not observe particle selection at the labial palps of oysters, although they did not rule out this possibility under 'more complex' feeding regimes. However, Cognie et al. (2003) have recently demonstrated that not only are oyster palps capable of such selection, but they are the only organs which can do so for those (often abundant) particles whose size is greater than the opening of the principal oyster gill filament (PF). It is not yet known whether the oyster's labial palps also participate in particle selection at sizes smaller than this threshold value. This 'PF constraint' on particle size is due to the fact that the opening of the oyster's PF is comparatively small (approx. 70 µm in *Crassostrea gigas* Thunberg) and, due to extensive tissue fusion, is not able to increase its gape through the muscular movements commonly seen in the almost completely unfused, larger-gape gills of pectinids.

The results of the present study have shown that although the PF opening presents virtually no particle size restriction in *Pecten maximus*, the labial palps nonetheless participate in qualitative selection.

Implications for ecology and evolution

The experiments reported here demonstrate that qualitative selection is a 2-stage, 2-site process in *Pecten maximus*. This suggests that although the heterorhabdic gill is capable of performing qualitative selection, the demands of processing are highly variable, and the often large numbers of heterogeneous particles per second (Bayne et al. 1988, 1993, Barillé et al. 1997, Navarro & Widdows 1997, Bacon et al. 1998) are too high for a single pallial organ of the heterorhabdic filibranch system to approach the necessary level of efficiency. Such multi-stage particle-processing was proposed earlier by Foster-Smith (1978), although he did not distinguish between homorhabdic and heterorhabdic conditions. Most data so far have indicated that the homorhabdic gill (typical of most bivalve species) is incapable of qualitative particle selection, and that in this system the labial palps comprise the only selection sites (Beninger & St-Jean 1997, Beninger et al. 1997, Ward et al. 1998b). A possible exception to this rule may be the freshwater venerid *Dreissena polymorpha* (Baker et al. 2000), whose homorhabdic gill may be modified and capable of some qualitative particle selection.

In the present study, qualitative particle selection by *Pecten maximus* has been shown to operate even when the proportions of inert particles are low (approx. 10%). This is in agreement with the general observations of Macdonald & Ward (1994) and Bacon et al. (1998), who showed that in scallops there is no 'threshold' level of particle concentrations for pseudofeces production.

Taken together, the results of the present study and those of Ward et al. (1998b) and Cognie et al. (2003) show that the heterorhabdic gill, either filibranch (*Pecten maximus*) or pseudolamellibranch (*Crassostrea virginica*, *C. gigas*) is capable of qualitative particle selection. In the case of *P. maximus*, this selection is unambiguous, since material at the gill ventral bend does not enter a particle groove (as in the oyster), and is thus definitively rejected during valve-clapping. The oyster presents a much more complex system, with the potential for both rejection of material in the ventral particle groove (Beninger & Veniot 1999) and for reprocessing on the labial palps (Ward et al. 1994).

Why species with a heterorhabdic gill are capable of particle selection on the gill, while most homorhabdic species are not, is an intriguing question. One possible explanation is that heterorhabdic bivalves evolved under conditions of higher seston load than homorhabdic species; indeed, contemporary oysters typically inhabit high-turbidity ecosystems. Another possible explanation is that the most widespread gill type, the homorhabdic eulamellibranch, evolved primarily in the direction of increased water-flow efficiency for smaller gills (Beninger et al. 1997), reducing the cost of processing seston and hence allowing the labial palps alone to cope with qualitative selection.

Qualitative selection appears to operate in *Pecten maximus* even when the scallop has been purged and is offered only non-nutritive particles of the same size and shape as nutritive particles (Condition 2), as shown by the successive accumulation in the ventral gill tract and labial palp pseudofeces of empty cells, compared to the numbers of empty cells rejected at these sites in Condition 1 (mixed-suspension). The heterorhabdic filibranch system of *P. maximus* therefore seems to reject non-nutritive particles even when there is no other particle choice. It should be noted that, since the empty frustules of Condition 2 were cleaned with hydrogen peroxide, there was probably very little post-cleaning bacterial/organic film establishment prior to particle-processing, and that these particles were thus of extremely low nutritive value. Rejection under these conditions could reflect an adaptation to avoid the energetic cost of processing low- or non-nutritive particles.

Conclusion

The present study has demonstrated that not only is the scallop heterorhabdic filibranch gill capable of qualitative selection, i.e. rejection of non-nutritive particles, it is able to do so both in the presence of other particles with higher nutritive value, and also when no other particle choice is available. In addition, the labial palps are also capable of such selection under simi-

lar conditions, demonstrating that qualitative particle selection is a 2-stage process in *Pecten maximus*. Localization of selection sites will allow detailed features of qualitative selection to be investigated, such as the key particle characteristics upon which selection is based, as well as the broader issues concerning functional correlates to the evolutionary trajectories of different bivalve gill types.

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