Marine Biology (1998) 131: 283–292

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Particle sorting in bivalves: in vivo determination of the pallial organs of selection

Received: 4 June 1997 / Accepted: 23 January 1998

Abstract Benthic particle feeders are exposed to a food supply varying in both quantity and quality. Previous studies have shown that bivalve molluscs deal with such fluctuating particle regimes in a variety of ways, including adjustments in pumping and ingestion rates, and selective rejection of non-nutritive particles as pseudofeces. The actual site of particle selection within the pallial cavity, however, has remained a topic of speculation. During August 1995 and January and August 1996, we exposed the oysters *Crassostrea virginica* (Gmelin) and *C. gigas* (Thunberg), and the mussel *Mytilus trossulus* Gould to a mixture of ground, aged *Spartina alterniflora* Loisel and similar-sized phytoplankton at three concentrations (10^3, 10^4, 10^5 particles ml^{-1}). We then examined the ctenidia and labial palps by means of endoscopy and sampled, in vivo, the particulate material from various ciliated tracts, and analyzed the samples with a flow cytometer. We found that in oysters, the ctenidia are responsible for particle sorting, whereas the labial palps play an accessory role in particle selection, or function to control the volume of material to be ingested. In mussels, however, the ctenidia play little role in particle selection and simply transport particulate matter to the palps for further processing. We suggest that selection by the ctenidia of oysters is a function of their architecture (plicate, heterorhabdic).

Communicated by J.P. Grassle, New Brunswick

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Introduction

Suspension feeders are exposed to a food supply varying in quality and quantity on both spatial and temporal scales. They encounter food particles that are small, easily dispersed, not much denser than the surrounding medium, and often mixed with non-nutritious or sometimes toxic particles of the same size. Past studies have demonstrated that many groups of benthic and planktonic particle feeding organisms (e.g., polychaetes, crustaceans, bryozoans, echinoderms, bivalves) may respond to such particle mixtures by altering capture and ingestion rates, and rejecting undesirable particles prior to ingestion (Taghon 1982; Miller 1984; Taghon and Jumars 1984; Rassoulzadegan et al. 1984; Strathmann 1987; Cowles et al. 1988; Gallager 1988; Okamura 1990). In particular, the compensatory strategies used by adult bivalve molluscs to maximize energy gain under variable seston conditions have been well studied. For example, previous studies on bivalve molluscs have documented selective ingestion of particulates based on particle size (Defossez and Hawkins 1997) and quality (e.g., Loosanoff 1949; Kiørboe and Møhlenberg 1981; Newell and Jordan 1983; Shumway et al. 1985; Newell et al. 1989; Prins et al. 1991; MacDonald and Ward 1994; Pastoureaud et al. 1996; Bougrier et al. 1997). Mechanisms for particle selection are thought to include one or more of the following: preferential retention on the ctenidia, preferential sorting on the ctenidia or labial palps, and preferential sorting in the gut (e.g., Newell and Jordan 1983; Shumway et al. 1985; Newell et al. 1989).

Although previous reports have demonstrated that many bivalve species can sort and preferentially ingest particulate matter, several aspects of particle selection remain undefined such as the actual site of selection, or which pallial organs are involved in the selective process. In all previous studies, selection was determined by

collecting bivalve pseudofeces after it was expelled from the pallial cavity so the organs responsible for its production could not be determined. Histological studies and observations of surgically altered specimens suggest that the labial palps are the major site of particle selection in bivalves (Kellogg 1915; Menzel 1955; Nelson 1960; Galtsoff 1964; Jørgensen 1966). The ctenidial filaments of some species bear frontal cilia that beat in opposite directions, however, and some workers have speculated that the ctenidia are also involved in particle selection (Allen 1921; Atkins 1937; Nelson 1960; Ribelin and Collier 1977; Barillé 1994). No studies have conclusively determined the location of particle selection in any living, intact species.

In the present study we examined particle selection in three species of bivalves, the oysters *Crassostrea virginica* and *C. gigas*, and the mussel *Mytilus trossulus*. We chose these species because their ctenidial structures allowed us to make predictions about particle selection. Mussels have a flat, homorhabdic ctenidium. Their ctenidial filaments bear frontal cilia that, for the most part, transport particles ventrally (Ward et al. 1993). Therefore, we hypothesized that in mussels the ctenidia contribute little to the selective process and the labial palps are the main organ of selection. In contrast, oysters have a plicate, heterorhabdic ctenidium with principal and ordinary filaments. The principal filaments bear cilia that transport particles towards the dorsal ciliated tracts. The ordinary filaments bear two types of frontal tracts, (1) coarse frontal cirri that transport particles towards the ventral ciliated grooves, and (2) fine frontal cilia that transport particles towards the dorsal ciliated tracts (Ribelin and Collier 1977). Particle processing is more extensive on the ctenidia of oysters, and particles can be transported in opposite directions on the same filament (Ward et al. 1994). Therefore, we hypothesized that in oysters the ctenidia contribute significantly to the selective process; the labial palps could also be an organ of selection.

Our goals were to define which pallial organs (ctenidia, labial palps) are involved in particle selection and to determine the relative contribution of these organs to the selective process for two natural particles at different concentrations. Unlike previous studies, we examined particle selection in vivo in intact bivalves making it possible to sample various locations in the pallial cavity while the bivalve was actively feeding.

Materials and methods

Crassostrea virginica (Gmelin) were obtained from the Cornell Cooperative Extension Hatchery, Southold, New York, USA during September 1995 and transported to sea water facilities at Southampton College, New York. *Crassostrea gigas* (Thunberg) and *Mytilus trossulus* Gould were obtained from Westcott Bay Farms, San Juan Island, Washington, USA, during July 1995 and 1996, respectively, and were transported to sea water facilities at Friday Harbor Laboratories, Washington. Bivalves were maintained in ambient, flowing sea water until they were prepared for observation.

In order to investigate the site of particle selection within bivalves, two different methods were used: (1) traditional particle depletion experiments, and (2) feeding assays combined with endoscope-directed, in vivo sampling. In all experiments, two different natural particle types were delivered to the bivalves. The first was the cryptophyte *Rhodomonas lens* Pascher et Ruttner (6 to 13 μm in length). This microalga was obtained from the Provasoli-Guillard Center for Culture of Marine Phytoplankton (clone CCMP-739), and was grown in *f*/2 media at 18 °C under constant light (Guillard 1975). The second particle type was ground, aged *Spartina alterniflora* Loisel. Dried *S. alterniflora* wrack was collected from beaches above mean high water around Flax Pond marsh, located in Old Field, Long Island, New York, USA. Individual shoots were cleaned of debris, cut into 20 to 25 cm pieces, placed in a blender with 0.45 μm filtered (Millipore) sea water and processed for 10 to 15 min. The resulting suspension was sieved through a 20 μm, nylon screen to obtain a final particle distribution (3 to 20 μm) overlapping that of the *R. lens* cultures. Particle suspensions were prepared by diluting the stock solutions of microalgal cultures and ground *S. alterniflora* with filtered sea water (0.45 μm) to form suspensions with a total particle concentration of 10^3, 10^4 and 10^5 particles ml^{-1} (0.6 to 26.0 mg l^{-1}). In some experiments, the bivalves were delivered only one particle type at a time (no choice), whereas in other experiments the bivalves were delivered a mixture of the two particle types in near equal proportions (choice). Particle suspensions were maintained at the same temperature as that in the ambient, flowing seawater system.

Particle depletion assays

In order to compare our results to those of previous studies on particle selection in bivalves, we conducted a series of particle depletion experiments using traditional methods (e.g., Shumway et al. 1985). Juvenile bivalves (2 to 4 cm in shell height) were cleaned of fouling material and placed in individual plastic containers (500 to 700 ml). The containers were filled with the mixed *Rhodomonas lens*/*Spartina alterniflora* particle suspension at concentrations of 10^3 to 10^5 particles ml^{-1}. The suspension in each container was gently aerated and maintained at ambient temperature conditions (12 to 20 °C, depending on season). Control vessels were left without bivalves to correct for microalgal cell division and settling during experiments. Experiments lasted for 0.5 to 1.0 h, depending upon the size of the bivalve and the volume of the container. During the experiments, water samples were taken periodically and analyzed using flow cytometry to determine feeding activity. Particle concentrations were not allowed to fall below approximately 70% of the original value. At the end of the experiment, pseudofeces were collected and analyzed using flow cytometry (see below). Experimental bivalves were then opened, their tissues were separated from the shell, blotted with a paper towel, and dried to constant weight at 65 °C.

Endoscopic observations and in vivo sampling assays

Adult bivalves (9 to 13 cm in shell height) were prepared for endoscopy using methods of Ward et al. (1993) and Ward et al. (1994). Specimens were scrubbed to remove debris and encrusting organisms from their valves. A small section of shell was trimmed from the inhalant margin of the upper and lower valves without damaging the underlying mantle margins. Trimming produced a narrow opening in the shell, which provided more freedom of movement for the optical insertion tube (OIT) of the endoscope and prevented the shell edges from damaging the OIT when the specimen adducted its valves. After preparation, the bivalves were isolated from other specimens and supplied with flowing or static, aerated sea water at the same temperature and salinity as that of the primary holding system. In static systems, sea water in each container was replaced regularly, and bivalves were fed a daily maintenance ration of cultured microalgae. Bivalves were allowed to recover for at least 1 d after preparation, and they usually began repairing their shells shortly after the recovery period.

Endoscopy was performed following methods described previously (Ward et al. 1991; Beninger et al. 1992). During endoscopic examination, bivalves were placed in an aerated, assay chamber (1.0 liter) filled with sea water at ambient temperature. Individual bivalves were exposed, in succession, to each of the three particle concentrations for 0.5 to 1.0 h depending on the feeding activity of the specimen. Particle concentration in the assay chamber was monitored during the exposure period using an electronic particle counter, and the chamber flushed with additional volumes of the appropriate suspension when needed.

To control for changes in gut fullness as the bivalves were exposed to successive particle concentrations, the order of delivery of the three concentrations was changed for every other specimen. To increase particle concentration, the assay chamber was flushed with the next higher concentration suspension. To decrease particle concentration, the assay chamber was first flushed with 0.45 μm filtered sea water before adding the next lower concentration suspension.

During the assays, the ventral ciliated grooves and dorsal ciliated tracts of the ctenidia were observed, as well as the junction between the ctenidia and labial palps. In addition, we collected samples in vivo using a micropipet connected to a low-flow peristaltic pump (ca. 50.0 μl min^{-1}). The sampling pipet was mounted on a micromanipulator and positioned with the aid of the endoscope. In this way, samples could be taken from various locations on the ctenidia and labial palps, and the sampling process could be observed directly and recorded on videotape. Samples of post-capture particulate material were collected from the ventral grooves and dorsal tracts of the ctenidia, and from between the lamellae of the labial palps (palp slurry). In addition, pseudofeces were collected from the bottom of the assay chamber following procedures of traditional selection experiments (e.g., those described below). This material may have been produced by the ctenidia, labial palps, or both organs. Finally, water samples were taken periodically from the assay chamber during in vivo sampling to determine the proportion of particle types available to the bivalve. At the end of the experiment, samples were analyzed using flow-cytometry (see below).

Analyses of samples and data

Flow-cytometry was used to enumerate particle abundance in all samples (water, pseudofeces, ventral grooves, dorsal tracts, etc.). Particles were differentiated based on their optical properties by means of a FACScan bench top flow-cytometer (Becton Dickinson, San Jose, California) equipped with a 15 mW, 488 nm, air-cooled argon laser. Detection of microalgal cells was derived from chlorophyll fluorescence (>650 nm) and phycoerythrin fluorescence (560 to 590 nm) emissions. All other particles (i.e., *Spartina alterniflora*) were detected by the simultaneous measurements of their forward scatter (FSC) and 90° light scatter (SSC) optical properties. All particles between 3 and 35 μm were enumerated based on the forward scatter (a sizing parameter) signal from polystyrene microspheres (3.15 μm). Prior to analysis, each sample was vigorously agitated on a vortex mixer to disrupt particle aggregates. The volume of sample analyzed was calculated gravimetrically by weighing the sample (mg) immediately before and after analysis. Samples were run at low (ca. 30 μl min^{-1}) or high (ca. 50 μl min^{-1}) flow rates, depending on initial particle concentration. The photomultiplier detectors were in logarithmic mode, providing four decades of signal detection, and signal peak integrals were measured.

Data obtained from flow cytometry allowed us to determine the proportion of *Rhodomonas lens* and *Spartina alterniflora* particles in the samples. Particle depletion data were used to calculate clearance rates of the bivalves after methods of Coughlan (1969). Rates were then standardized to a 1.0 g dry tissue mass. Relative clearance rates for *S. alterniflora* particles and *R. lens* cells were calculated separately for each specimen. Clearance rates for the two particle types were then compared using paired *t*-tests (Zar 1984).

In order to examine particle selection for or against the two particle types, we calculated a modified electivity index (EI) (Jacobs 1974; Bayne et al. 1977). This index was defined as:

$$EI = \frac{r - p}{(r + p) - (2rp)} ,$$

where r is the proportion of *Rhodomonas lens* cells in the post-capture samples (ventral groove, dorsal tracts, pseudofeces, etc.), and p is the proportion of cells in the water samples (pre-capture, food supply). A positive EI indicates an enrichment of *R. lens* in the sample compared to the water, whereas a negative EI indicates an enrichment of *Spartina alterniflora* particles in the sample compared to the water. In order to better demonstrate the efficiency of particle selection, sorting efficiency (SE; Iglesias et al. 1992; MacDonald and Ward 1994) was calculated as:

$$SE = (S_F/W_F) - 1 ,$$

where S_F is the fraction of *R. lens* cells in the sample and W_F is the fraction of *R. lens* cells in the water. This index represents the percentage increase or decrease in *R. lens* cells of the samples compared to that of the food supply.

Calculated EIs for samples were compared for each particle concentration using Kruskal–Wallis tests followed by appropriate nonparametric multiple comparison tests (Tukey, Dunnett; Zar 1984). Nonparametric procedures were used because data were not normally distributed. The null hypothesis for choice assays was that the EIs did not vary significantly among samples. Electivity indices obtained for pseudofeces from depletion experiments were compared to zero using a one-sample, *t*-test (two-tailed; Zar 1984). The null hypothesis for depletion assays was that the electivity indices of pseudofeces were equal to zero (i.e., no selection). In all statistical tests a significance level of $\alpha = 0.05$ was used.

Results

Data from the particle depletion experiments indicated that clearance rates of *Mytilus trossulus* for both particles were not statistically different when delivered a mixed particle regime (Fig. 1A; $n = 5$ to 7, $p > 0.05$). In contrast, *Crassostrea gigas* had significantly lower clearance rates for *Spartina alterniflora* particles than for *Rhodomonas lens* cells (Fig. 1B; $n = 6$ to 10, $p < 0.02$). Based on these results, electivity indices and sorting efficiencies were calculated using water samples taken periodically from the experimental chambers during in vivo sampling, or taken at the end of the depletion experiments. Using this type of sampling scheme, we controlled for changes in the proportion of *S. alterniflora* and *R. lens* particles in the chambers due to differential retention of these two particle types by the bivalves.

Analysis of the pseudofeces produced by juvenile bivalves during depletion experiments at 10^5 particles ml^{-1} confirmed that *Mytilus trossulus* and *Crassostrea gigas* have a similar capacity for particle selection. Pseudofeces from both species were selectively depleted of *Rhodomonas lens* cells compared to the food supply, and calculated EIs were significantly less than zero (*M. trossulus*: mean EI = −0.52 ± 0.26 SD; *C. gigas*: mean EI = −0.55 ± 0.11 SD; $n = 6$ to 7, $p < 0.01$).

Obvious differences existed in the way in which the two particle types were transported by the ctenidia of oysters. Material in the dorsal tracts appeared to be red-brown in color, whereas material in the ventral grooves was more straw colored. These observations suggested that a higher proportion of *Rhodomonas lens* cells were present in the dorsal tracts, and a higher proportion of

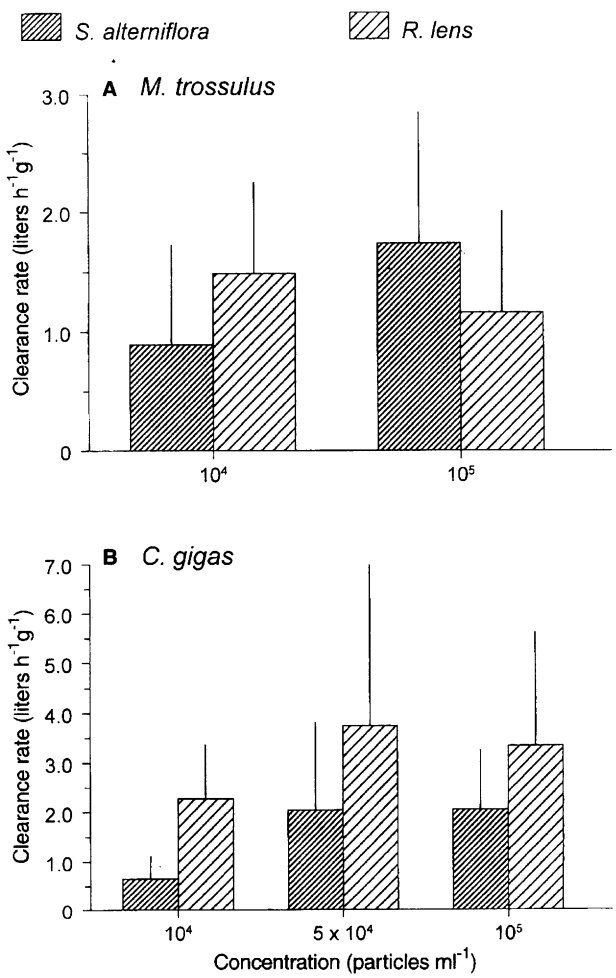

Fig. 1 *Mytilus trossulus*, *Crassostrea gigas*. Clearance rates (mean ± SD) of bivalves feeding on a mixture of *Spartina alterniflora* particles and *Rhodomonas lens* cells at different concentrations. **A** *M. trossulus*. Relative clearance rates for the two particles were not statistically different at either concentration ($p > 0.05$). **B** *C. gigas*. Relative clearance rates for *S. alterniflora* particles were significantly lower than clearance for *R. lens* cells at all concentrations ($p < 0.02$)

Spartina alterniflora particles were present in the ventral grooves (Fig. 2). *Crassostrea gigas* appeared to show a similar sorting preference when *R. lens* cells or *S. alterniflora* particles were delivered separately. Qualitatively, it appeared that a higher concentration of *R. lens* cells was transported in the dorsal tracts when delivered alone, and a higher concentration of *S. alterniflora* particles was transported in the ventral grooves when delivered alone. In the mussel, *M. trossulus*, there was no obvious color difference in the transport tracts; the dorsal tracts transported very little material, and the ventral groove appeared to transport a mix of both particles.

Analysis of samples by flow-cytometry confirmed our qualitative observations. Electivity indices of in vivo and pseudofeces samples from *Crassostrea virginica* and *C. gigas* were very similar; dorsal tract samples were enriched with *Rhodomonas lens*, whereas ventral groove and pseudofeces samples were enriched with *Spartina alterniflora* particles (Fig. 3). For both oyster species, at all assay concentrations, mean EIs of material in the dorsal tracts were significantly higher than mean EIs in the ventral grooves (Fig. 3; *C. virginica*: $n = 4$, $p < 0.05$; *C. gigas*: $n = 6$, $p < 0.05$). Electivity indices of pseudofeces samples from both species, at 10^4 and 10^5 particles ml^{-1}; were statistically indistinguishable from the ventral groove samples (Fig. 3; $n = 4$ to 6, $p > 0.05$). Mean sorting efficiencies for *C. virginica* indicated an enrichment of *R. lens* cells in the dorsal tracts of 50 to 60%, and a depletion of *R. lens* cells in the ventral grooves of 45 to 77% (Table 1). Similarly, mean sorting efficiencies for *C. gigas* indicated an enrichment of *R. lens* cells in the dorsal tracts of 47 to 187%, and a depletion of *R. lens* cells in the ventral grooves of 64 to 82% (Table 1).

In contrast, EIs of samples from the ventral grooves of *Mytilus trossulus* were close to zero at all three particle concentrations (Fig. 4), suggesting that the composition of material in the groove was similar to that of the food supply. Selection by the ctenidia of *M. trossulus* was examined further by comparing mean EI of the ventral groove material among all three bivalve species. Electivity indices of oysters were significantly different from those of the mussel at all concentrations (Fig. 4; $n = 4$ to 6, $p < 0.05$), except for *C. virginica* at 10^5 particles ml^{-1} (Fig. 4; $n = 4$ to 6, $p > 0.05$).

To further examine particle selection in *Crassostrea gigas*, exposed to 10^4 to 10^5 particles ml^{-1}, samples of material taken from between the labial palp lamellae (posterior region) were compared to material from the dorsal tracts of the ctenidia and material rejected as pseudofeces. Electivity indices of the palp slurry samples indicated an enrichment of *Rhodomonas lens* in this area relative to the food supply (Fig. 5). Statistical comparison of EIs of the three samples indicated that the composition of material between the palps and in the dorsal tracts was significantly different from that in the pseudofeces (Fig. 5; $n = 5$ to 7, $p < 0.05$). There was no significant difference in EIs, however, between material in the palp slurry and material in the dorsal tracts (Fig. 5; $n = 5$ to 7, $p > 0.05$).

Discussion

Particle depletion experiments provided two important pieces of information about our experimental protocol. First, *Crassostrea gigas* has significantly lower clearance rates for *Spartina alterniflora* particles than for *Rhodomonas lens* cells (Fig. 1). This difference was probably caused by a lower retention efficiency for the *S. alterniflora* particles, which had a broader size distribution (3 to 20 μm) than that of *R. lens* (6 to 13 μm). In general, particle retention efficiency of oysters decreases rapidly from about 100% for 6 μm diameter particles to about 50% or less for 2 μm particles (see Newell and Langdon 1996), although this can be adjusted by the oyster in

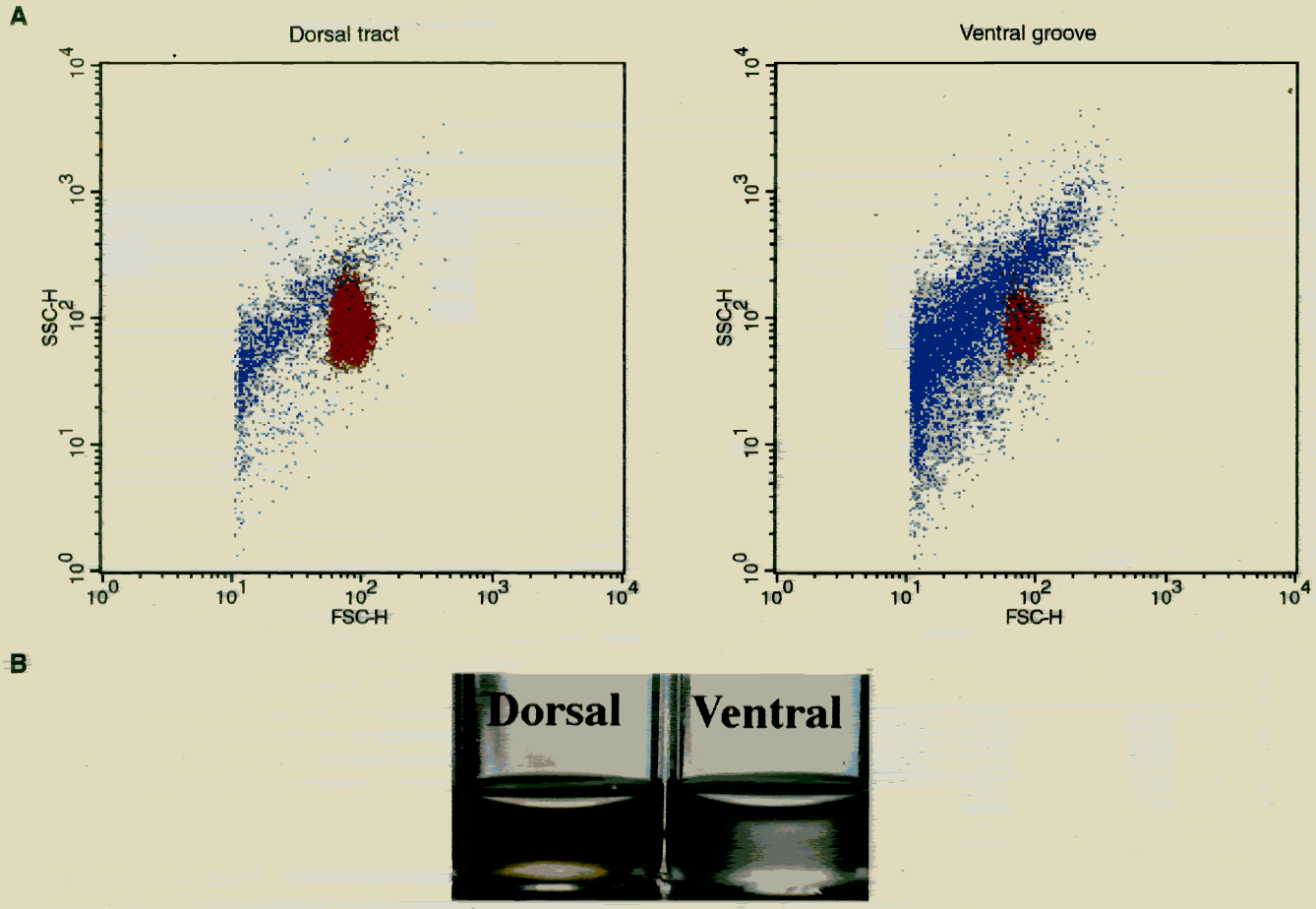

Fig. 2 *Crassostrea gigas*. **A** Flow cytometry analyses showing side scatter (*SSC*) and forward scatter (*FSC*) discrimination of particles in two different samples (dorsal tract, ventral groove) taken during a typical feeding assay. The oyster was feeding on a mixture of *Spartina alterniflora* particles and *Rhodomonas lens* cells at 10^5 particles ml^{-1}. Note that the distribution of *S. alterniflora* particles (*blue dots*) overlaps the distribution of *R. lens* cells (*red dots*), and the relative distribution of *S. alterniflora* particles is similar in both samples. Also notice the enrichment of *R. lens* cells in the dorsal tract sample and enrichment of *S. alterniflora* particles in the ventral groove sample. **B** Photograph of samples taken in vivo from living, actively feeding oysters. Note the red color of the dorsal tract sample indicating an increased number of *R. lens* cells (red-brown) compared to the ventral groove sample

response to changes in seston quantity (Barillé et al. 1993). In contrast, *Mytilus trossulus* demonstrated no significant difference in clearance rates, and thus none for retention efficiency of either particle type (Fig. 1), which agrees with previous reports. Mussels have a higher retention efficiency for smaller particles than oysters, demonstrating about 80% efficiency for 2 µm diameter particles and 100% efficiency for particles 4 µm and above (Møhlenberg and Riisgård 1978; Riisgård 1988). Our data indicated that calculations of selection parameters had to be based on the proportion of *S. alterniflora* and *R. lens* in water samples taken at the time of in vivo sampling, and not based on the proportion of particles in the working suspension.

Particle depletion experiments also demonstrated that both *Crassostrea gigas* and *Mytilus trossulus* are able to sort and selectively reject *Spartina alterniflora* particles in the pseudofeces. Such rejection of low-quality detrital particles over higher-quality microalgal cells has been demonstrated by previous workers using methods similar to our depletion experiments (Newell et al. 1989; Newell and Shumway 1993). In all previous studies, however, the origin of the analyzed pseudofeces was undetermined, possibly being produced by the labial palps, ventral or dorsal margin of a ctenidium, or a combination of all pallial organs. Therefore, the anatomical location of particle selection remained uncertain.

Endoscope-directed, in vivo sampling demonstrated that the ctenidia of *Crassostrea virginica* and *C. gigas* function in particle selection. At all concentrations tested, for both oyster species, there was a significantly lower proportion of *Rhodomonas lens* cells in the ventral groove material compared to the dorsal tract material (Figs. 2, 3; Table 1), a consequence of particle selection by the ctenidia. In contrast, the ctenidia of *Mytilus trossulus* does not seem to be involved in particle selection. The proportion of *R. lens* cells in the ventral grooves, at all concentrations tested, was similar to that in the food supply (EI close to zero, Fig. 4). In addition, EIs of material in the ventral groove of *M. trossulus* were significantly different from those of the two oyster

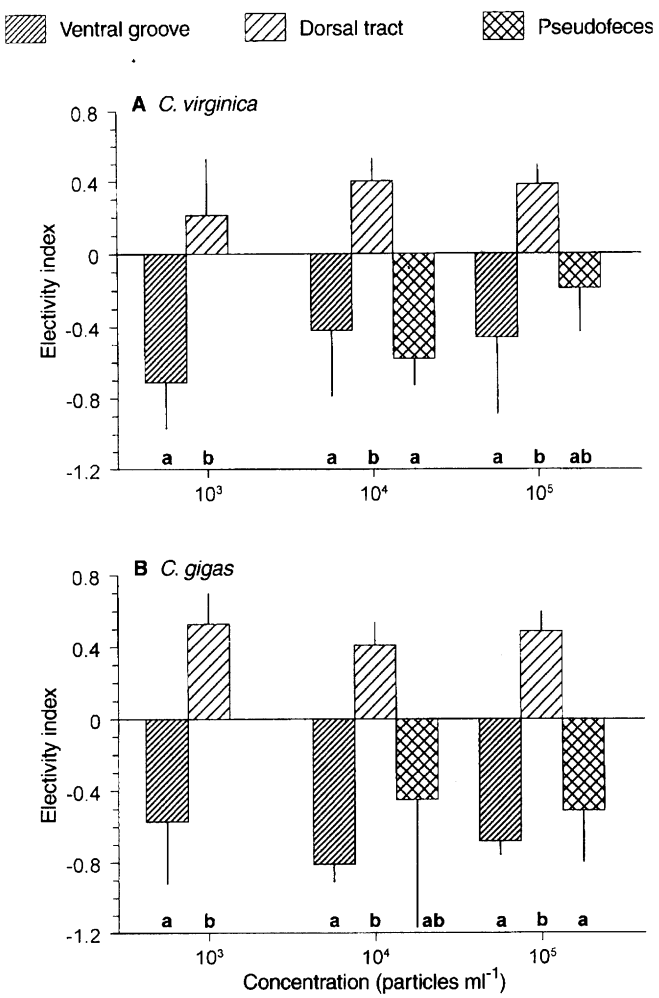

Fig. 3 *Crassostrea virginica*, *C. gigas*. Electivity indices (EI, mean ± SD) of samples taken in vivo from the ctenidia (ventral groove, dorsal tract), and from the pseudofeces of two oyster species feeding on a mixture of *Spartina alterniflora* particles and *Rhodomonas lens* cells at three concentrations. Within each concentration, sample indices with the same lower case letter are not significantly different ($p > 0.05$), whereas different letters indicate a significant difference between samples ($p < 0.05$). A negative EI indicates a depletion of *R. lens* cells, whereas a positive EI indicates enrichment of *R. lens* cells in the sample. **A** *C. virginica*. At all concentrations, EIs of the ventral groove were significantly different from those of the dorsal tract. EI of pseudofeces at 10^4 was significantly different from that of the dorsal tract. **B** *C. gigas*. At all concentrations, EIs of the ventral groove were significantly different from those of the dorsal tract. EI of pseudofeces at 10^5 was significantly different from that of the dorsal tract

species. Our data suggest that the ctenidia of *M. trossulus* non-selectively transport particles to the ventral grooves.

The role of the ctenidia of bivalves in particle selection has been debated for many years (Nelson 1923, 1960; Yonge 1926; Atkins 1937; Menzel 1955; Newell and Jordan 1983; Ward et al. 1994). In oysters, sorting of particles based on size and other criteria has been inferred from the heterorhabdic nature of the ctenidium (principal and ordinary filaments) and the two ciliated tracts on the frontal surface of the ordinary filaments

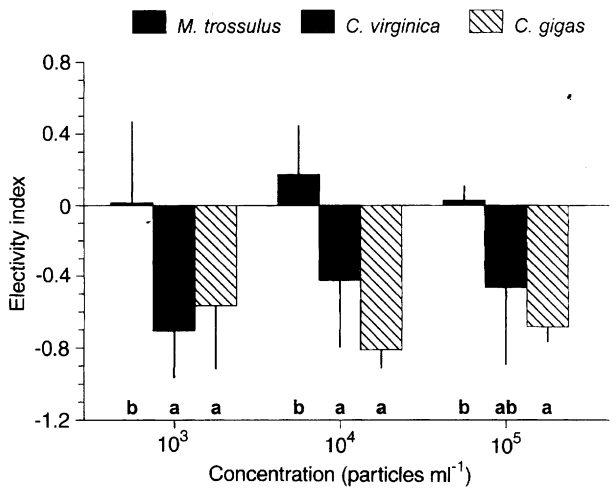

Fig. 4 *Mytilus trossulus*, *Crassostrea virginica*, *C. gigas*. Electivity indices (EI, mean ± SD) of samples taken in vivo from the ventral grooves of the ctenidia of three bivalve species delivered a mixture of *Spartina alterniflora* particles and *Rhodomonas lens* cells at three different concentrations. Within each concentration, sample indices with the same lower case letter are not significantly different ($p > 0.05$), whereas different letters indicate a significant difference between samples ($p < 0.05$). A negative EI indicates a depletion of *R. lens* cells, whereas a positive EI indicates enrichment of *R. lens* cells in the sample. EIs of material in the ventral groove of *C. virginica* and *C. gigas* were significantly different from those of *M. trossulus* at all concentrations, except for *C. virginica* at 10^5 particles ml^{-1}

that beat in opposite directions. Manipulative studies – using juvenile oysters with transparent shells, surgically altered adults, or isolated ctenidia – have produced

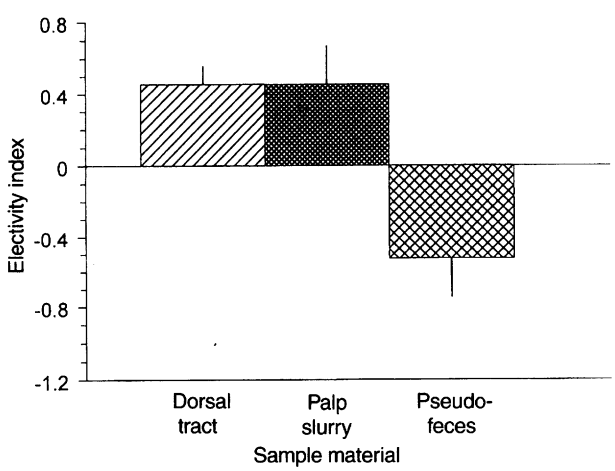

Fig. 5 *Crassostrea gigas*. Electivity indices (EI, mean ± SD) of samples taken in vivo from the ctenidia (dorsal tract) and labial palp (palp slurry), and from the pseudofeces. Oysters were delivered a mixture of *Spartina alterniflora* particles and *Rhodomonas lens* cells at 10^4 to 10^5 particles ml^{-1}. A negative EI indicates a depletion of *R. lens* cells, whereas a positive EI indicates enrichment of *R. lens* cells in the sample. EIs of the dorsal-tract and palp-slurry samples were significantly different from EI of the pseudofeces ($p < 0.05$). No significant difference was found between EIs of the dorsal-tract and palp-slurry samples ($p > 0.05$)

Table 1 *Crassostrea virginica*, *C. gigas*. Sorting efficiencies calculated for samples taken in vivo from the ctenidia (ventral groove, dorsal tract), and from the pseudofeces of oysters feeding on a mixture of *Spartina alterniflora* particles and *Rhodomonas lens* cells at three concentrations. Sorting data are presented as means ± SE. *C. virginica*, $n = 4$; *C. gigas*, $n = 6$

Species, sample type	Sorting efficiencies (%)		
	10^3 (particles ml^{-1})	10^4 (particles ml^{-1})	10^5 (particles ml^{-1})
C. virginica			
Ventral groove	−77 ± 11	−45 ± 20	−48 ± 24
Dorsal tract	50 ± 35	59 ± 14	53 ± 10
Pseudofeces	–	−62 ± 8	−22 ± 14
C. gigas			
Ventral groove	−64 ± 14	−82 ± 4	−67 ± 3
Dorsal tract	187 ± 58	52 ± 11	47 ± 8
Pseudofeces	–	−42 ± 34	−48 ± 11

conflicting results concerning particle selection by the ctenidium (cf., Allen 1921; Nelson 1923; Menzel 1955; Bernard 1974). In addition, using flow-cytometry techniques several workers have reported that *Ostrea edulis* (Shumway et al. 1985), *Crassostrea virginica* (Bougrier et al. 1997), and *Mytilus edulis* (Newell et al. 1989; Bougrier et al. 1997) preferentially retain certain species of microalgae on the ctenidia, sometimes at particle concentrations below the levels known to induce pseudofeces production. The mechanisms involved in this discrimination, however, have yet to be determined. Despite inconsistent observations of particle selection by the ctenidia, current thinking suggests that in oysters, small, more-desirable particles enter the plical troughs and are carried by the principal filaments to the dorsal ciliated tracts; larger, less-desirable particles are more likely to be captured by the ordinary filaments. On the ordinary filaments, the fine frontal cilia are thought to trap smaller, more-nutritious particles and to transport them to the dorsal tracts, whereas large, less-nutritious particles are transferred to the coarse frontal cirri and transported to the ventral grooves. Based on in vivo observations, Ward et al. (1994) found no evidence for particle selection by ctenidium of the oyster *C. virginica*, but this study used polystyrene beads treated with microalgal metabolites, and not natural particles. The present study clearly indicates that selection of natural particles by the ctenidium does occur.

Our study is the first to support conclusively the concept of particle selection by the ctenidia of oysters. Although particle size may be a factor in sorting by the ctenidium (see Allen 1921; Yonge 1926; Nelson 1960), our results suggest that size played little role in the separation of *Spartina alterniflora* particles from *Rhodomonas lens* cells in either oyster species. The size distribution of *S. alterniflora* completely overlapped the distribution of *R. lens* cells, and the relative distribution of *S. alterniflora* particles in the water, dorsal tracts, and ventral grooves was similar. These results indicate that the same proportions of small, medium, and large *S. alterniflora* particles were found in both tracts and the water.

Defossez and Hawkins (1997) have demonstrated that particle size can be a factor in selective rejection of particulate matter by bivalves, and suggest that size-dependent rejection may be the factor controlling selection between organic (typically smaller) and inorganic (typically larger) particles by bivalves. They also suggest that incomplete separation of particles in pseudofeces has confounded the issue of size selection in previous studies. In our study we obtained excellent disaggregation of particles in our samples, which yielded size–frequency distributions similar to those in the water at the time of sampling. Complete disaggregation of particle masses in our study was facilitated by two factors. First, material in the dorsal tract is not bound in cohesive mucus and is easily dispersed. Second, flow-cytometry requires only small amounts of material for analyses, so mucous-bound material (e.g., ventral groove material and pseudofeces) can easily be diluted and dispersed.

In the mussel, *Mytilus trossulus*, the ctenidia seem to play little or no role in particle selection, but the pseudofeces were significantly depleted of *Rhodomonas lens* cells. These results suggest that the labial palps are the main sorting organ in mussels. The role of the labial palps in particle selection by oysters is less clear. When oysters were delivered 10^4 and 10^5 particles ml^{-1}, there was no significant difference between EIs of the ventral groove material and EIs of the pseudofeces. This indicates that the proportional depletion of *R. lens* cells in the pseudofeces was similar to that in the ventral groove, and suggests that no further selection of material occurred (Fig. 3; Table 1). Similarly, EIs of particulate matter between the labial palps (palp slurry) were not significantly different from EIs of material in the dorsal tracts. This indicates that the proportional enrichment of *R. lens* cells in the dorsal tract material was similar to that between the palps, and again suggests that no further selection occurred (Fig. 5).

Our results imply that the labial palps of oysters play little role in particle sorting, and are contrary to the currently accepted view of the labial palps as organs of selection (e.g., Kiørboe and Møhlenberg 1981; Newell

and Jordan 1983; Newell and Langdon 1996). There are several possible reasons, however, why we did not observe an enhancement of the quality of material by the labial palps. First, the pseudofeces of oysters collected from the bottom of the assay chambers could have been produced by complete rejection of mucous strings from the ventral grooves of the ctenidia, as described by Ward et al. (1994). Second, the slurry sampled from between the palp lamellae was collected near the posterior portion of the labial palps. It is possible that particle selection (e.g., enrichment of *Rhodomonas lens* cells) occurs gradually along the entire length of the palps, resulting in enhanced quality near the buccal region. Unfortunately, collection of material from the buccal region resulted in excessive disturbance of the oyster and could not be accomplished. Based on our findings, we suggest several possible functions of the labial palps in oysters, and perhaps other bivalves with heterorhabdic ctenidia: (1) the labial palps are not involved in particle selection, but act to reduce the volume of material ingested, as proposed by Bernard (1974) and Foster-Smith (1978); (2) the palps are an accessory sorting organ enhancing the quality of material to be ingested via continual, but gradual selective rejection of particles as material is carried anteriorly towards the buccal region; or (3) the palps play a more significant role in particle selection under particle regimes more complex than those tested in our study.

Although we determined the anatomical sites of particle selection in several bivalve species, the actual mechanism(s) of selection and the criteria upon which particles are sorted, remain unknown. For particles of the same size, hypothesized criteria include particle shape, density, chemical composition, or surface properties. Particle chemistry can play a significant role in triggering acceptance or rejection of particulate matter in the blue mussel, *Mytilus edulis* (Ward and Targett 1989). This suggests that contact chemoreception of epiparticulate metabolites is involved in the observed particle selection by bivalves. Such chemically mediated particle selection has been demonstrated for certain species of zooplankton (Poulet and Marsot 1978; Rassoulzadegan et al. 1984; Huntley et al. 1986; Van Alstyne 1986; Cowles et al. 1988; Uye and Takamatsu 1990). In bivalves, however, chemosensory cells have yet to be conclusively identified on the pallial organs (i.e., ctenidia and labial palps) involved in the selection process. Surface properties of particles, such as electrostatic charge, also could account for some of the selection of particles by bivalves. The electrostatic charge and density of particles has been shown to affect capture efficiency in bivalve larvae (Gallager et al. 1988; Solow and Gallager 1990), and in other zooplankton that capture particles at low Reynolds numbers (Gerritsen and Porter 1982; LaBarbera 1984; Monger and Landry 1990). The role of electrostatic charge, density, and wettability of particles in the selection process of adult bivalves is not clear (N.M. Targett, N.H. Vrolijk, and J.E. Ward, unpublished data).

Suspension-feeding bivalves process and ingest thousands of particles per second (Jørgensen 1966; Foster-Smith 1975), and most species retain particles with a diameter of ≥5 μm with 90 to 100% efficiency (Møhlenberg and Riisgård 1978; Jørgensen et al. 1984; Riisgård 1988). Therefore, particle selection must be a rapid, continual process operating on various functional levels. We suggest that morphological differences in the ctenidia of different bivalve species reflect functional differences in the ability for particle selection. Species whose ctenidia possess ciliary tracts that beat in opposing directions (e.g., heterorhabdic ctenidia of oysters and scallops) have evolved a mechanism for bi-directional transport of material and particle selection on the ctenidia. Species whose ctenidia lack such an arrangement of ciliary tracts must rely exclusively on the labial palps for particle selection.

Endoscope-directed, in vivo sampling combined with flow-cytometry is a powerful technique for elucidating the mechanisms of selection in living, intact bivalves. In future studies we will examine further the role of the ctenidia and labial palps of bivalves in particle selection of a wider range of natural particulates, and determine some of the physical and chemical factors mediating the selection process.

Acknowledgements We thank G. Rivara and the Cornell Cooperative Extension Hatchery for the supply of *Crassostrea virginica*, Westcott Bay Farms for the supply of *Crassostrea gigas* and *Mytilus trossulus*, and G. Wikfors (NMFS) for supplying us with various batches of microalgal cultures. This research was funded by a grant from the National Science Foundation (OCE-9416943 and Research Experiences for Undergraduates supplemental funds), and a Faculty Research Grant from Salisbury State University (JEW). We appreciate this support.

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