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Particle Transport in the Zebra Mussel, *Dreissena polymorpha* (Pallas)

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Abstract. The capture, transport, and sorting of particles by the gills and labial palps of the freshwater mussel *Dreissena polymorpha* were examined by endoscopy and video image analysis. More specifically, the morphology of the feeding organs in living zebra mussels was described; the mode and speeds of particle transport on the feeding organs was measured; and the sites of particle selection in the pallial cavity were identified. Particle velocities (outer demibranch lamellae, 90 $\mu m\ s^{-1}$; inner demibranch lamellae, 129 $\mu m\ s^{-1}$; marginal food groove of inner demibranchs, 156 $\mu m\ s^{-1}$; dorsal ciliated tracts, 152 $\mu m\ s^{-1}$), as well as the movement of particles on the ctenidia and labial palps of *D. polymorpha*, are consistent with mucociliary, rather than hydrodynamic, transport. Particles can be sorted on the ctenidia of zebra mussels, resulting in a two-layer transport at the marginal food groove of the inner demibranch. That is: preferred particles are transported inside the marginal groove proper, whereas particles destined for rejection are carried superficially in a string of mucus. Sorting also occurs at the ventral margin of the outer demibranch; desirable particles are retained on the outer demibranch, whereas unacceptable particles are transferred to the inner demibranch and ultimately excluded from ingestion. We suggest that the structure of homorhabdic ctenidia does not preclude particle sorting, and that some ecosystem modifications attributed to zebra mussels may ultimately be due to ctenidial sorting mechanisms.

Introduction

Many suspension-feeding organisms, including bivalves, sort food particles on the basis of size (Vahl, 1972; Stenton-Dozey and Brown, 1992; Defossez and Hawkins, 1997) and quality (MacDonald and Ward, 1994; Arifin and Bendell-Young, 1997; Ward *et al.*, 1997). Moreover, endoscopic examination and video image analysis are now frequently used to directly observe particle capture, transport, and sorting in marine bivalves (Ward *et al.*, 1991, 1998; Beninger *et al.*, 1992; Ward, 1996). With this method, pallial structures can be observed *in vivo* in relatively undisturbed specimens, so that the direction, velocity, fate, and hydrodynamic mechanisms of ciliary transport of particles within the pallial cavity can be determined directly (Ward *et al.*, 1993). The endoscope does not disturb the morphological and hydrodynamic relationships of pallial organs, an advantage over the usual techniques of excision and surgical alteration of living bivalves (Nelson, 1960; Galtsoff, 1964; Jørgensen, 1966).

In previous studies, Ward *et al.* (1998) found that particle sorting in oysters (subclass Pteriomorphia; psuedolamellibranch, heterorhabdic, plicate gills) takes place on the ctenidia; particles of differing food qualities are partitioned between the marginal groove and the dorsal ciliated tract. In contrast, the ctenidia of marine mussels (subclass Pteriomorphia; filibranch, homorhabdic, nonplicate gills) play little role in particle selection. Ward *et al.* (1998) suggested that selection by the oyster ctenidia reflects the greater complexity of those organs. (For review of bivalve gill anatomy, see Atkins, 1937a,b; Ruppert and Barnes, 1994.)

Zebra mussels [*Dreissena polymorpha* (Pallas, 1771)] are freshwater suspension-feeding bivalves in the subclass Heterodonta. Like marine mussels, zebra mussels have homorhabdic, nonplicate ctenidia. But, unlike marine mussels, they are eulamellibranchs; ctenidial filaments are connected by interfilamentous tissue junctions. This ctenidial condition is more similar to that of the pseudolamellibranch oysters in which the filaments are connected by some,

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Abbreviations: OIT, optical insertion tube of an endoscope.

although not extensive, interfilamentous tissue junctions. An examination of zebra mussel ctenidia, therefore, might indicate whether ctenidial sorting is related to morphology or to phylogeny. Indeed, recent flow-cytometry studies by Baker *et al.* (1998) demonstrate that the zebra mussel pallial organs effectively sort particles. In this investigation, we use endoscopic examination to observe (1) the morphology of feeding organs in living zebra mussels, (2) the mode and speeds of particle transport on the feeding organs, and (3) sites of particle selection in the pallial cavity. We compare our endoscopic examination of zebra mussels with previous reports of feeding processes in both zebra mussels and marine bivalves.

Zebra mussels have invaded many freshwater localities in Europe and North America. In systems where large populations of these mussels have become established, not only has phytoplankton biomass declined (Holland, 1993; Fahnenstiel *et al.*, 1995; Caraco *et al.*, 1997), but seston composition has changed as well (Vanderploeg *et al.*, 1996; Smith *et al.*, 1998; Strayer *et al.*, 1999). Understanding the form and function of feeding structures in zebra mussels and their mechanisms of particle selection will contribute to a better understanding of these effects on ecosystems.

Materials and Methods

Phytoplankton cultures were obtained from the University of Texas Culture Collection and grown in a freshwater enrichment medium WCL1 (Guillard, 1983; Guillard and Hargraves, 1993). Cultures were grown at room temperature, under a 16:8 h light:dark regime. Species of phytoplankton that were cultured included *Cyclotella meneghiniana* (LB 2455; barrel-shaped, 18 × 6 μm) (Bacillariophyceae), *Crucigenia tetrapedia* (63; disk-shaped, 5 × 11 μm), *Scenedesmus quadricauda* (LB 614; four cells stacked, total 25 × 10 μm) (Chlorophyceae), and *Microcystis aeruginosa* (LB 2386; spherical, 4 μm) (Cyanophyceae). Cell dimensions were measured with a compound microscope and calibrated ocular micrometer.

Nonliving particles were also used in endoscopic observations. Polystyrene beads (Polysciences, Inc., Warrington, PA) of 1, 10, or 22 μm were often used as tracer particles. Dead cattail (*Typha* sp.) leaves from the previous growing season were collected for use as detrital material. The leaves were washed of debris and processed in a blender with distilled water for 5 min. The resulting suspension was sieved through a 20-μm nylon screen, and the retained particles (>20 μm) were discarded; 90% of the particles in the remaining suspension were ≤3.5 μm, as measured by an electronic particle counter (Coulter Electronics, Multisizer).

Specimens of *Dreissena polymorpha*, about 20 mm in length, were collected from the Hudson River at Tivoli, New York, or from the Huron River, Ann Arbor, Michigan. Mussels were maintained in 40-l aquaria at 16°C and fed a daily ration of cultured phytoplankton plus a mixture of preserved diatoms (Diet C, Coast Seafoods, Co., Quilcene, WA). Partial water changes (*ca.* 20%) were performed on alternating days; freshwater was prepared according to Sprung (1987).

We prepared zebra mussels for endoscopy by drilling a small hole (<2 mm in diameter) in one valve with a rotary tool (Dremel, Racine, WI) and cauterizing the underlying mantle tissue. The hook side of a piece of hook and loop fastener (Velcro brand) was cemented with epoxy to the valve opposite the drilled hole for later use in positioning the animal for examination. The mussels were allowed to recover for at least one day. This treatment caused no apparent adverse change in the behavior of the mussels, and shell and mantle repair at the site of the drilled hole often began within several days.

Endoscopic examinations were performed according to Ward *et al.* (1991, 1993, 1994). An endoscope (K12-09-15-53, Olympus Corp., Lake Success, NY), with an optical insertion tube (OIT) of 1.2 mm diameter, was connected to an optical zoom-adaptor (Schölly Fiberoptic, Denzlinger, Germany) and attached to a monochrome or color CCD camera (4990 or 8280, Cohu Electronics, San Diego, CA). A halogen (HLS24-0, Welch Allyn, Skaneateles Falls, NY) or xenon lamp (ALS-6250U, Olympus High Intensity Helioid Light Source) provided light to the OIT. The camera and endoscope were mounted on a macro-focusing rail, allowing fine adjustments of the OIT. Video was recorded at 30 frames s^{-1} on an sVHS videocassette recorder (VCR) (AG-1960, Panasonic Industrial Company, Secaucus, NJ).

For endoscopic examination, mussels were placed in a 500-ml plastic container set in a 15-l water bath with its temperature maintained between 16° and 18°C. A dome inside the plastic container had been covered with the loop side of Velcro-brand fastener, allowing rapid mounting and precise positioning of mussels. The OIT was inserted into the pallial cavity of the mussel through the inhalent siphon, the pedal gape, or the drilled and cauterized hole. Recordings were made after the mussel showed active feeding behavior, as indicated by extension of the mantle and siphons and by the intake of particles. Mussels were exposed to suspensions of one or two particle types at concentrations of 10^4, 10^5, and 10^6 particles ml^{-1}. Particle suspensions were delivered to the plastic container by gravity from a 4-l carboy. The container was frequently flushed to maintain particle concentration, which was also monitored with a Coulter Multisizer.

We observed and recorded the positions and movements of the ctenidia and labial palps, as well as the movement of particles on these organs. The best observations were made when the OIT was inserted into the pallial cavity through the drilled hole in the shell and mantle. In this position, the mussels fed normally, uninterrupted by movements of the OIT. Although the pallial cavity could be entered through

the inhalent siphon, any movement of the OIT resulted in the cessation of feeding. And when the pallial cavity was entered through the pedal gape, the foot usually touched the OIT, coating it with mucus. Results were based on the examination of 21 mussels.

Particle velocities on feeding structures were determined from the number of video frames required for a particle to traverse a known distance. Distances were calibrated according to Ward (1996): *i.e.*, the pallial organs were dissected from several mussels, and the widths of the ctenidial filaments, palp ridges, and marginal grooves were measured with a compound microscope equipped with a calibrated ocular micrometer. Velocities ($\mu m\ s^{-1}$) are presented as means ± 1 standard deviation.

Results

When observed by endoscopy, the positions of the ctenidia within the pallial cavity are different from those that might be expected from dissected specimens (Fig. 1). The demibranchs are held curved towards the visceral mass, and the ventral margin of the outer demibranchs is particularly bent inward (Fig. 2). These gill postures are maintained despite variation in the overall orientation of the mussels.

Through the relatively transparent ciliated epithelia of the ctenidia, we observed internal bands of muscular cross-struts (Medler and Silverman, 1997) that are perpendicular to the ctenidial filaments and 60–80 μm apart. Ostia, located in the epithelium of the interfilamentary spaces, are lacking directly above the struts (Medler and Silverman, 1997).

The inhalent flow of suspended particles sometimes stops, or even reverses momentarily, especially under high particle concentrations. In addition, the ctenidia often contract during active feeding; the interfilamentary spaces, where the ostia are located, alternately flare and close at a rate of 1 cycle s^{-1}. The extension of the mantle and siphons, often used as an indication of steady feeding, does not change during flow cessation and reversals, or during pulsation of the ctenidia.

Particles captured by the ctenidia move smoothly along the frontal surfaces of the ctenidial filaments, and particles of different types and sizes maintain their distance from each other. Mucous strings were observed on the frontal surfaces of the filaments only when the particle concentration was extremely high.

Outer demibranchs and their ventral margins

The outer demibranchs and their ventral margins were observed in seven specimens, on 42 occasions, for 15.5 h of total observation time and 1.7 h of video recording. The outer demibranchs are held relatively straight, but with an inward bend, especially of the ascending lamella, near the ventral margin (Fig. 2). The position of the outer demibranch changes with pumping activity: the outer demibranch is positioned near the inner demibranch when inhalent flow speeds are low; as flow speeds increase, the outer demibranch moves laterally away from the inner demibranch (Fig. 1). Particles captured on the descending lamella of the outer demibranch are transported dorsally to the

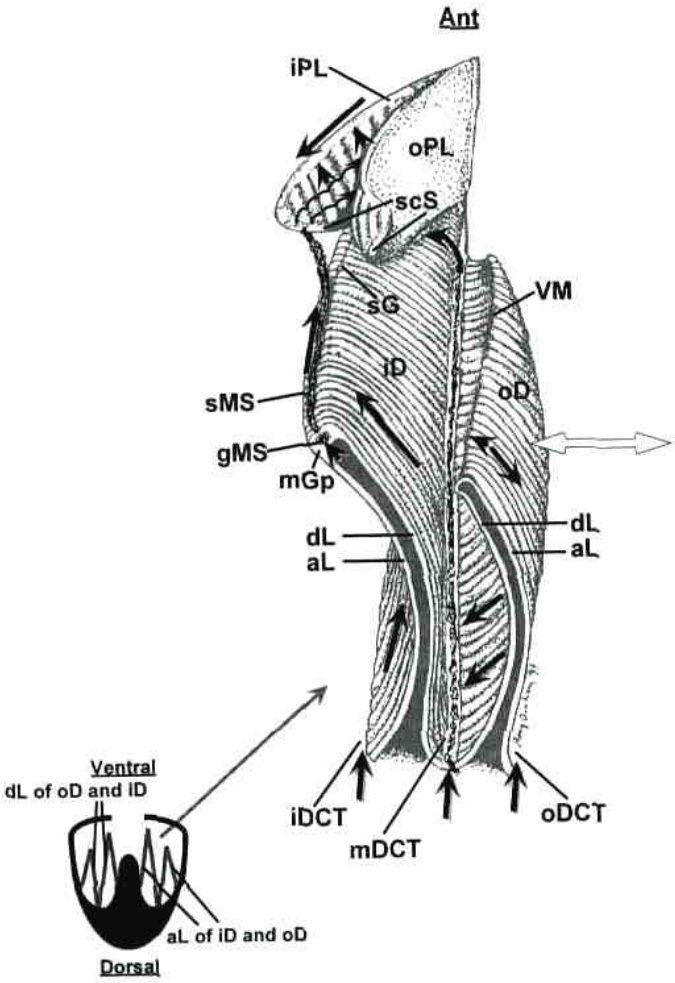

Figure 1. Diagram of labial palps and anterior portions of the inner and outer demibranchs of *Dreissena polymorpha* as observed through the endoscope (looking dorso-anteriorly). Palp lamellae are shown slightly spread apart, with the outer Palp lamella (oPL) curled back. Solid arrows indicate particle paths after capture. The white arrow indicates movement of the outer demibranch (oD). Particles in the medial dorsal ciliated tract (mDCT) are transported in mucous clumps and strings. Particles are transported at the ventral margin of the inner demibranch (iD) as both a groove mucous string (gMS) in the marginal groove proper (mGp) and as a superficial mucous string (sMS). The palps (iPL and oPL) enclose the inner demibranch only, drawing in the superficial mucous string (sMS) from the inner demibranch (iD) (see text for details). (aL = ascending lamella, Ant = anterior, dL = descending lamella, gMS = groove mucous string, iD = inner demibranch, iDCT = inner dorsal ciliated tract, iPL = inner palp lamella, mDCT = medial dorsal ciliated tract, mGp = marginal groove proper, oD = outer demibranch, oDCT = outer dorsal ciliated tract, oPL = outer palp lamella, scS = smooth ciliated surface, sG = superficial groove, sMS = superficial mucous string, VM = ventral margin.) See *Video Note*, p. 124.

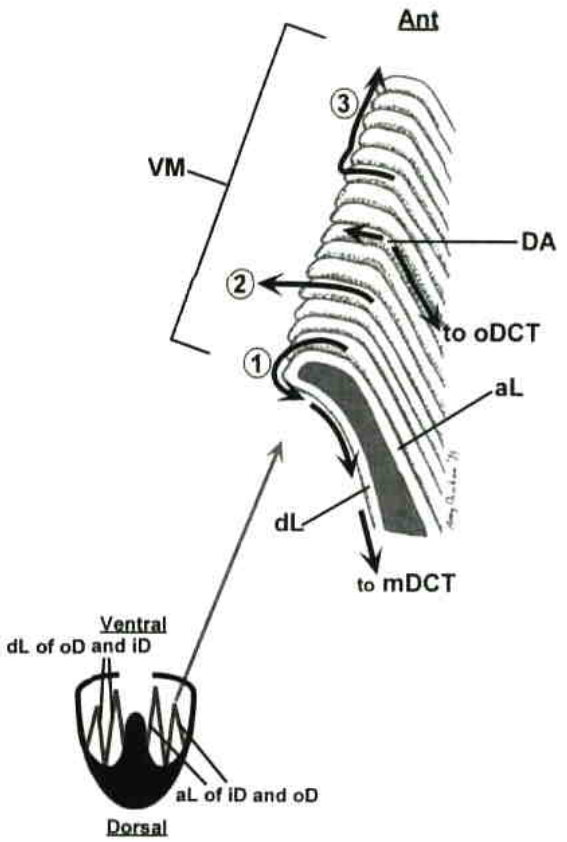

Figure 2. Diagram of the ventral margin (VM) of the outer demibranch (oD) of *Dreissena polymorpha* as observed through the endoscope (looking dorso-anteriorly). Solid arrows indicate particle paths after capture. Particles on the ascending lamella (aL) are transported either dorsally or ventrally, depending on whether they are above or below a divergence area (DA) when captured. Upon reaching the ventral margin (VM), particles traveling ventrally either (1) move over the ventral margin and proceed dorsally on the descending lamella (dL); (2) continue moving ventrally, leaving the surface of the ctenidia and becoming resuspended; or (3) make a right angle turn and begin moving anteriorly on the ventral margin (VM) (see text for details). (aL = ascending lamella, Ant = anterior, DA = divergence area, dL = descending lamella, mDCT = medial dorsal ciliated tract, oDCT = outer dorsal ciliated tract, VM = ventral margin.) See *Video Note*, p. 124.

medial dorsal ciliated tract (Fig. 2). On the ascending lamella of the outer demibranch, particles are transported either dorsally or ventrally, depending on whether they were captured above or below a divergence area located near the bend of the demibranch (Figs. 1, 2). When particles are captured directly at the divergence area, they oscillate in place for several seconds before proceeding either ventrally or dorsally. The position of the divergence area can shift ventrally or dorsally by about 1 mm. This shift does not appear to be correlated with any particular stimulus, such as particle type or concentration. Mean particle velocity on the frontal surfaces of the ascending lamella of the outer demibranch is 90 μm s^{-1} (Table 1).

Due to the bend of the outer demibranch, the ventral margin is pointed towards the inner demibranch, and the space between the two demibranchs is small (Fig. 1). Particles captured ventral to the divergence line on the ascending lamella of the outer demibranch move ventrally. Upon reaching the ventral margin, one of the following three behaviors occurs (Fig. 2): (1) The particles move over the ventral margin and proceed dorsally on the descending lamella of the demibranch. (2) The particles continue moving ventrally, leave the surface of the ctenidia, and become resuspended. Most often, these resuspended particles are then recaptured by the descending lamella of the inner demibranch and continue moving ventrally. The majority of particles that leave the ventral margin of the outer demibranch are large, like *Scenedesmus*. (3) The particles make a right angle turn and begin moving anteriorly on the ventral margin. Individual particles bounce along the ventral margin from filament to filament at a mean velocity of 65 μm s^{-1} (Table 1). At high concentrations ($>10^6$ ml^{-1}), particles are sometimes carried along the ventral margin in clumps of mucus. As a result of these three particle trajectories, desirable particles are retained on the outer demibranch, while unacceptable particles are transferred to the inner demibranch and ultimately excluded from ingestion.

Inner demibranchs and their marginal grooves

The inner demibranchs and their marginal grooves were observed in seven specimens, on 60 occasions, for 29.7 h of total observation time and 2.9 h of video. Particles captured on either descending or ascending lamellae of the inner demibranch are transported toward the ventral margin, whose mean width is 276 μm (n = 7; Fig. 1). Mean particle velocities on the ascending and descending lamellae of the inner demibranch are 129 μm s^{-1} (Table 1).

Material at the ventral margin of the inner demibranch is transported anteriorly in one of two channels, one deep and one superficial (Figs. 1, 3). The deep channel, the marginal groove proper, is nearly enclosed by the projection of the ventral tips of the filaments over the groove. The superficial

Table 1

Particle velocities (22-μm polystyrene beads) on the pallial organs of zebra mussels, Dreissena polymorpha, *at 18°C*

Location	Mean velocity ± SD (μm s^{-1})	Range (μm s^{-1})	n
Outer demibranch			
Frontal surface	90 ± 22	60–123	9
Ventral margin	65 ± 23	24–104	19
Inner demibranch			
Frontal surface	129 ± 54	42–251	33
Ventral food groove	156 ± 53	45–354	106
Dorsal ciliated tract	152 ± 62	41–305	50
Labial palps			
Frontal surfaces	94 ± 34	54–150	10
Ventral margin	54 ± 21	16–113	33

channel or groove is a depression at the center of the ventral margin, with openings between adjacent and opposite filament tips leading to the marginal groove proper (Fig. 3). The superficial groove is entirely exposed to the inhalent flow.

Particles transported in the superficial groove are embedded in a string of mucus, up to 80 μm thick (Figs. 1, 3), which moves at a mean velocity of 156 μm s^{-1} (Table 1). The presence of a superficial mucous string, as opposed to a particle slurry, was confirmed by observing the dislodgment of strings from the ventral margin when the valves were rapidly adducted, or when a jet of water was pipetted into the hole through which the OIT was inserted. After dislodgment, the superficial mucous string remains unbroken and returns to the ventral margin of the demibranch. Polystyrene spheres (1 μm) and the large green alga *Scenedesmus* are incorporated into the superficial mucous string and are eventually rejected as pseudofeces.

Particles transported anteriorly inside the marginal groove proper are also embedded in a string of mucus (Figs. 1, 3). These particles appear to be those that are eventually ingested. This observation was confirmed when specimens were fed a combination of *Microcystis*, which is preferentially ingested, and *Scenedesmus* or *Typha* detritus, which are both rejected (Baker *et al.*, 1998). The resulting colors of the mucous strings, as well as the relative particle sizes, indicate that *Microcystis* is incorporated into the groove mucous string, whereas *Scenedesmus* or *Typha* is incorporated into the superficial mucous string. Two other observations suggest that particles moving inside the marginal groove proper are also embedded in mucus. First, particles inside the marginal groove proper move at the same velocity as particles transported in the superficial mucous string. Second, particles both inside and outside the marginal groove maintain positions relative to each other as they move anteriorly. Additional observations suggest that the deep and superficial mucous strings are not continuous with each other, but are physically separate. For example, when the superficial mucous string is dislodged from the superficial groove, the groove mucous string is not disturbed and remains within the marginal groove proper.

Particles approaching the ventral margin of the inner demibranch have three potential fates (Fig. 3): (1) As particles round the crest of the ventral margin, some move anteriorly and diagonally, bouncing from filament to filament, before being entrained in the superficial mucous string. These particles join the more ventral portion of the superficial mucous string, and move in a uniform, smooth manner (Fig. 3, path 1). (2) Other particles move laterally prior to rounding the crest of the ventral margin, and enter the interfilamentary space between two adjacent filaments. Some of these particles stall, oscillate for several seconds, and then disappear from view, possibly lost to an underlying water tube through an ostial opening. Many particles, however, continue moving toward the superficial groove and became entrained in the more dorsal portion of the superficial mucous string. These particles do not move smoothly but follow the contours of the filament tips, bouncing as they move anteriorly (Fig. 3, path 2). (3) Still other particles appear to remain on the frontal surfaces of the filaments until reaching the tips, where they move laterally into the

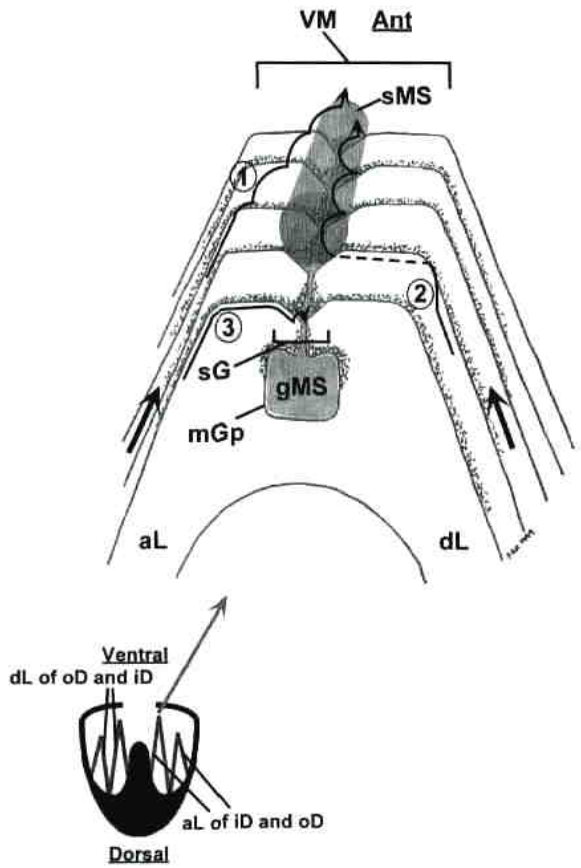

Figure 3. Diagram of the ventral margin of the inner demibranch of *Dreissena polymorpha* as observed through the endoscope (looking dorso-anteriorly). The groove mucous string (gMS) and a portion of a superficial mucous string (sMS) are shown. Solid arrows indicate particle paths after capture. Shaded arrows indicate particle paths behind a filament. Particles approaching the ventral margin (VM) of the inner demibranch (iD) enter the mucous strings in three ways: (1) As particles round the crest of the ventral margin (VM), they move anteriorly and diagonally, bouncing from filament to filament. These particles are entrained in the top (ventral side) of the superficial mucous string, where they move smoothly towards the anterior of the ctenidium. (2) Particles move laterally prior to rounding the crest of the ventral margin and enter the furrow between two adjacent filaments (dashed arrow). These particles become entrained in the bottom (dorsal side) of the superficial mucous string and follow the contours of the filament tips as they move anteriorly. (3) Particles remain on the frontal surfaces of the filaments until reaching the tips, where they move laterally and down (dorsally) into the marginal groove proper (see text for details). These particles move smoothly towards the anterior in the groove string. (aL = ascending lamella, Ant = anterior, dL = descending lamella, gMS = groove mucous string, mGp = marginal groove proper, sG = superficial groove, sMS = superficial mucous string, VM = ventral margin.) See *Video Note*, p. 124.

marginal groove proper through gaps between adjacent and opposing filament tips (Fig. 3, path 3). Those particles transported in the groove proper are alternately seen through the gaps between adjacent filament tips and, faintly, as they pass behind the relatively transparent filament tips.

Because the superficial mucous string is sometimes opaque, we were unable to observe the marginal groove proper at all particle concentrations and types. Therefore, it is unclear whether the filament tips forming the marginal groove proper flare "open" and "closed." Although the gaps through which particles enter into the marginal groove proper appear absent at times and large at others, the overall width of the superficial groove does not change markedly, ranging from 17 to 38 μm wide (n = 10).

At high particle concentrations, the ventral margin of the inner demibranch occasionally presses against the visceral mass for several seconds. In these cases, the superficial mucous string is transferred to ciliated tracts on the visceral mass and is transported posteriorly, presumably to the inhalent siphon for rejection. Movement of the mucous string inside the marginal groove proper does not appear to be interrupted.

Dorsal ciliated tracts

The dorsal ciliated tracts were observed in three specimens, on 12 occasions, for 11.4 h of total observation time and 47 min of video. There are three dorsal tracts on each side of the visceral mass: at the junction of the viscera and inner demibranch (inner dorsal ciliated tract), between the two demibranchs (medial dorsal ciliated tract), and at the junction of the outer demibranch and the mantle (outer dorsal ciliated tract) (Fig. 1). Particles enter the medial dorsal ciliated tract from the descending lamella of the outer demibranch (Figs. 1, 2); they are carried anteriorly as individuals at low particle concentrations, or in mucous clumps and discrete strings at higher concentrations (Fig. 1). Particles moving in the medial dorsal ciliated tract sometimes stop or reverse for several seconds, and this behavior is associated with extreme flaring of the interfilamentary spaces on the adjacent demibranchs. In addition, quick successive contractions by the adjacent demibranchs seem to make the mucous clumps less cohesive. At high particle concentrations, the two demibranchs occasionally contract strongly, and a slurry of particles becomes resuspended in the pallial cavity. It was not possible to determine whether these particles are recaptured. Particles in the medial dorsal ciliated tract move at a mean velocity of 152 μm s^{-1} (Table 1).

A few particles are also transported anteriorly in the inner and outer dorsal ciliated tracts (Fig. 1). Particles enter these tracts not only from the demibranchs, but also from the mantle or body, suggesting that cilia on these surfaces can trap some particles.

Labial palps

The labial palps were observed in four specimens, on 14 occasions, for 11 h of total observation time and 2.2 h of video. Two pair of palp lamellae lie at the anterior end of the ctenidia, one pair on each side of the mouth. A pair of palps forms a functional unit consisting of one inner and one outer palp lamella (Figs. 1, 4). The apposing surfaces of each pair of palp lamellae are highly ciliated and folded into deep grooves and ridges (see Galtsoff, 1964; Ward *et al.*, 1994). The labial palps transport material from the ctenidia to the mouth, control the volume of food ingested, and may also

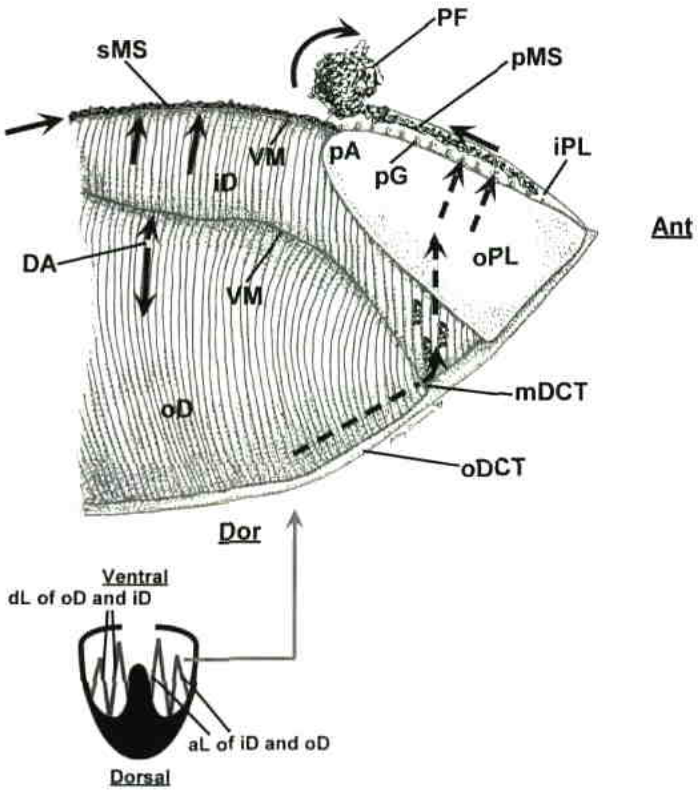

Figure 4. Diagram of the labial palps and the anterior portions of the inner and outer demibranchs of *Dreissena polymorpha* as observed through the endoscope (looking medially). Solid arrows indicate particle paths after capture. Dashed arrows indicate particle paths behind the outer demibranch (oD) and outer palp lamella (oPL). At the anterior termination of the outer demibranch (oD), mucous clumps and strings from the medial dorsal ciliated tract (mDCT) move ventrally onto the inner demibranch (iD) and enter between the palp lamellae (PL). The superficial mucous string (sMS) is drawn from the ventral margin (VM) of the inner demibranch (iD) and between the palp lamellae (PL). The material disperses on the palp lamellae (PL), and rejected particles move ventrally from the palp grooves (pG). Rejected particles are incorporated into a mucous string (pMS) that moves posteriorly and forms an irregular ball at the palp apices. This ball is expelled as pseudofeces (PF) (see text for details). (Ant = anterior, DA = divergence area, Dor = dorsal, iD = inner demibranch, iPL = inner palp lamella, mDCT = medial dorsal ciliated tract, oD = outer demibranch, oDCT = outer dorsal ciliary tract, oPL = outer palp lamella, pA = palp apex, PF = pseudofeces, pG = palp groove, pMS = palp mucous string, sMS = superficial mucous string, VM = ventral margin.) See *Video Note*, p. 124.

sort particles before ingestion (Yonge, 1926; Menzel, 1955; Newell and Jordan, 1983).

In zebra mussels, the labial palps enclose the inner demibranch only. Along the entire free dorsal edge of each palp lamella is a smooth ciliated surface that rests against the demibranch lamellae. These smooth ciliated surfaces are narrow at the distal apices of the palp lamellae and increase in width anteriorly, up to four palp ridges wide. The distal apex of the inner palp lamella often sweeps from the ascending lamella of the associated demibranch, across the superficial groove, and back, drawing in the superficial mucous string (Fig. 1).

Although the superficial mucous string is drawn between the palp lamellae at their distal apices, the superficial groove extends the entire length of the inner demibranch, ending at the oral groove between the palp pair. It was unclear whether, or at what point, the mucous string is removed from the marginal groove proper. This material may remain within the marginal groove proper to be deposited directly into the oral groove, without processing by the palps.

At the anterior terminus of the outer demibranch, mucous clumps and discrete strings from the medial dorsal ciliated tract move ventrally on the filaments of the descending lamella of the inner demibranch (Figs. 1, 4). The superficial mucous string is drawn between the palp lamellae at a point posterior to this location (Fig. 1). Rather than proceeding anteriorly in the superficial groove, the mucous clumps from the dorsal tract continue moving in a ventral direction, *entering between the labial palp lamellae.*

Mucous strings or clumps from the superficial groove or the dorsal ciliated tract disperse on the palp lamellae. Individual particles bounce anteriorly over the ridges of the palps, slowing in the grooves and moving more quickly over the ridges to the next groove (Fig. 1); the mean velocity is 94 μm s^{-1} (Table 1). The palp lamellae alternate between being spread slightly apart and being closely appressed. When the palp lamellae part slightly, particles remain close to one or the other lamella and continue their bouncing motion. Occasionally, the palp lamellae spread wide apart, and particles can be seen suspended between the apposing palp surfaces and moving posteriorly. This material may include particles that are moving ventrally from the dorsal ciliated tract. When appressed, the lamellae rub together with an anterior-posterior displacement of the width of one to two palp ridges (one palp ridge = 125 μm), and the smooth outer surfaces of the palp lamellae undulate in waves from dorsal to ventral.

Rejected particles move ventrally from the palp grooves (Figs. 1; 4). At the ventral margins of the palp lamellae, these particles form a mucous string which then moves posteriorly at a mean velocity of 54 μm s^{-1} (Table 1). The palp mucous string moves toward the distal apices of the palps, where it forms an irregular ball (Fig. 4). Once the ball of mucus and particles reaches a particular size, the palps push or "clap" the ball away. In this manner, the ball is transferred to ciliated tracts on the mantle, presumably to be expelled from the inhalent siphon or pedal gape as pseudofeces. Sometimes the palp mucous strings are transferred to the mantle before reaching the palp apices.

During exposure to high particle concentrations (10^6 ml^{-1}), the processing of particles by the labial palps changes according to the acceptability of the particles. When mussels were fed high concentrations of a combination of both desirable and unacceptable particles (*Microcystis* and *Scenedesmus*), the ball of mucus that forms near the palp apices was drawn back between the palp lamellae and once again dispersed. We observed mucous balls being reprocessed by the palps up to four times before finally being rejected. In contrast, when mussels were fed high concentrations of primarily unacceptable particles (*Scenedesmus* alone), the superficial mucous string from the inner demibranch sometimes by-passed processing by the labial palps. In this case, the superficial mucous string does not disperse on the ridged surfaces of the palp lamellae but is transferred from the marginal groove of the inner demibranch directly to the palp apices by the extreme posterior section of the smooth ciliated surface (see Fig 1). At the palp apices, the material is formed into a mucous ball and rejected.

Discussion

The observations reported here explain the efficient selection of particles measured in our previous work (Baker *et al.*, 1998) with *Dreissena polymorpha.* Particles are sorted on the ctenidia of zebra mussels, and more specifically, at the marginal food groove of the inner demibranch. We observed a two-layer transport at the marginal food groove: desirable particles appear to be transported inside the groove proper, while unacceptable particles are carried superficially. We also observed sorting at the ventral margin of the outer demibranch: desirable particles are retained on the outer demibranch, while unacceptable particles are transferred to the inner demibranch and ultimately rejected. Here, we compare and contrast our observations with previous reports of feeding processes in both zebra mussels and marine bivalves. We suggest that the structure of homorhabdic ctenidia does not preclude particle sorting, and that the changes in seston composition attributed to zebra mussels may ultimately be due to the ctenidial sorting mechanisms observed in this study.

Foster-Smith (1975) proposed that three conditions must be met for particle selection to take place at the marginal groove of bivalve ctenidia (*i.e.*, in *Mytilus edulis, Cerastoderma edule, Venerupis pullastra*): (1) some particles must be able to enter the deep area of the marginal groove; (2) particles in the deep area of the marginal groove must be physically separate from the superficial material; and (3) the

superficial material must be rejected, while the material in the deeper area of the marginal groove is accepted. The two-layer transport that we observed at the marginal groove of zebra mussels meets these requirements for particle selection.

Two-layer transport has previously been described for filibranchs and pseudolamellibranchs, but does not necessarily indicate the capacity for particle selection. Foster-Smith (1975) reported two-layer transport in *M. edulis* (filibranch), with the particles in the deep region of the marginal groove tending to be small, and those in the superficial material tending to be larger. But the two layers are contiguous, precluding particle selection. In *M. edulis,* partitioning between the two layers may be temporal, rather than physical. Jørgensen (1975) reported that particles arriving at the marginal groove might either enter the groove between the bases of the filament tips or pass outside, depending on whether the groove is open or closed. We never observed the marginal groove in zebra mussels to be "open" with filament tips flared, as Jørgensen (1975) illustrated for *M. edulis,* although our observations suggest that there may be some regulation of the amount of material allowed to enter the marginal groove proper.

Two-layer transport at the marginal groove, in combination with particle sorting, has previously been reported only for pseudolamellibranchs. Atkins (1937a) described both two-layer transport and the potential for size sorting at the marginal groove in *Pinna fragilis* and several *Pinna*-like species (pseudolamellibranchs). In these species, which have plicate heterorhabdic ctenidia, fine particles transported by the principal filaments are deposited into the depth of the marginal groove proper, while coarse particles transported by the ordinary filaments are deposited outside the groove and are usually rejected (Atkins, 1937a). Although the mode of particle introduction to the marginal groove of *D. polymorpha* differs from that observed in *Pinna* sp. due to the nonplicate nature of the zebra mussel ctenidia, the marginal groove appears to function similarly in both species.

Previous feeding studies have indicated that, in addition to selection by particle size in *D. polymorpha,* a chemical mechanism of selection is also present (Ten Winkel and Davids, 1982; Baker *et al.,* 1998). In the present study, the disparate sizes of particle types embedded in the superficial mucous string in *D. polymorpha* suggest that some factor other than size is important in the shunting of particles either to the marginal groove proper or to the superficial groove. The superficial mucous string is picked up by the apices of the palps, and much of the material is rejected. The arrangement of the ctenidium/palp junction suggests that material within the marginal groove proper may be transported to the anterior portion of the labial palps or directly to the oral groove. The differing degree to which the two mucous strings are processed by the palps suggests that the material in the superficial mucous string is of lower quality than that in the groove string. This two-layer transport at the marginal groove could potentially increase the rate of processing and decrease the possibility of sorting mistakes at the palps. Microscopic examination of the structure and function of cilia at the marginal groove may help elucidate the sorting mechanisms.

The labial palps of zebra mussels function very similarly to those of other bivalves, despite differences in demibranch structure and function. Zebra mussels have a smooth ciliated surface along the free dorso-posterior edge of the labial palp lamellae, similar to that of oysters (Ward *et al.,* 1994). Our observations of mucous ball formation near the apices of the labial palps are similar to those described for both oysters (Menzel, 1955; Galtsoff, 1964; Ward *et al.,* 1994) and marine mussels (Beninger and St-Jean, 1997a). As in oysters, *D. polymorpha* palp lamellae alternate between being appressed and being slightly separated. When separated, we observed off-surface posterior movements of particles like those reported by Galtsoff (1964) and Ward *et al.* (1994) for oysters. Ward *et al.* (1994) speculated that the posterior movement allows the particles to be cycled through the palps several times before being rejected or ingested. In addition to this type of reprocessing, we observed a second recycling method: the mucous ball forming near the palp apices is sometimes re-engulfed by the palps up to four times before finally being rejected.

Video endoscopy allowed us to observe, *in situ,* the position of the feeding organs within the pallial cavity of living zebra mussels. These observations build on previous reports of feeding organ functioning based on dissected specimens of zebra mussels (Atkins, 1937b; Morton, 1969). For example, like Atkins (1937b) and Morton (1993), we observed particles passing off the outer demibranch at the ventral margin and being transferred to the inner demibranch. Dissected preparations, however, did not allow the authors of previous studies to observe the bend in the outer demibranch and the curvature of the inner demibranch that occurs under natural feeding conditions. Our observations suggest that maintenance of the ctenidia in these positions may facilitate particle recapture; this natural ctenidial morphology enhances the transfer of some particles from the outer demibranch to the inner demibranch.

Our observations of particle transport in zebra mussels contradict some previous observations and corroborate others. For example, Atkins (1937b) described rare filaments of the descending lamella of the outer demibranch that transport particles ventrally; these particles are then passed to normal filaments that transport them dorsally. During our observations of this area (five specimens on 14 occasions, for 8.2 h total observation time), all filaments of the descending lamella of the outer demibranch transported particles dorsally. In addition, Atkins (1937b) did not report any anteriorly directed movement on the ventral margin of

the outer demibranch, such as we occasionally observed. That anterior movement is, however, similar to that of mucous-particle masses on the ventral bend of *Placopecten magellanicus* ctenidia, which also lack a ventral groove (Beninger *et al.*, 1992).

In greater contrast, both Atkins (1937b) and Morton (1969) reported ventral movement of particles on the ascending lamella of the outer demibranch, whereas we observed dorsally directed movement, above a divergence area. Atkins (1937b) reported dorsally directed currents on the ascending lamella of the outer demibranch of the Unionidae, another unrelated group of freshwater bivalves.

The dorsally directed movement on the ascending lamella of the outer demibranch allows some proportion of material to be directed to the outer dorsal tract, rather than to the medial dorsal ciliated tract between the two demibranchs, perhaps preventing overloading of the latter tract. Both Atkins (1937b) and Morton (1993) described the anterior movement in the dorsal tract at the junction of the mantle and ascending lamella of the outer demibranch as well; Atkins (1937b) noted that anterior movement in this outer tract usually occurs only in bivalves with heterorhabdic ctenidia. Partitioning material between two dorsal tracts may increase the rate of total particle transport.

Particle velocities, as well as the movement of particles on the ctenidia and labial palps of *D. polymorpha,* are consistent with mucociliary, rather than hydrodynamic, transport (Ward *et al.*, 1993; Beninger and St-Jean, 1997b). The velocities of particles transported on the frontal surfaces of the demibranchs overlap the ranges reported for *M. edulis* and for the plical crests of *C. virginica* (Ward *et al.*, 1993). In addition, the superficial mucous string at the marginal groove moves at a rate similar to the mucous strings observed in *C. virginica, M. edulis, Mya arenaria,* and *Placopecten magellanicus* (Ward *et al.*, 1993, 1994). Material in the dorsal ciliated tract, however, travels at a rate many times slower than it does in *C. virginica* or *P. magellanicus.* In *D. polymorpha,* material at the dorsal ciliated tract is embedded in mucous clumps and trains, but in the oyster and scallop, the material is in a slurry (Ward *et al.*, 1993). Transport rate is generally inversely correlated with the viscosity of the mucus (Menzel, 1955; Winet and Blake, 1980), and therefore, material in a slurry moves at a faster rate than material in more cohesive mucous clumps. The lack of hydrodynamic transport in zebra mussels may reflect a dorsal tract that is smaller and less well developed than that in oysters and scallops.

Zebra mussels have had major impacts on the freshwater systems in which they have become established. Because of the high clearance rates of these mussels, phytoplankton biomass has decreased by more than 60% in many of the invaded systems (Morton, 1971; Kryger and Riisgård, 1988; Holland, 1993; Fahnensteil *et al.*, 1995). In addition, seston composition has changed in some systems, including the Hudson River, New York, where the phytoplankton community has shifted from a prevalence of cyanobacteria to diatoms (Vanderploeg *et al.*, 1996; Smith *et al.*, 1998). Recent studies using flow cytometry (Baker *et al.*, 1998) have shown that zebra mussels can very effectively sort particles and preferentially accept the cyanobacterium *Microcystis.* In the present study we found that accepted particles were directed to the inside of the marginal groove of the inner demibranch and appear to be transported directly to the mouth for ingestion.

In summary, we observed pallial organ morphology, particle transport, and particle sorting in zebra mussels by using video endoscopy. These observations contribute to a growing body of information on the feeding dynamics of bivalves and suspension-feeding invertebrates. More importantly, our results suggest that particle sorting occurs on zebra mussel ctenidia, despite their homorhabdic nature and their lack of adjacent tracks of frontal cilia beating in opposing directions. Our direct observations of zebra mussel ctenidia provide an explanation for the efficient selection of particles measured by Baker *et al.* (1998) and, ultimately, for the role of zebra mussels in ecosystem modification. The role of ctenidial morphology in particle selection by zebra mussels exemplifies the direct link between the functioning of individual bivalves and ecosystem-level processes.

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Video Note

Supplementary video clips are available for viewing on *The Biological Bulletin* Website at ⟨http://www.mbl.edu/BiologicalBulletin/VIDEO/BB.video.html⟩.

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