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In vitro studies of particle capture and transport in suspension-feeding bivalves

Contribution of various techniques to our understanding of particle transport and selection by ctenidial organs of suspension-feeding bivalves—Particle processing mechanisms in suspension feeding bivalves can be divided into the following components: encounter, capture, transport, selection, and finally ingestion. Recent development of a number of new research tools or approaches has advanced understanding of the overall process. Three recent technical advances leading to better insights into particle processing have been the use of (1) endoscopic techniques (Bernard 1974; Ward et al. 1991, 1998*b*; Beninger et al. 1992, 1993; Ward 1996); (2) confocal laser microscopy (Silverman et al. 1996; Beninger et al. 1997); and (3) advanced modeling of isolated elements based on experimental video recording of the pallial organ system (Nielsen et al. 1993; Riisgård et al. 1996). These advances have driven modern reexaminations of pallial organ structure (Beninger and St. Jean 1997) and of ciliary movements (Silverman et al. 1997). In addition, numerical modeling (Nielsen et al. 1993) coupled with clearance studies provide a better understanding of the mechanisms underlying each of the component steps.

Visualization of the process in living, intact, bivalves has been impeded by the shell and specifically in the case of particle capture by the magnification and resolution necessary to critically observe the interaction of particles with gill elements or water currents created by those elements. The advent of endoscopy using a small-bore fiber-optics tube has provided valuable insight into the particle transport and particle selection mechanisms. Endoscopy allows visualization of net processes that are occurring. Endoscopy reveals the end result of some types of mucus secretions without observing the source of the secretion. Endoscopy also shows water currents and vectors produced by ciliary activity without seeing any particular cilia or cirral movement or their potential interactions with a particle (Beninger et al. 1992, 1993).

Endoscopy has afforded observations of large mucus strands (sometimes the width of a gill filament, 50–70 μm),

and also allowed tracking and sampling of material at various sites along the pallial organs indicating where selection has taken place (Ward et al. 1998*b*). These are elegant experiments and add much to knowledge of particle processing. They cannot, however, differentiate whether mucus has a role in the particle capture mechanism (Jørgensen 1996) because the endoscope cannot resolve the presence of mucus at the point of particle capture (Ward et al. 1998*b*).

Endoscopic observations are necessarily of low magnification, but allow visualization with limited resolution of large aggregate materials or slurries. The advantage is the ability to observe and only slightly disrupt the intact organism with experimental manipulations (Ward et al. 1998*b*). Understanding the handling of mucus by palps is another example of an advance achieved with endoscopy (Beninger and St. Jean 1997). Cutting of mucus strands by palp manipulation, and the movement of mucus from gills to palps as hypothesized earlier (Morton 1983 for review) has been confirmed using endoscopy (Ward et al. 1991; Beninger and St. Jean 1997). The scale of observation matches the magnification and resolution that endoscopy brings with its clear advantage of limiting disturbance to anatomical and functional relationships.

Thus, the contribution of endoscopy to understanding of particle transport mechanisms, particle selection mechanisms, and ingestion mechanisms is unique and provides new input that allows either confirmation of previously held hypotheses or their modification.

The endoscope as it is currently configured is less valuable with regard to particle capture mechanisms (Ward et al. 1998*a*). Particle capture by suspension-feeding bivalves is not generally considered to be done in aggregate as a slurry or as mucus-entrapped material. Instead, bivalves as a group are known to capture particles of many different sizes, ranging from >10 μm to 0.5 μm. These particles are captured individually and directed onto the gill surface. Those bivalve species that can capture particles in the smaller size ranges tend to have more complex latero-frontal cirri, with more cilia fused to form an individual cirrus (Riisgård 1988; McHenery and Birkbeck 1985; Silverman et al. 1997).

Laser confocal-microscopy observation of particle capture by cirri of Mytilus edulis—Laser confocal microscopy allows visualization of particles and resolution of individually beating ciliary organelles at the same time. The precise plane of focus obtainable allows observation of the particle and the cirri with a resolution better than 0.3 μm and approaching 0.1 μm (*see* Fig. 2 below; the free ciliary tips of a beating cirrus are resolved). The gill fragment or strip is alive, and cirri beat with a normal frequency ≤15 Hz. In addition, lateral cilia have a normal beat frequency ~20 Hz, and frontal cilia are motile. Thus, while this is not an in vivo condition, it is a viable in vitro preparation that allows high resolution observations. The preparation did not need to be and was not manipulated by any pharmacological agent (but would respond to serotonin). In our previous paper we noted that cirri appear to "trap" or deliver 0.75–1.0 μm fluorescent beads to the frontal surface of the filament (Silverman et al. 1996). Even with these extremely magnified and high-resolution images we concluded in 1996 that "these images by themselves do little to confirm whether the delivery mechanism is strictly hydromechanical or whether some particle contact with the cirri occurs." We can only indicate that the particle comes within a particle diameter, or less, of the cirrus. "Given the unsteady flow environment, only exacting measurements of fluid velocities at cirral sites themselves, identification of particle trajectories, and measurement of shear and drag forces will allow the exact mechanism of cirral interaction with the particles to be determined." Having identified the limitations of our own observations, where magnification and resolution are superb, it is difficult to envision what endoscopic observations add to a discussion at the level of "mechanisms" of particle capture. Small particles and cilia are below the resolution of the endoscope. Observations of a particle and the surface organelles on the gill or the periciliary space are not achievable. While apparent particle paths are visible, it is difficult if not impossible to see individual organelles in the endoscopy videos. The scale necessary and the instrument being used simply do not match.

Ward et al. (1998*a*) presented data from *Mytilus edulis* in their recent paper. The images presented in this commentary (Figs. 1–3) are from preliminary studies using living gill segments from *M. edulis.* While there is some variation in cirral size in *M. edulis* (Dral 1967), the cirri in this species are highly complex (~40–50 cilia per cirrus) (Owen 1974; in contrast to Ward et al. 1998*a*, fig. 6 depiction of 8 or 16?) and similar to *Dreissena polymorpha.* However, the *Mytilus* gill is filibranch. The size and orientation of latero-frontal cirri on the gill filaments of *M. edulis* are documented in Figs. 1, 2. Figure 3 shows serial images of a latero-frontal cirrus interacting to move a particle onto the frontal surface. Once on the frontal surface, the particle is transported rapidly toward the ventral groove.

There are two major hypotheses concerning the particle capture mechanism employed by bivalves. One suggests direct particle interaction with latero-frontal cirri that beat between filaments and "capture" particles by moving them onto the frontal surface of the filament where they are transported. A second suggests that the hydrodynamic forces created by the various gill cilia create water currents and that particles entrained in the currents are moved onto the frontal surface and then transported (Jørgensen 1996). Because hydrodynamic flows are in fact due to ciliary movements on all participating gill surfaces, both ciliary interactions and hydrodynamics must play a role in particle manipulation by the gill. Ward et al. (1998*a*) in their recent paper correctly indicate that the two theories derive largely from altered specimens, but go on to state in their introductory comments that none of the mechanistic studies to date take into account the variation of cirral (or in some cases latero-frontal cilia) structure among different bivalves. This is an inaccurate description of the extensive literature over the past 60 years on this topic (e.g., Atkins 1937, 1938). Numerous studies have shown that those bivalve species with more complex cirri (e.g., *M. edulis*) are more efficient in removing small (<2 μm) particles than those species with less complex cirri, or no cirri at all (Owen and McCrae 1976; Møhlenberg and Riisgård 1978; McHenery and Birkbeck 1985; Riisgård 1988; Silverman et al. 1995, 1997).

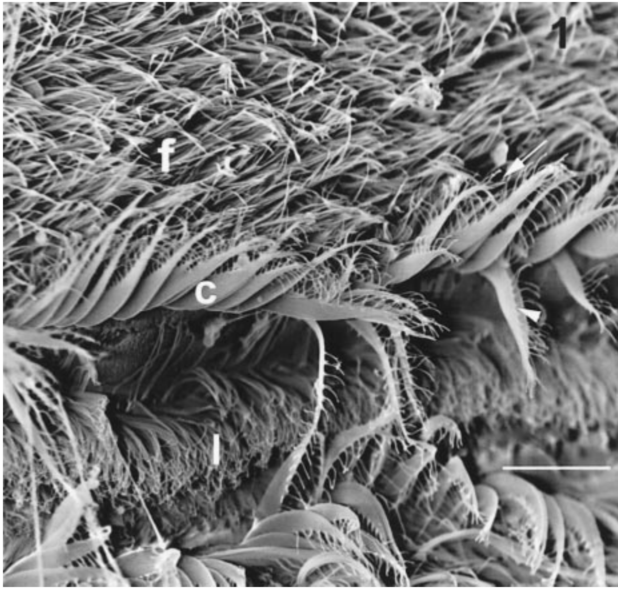

Fig. 1. Scanning electron micrograph (SEM) of gill filaments from *M. edulis.* Fixation was accomplished by slowly introducing drops of osmium (2%) into a beaker (approx. 100 ml) containing actively siphoning animals. The purpose was to rapidly fix surface features that were functioning in vivo when initial contact was made with fixative. The tissue was further processed with a standard glutaraldehyde protocol followed by osmium postfixation, dehydration, and coating for SEM observation (see Silverman et al. 1996). This image provides orientation for images in Figs. 2, 3. The ciliated frontal surface (f) of the filament is shown with most of the latero-frontal cirri (c) moving toward a fully flexed position with their free tips extending over the frontal surface of the filament. A few of the cirri are almost in the fully extended position (arrow-head). The tips of lateral cilia (l) are visible in the interfilament space. The two fused plates of cilia (not visible at this magnification; Silverman et al. 1996) that form the body of a cirrus, have free-tips (~2 μm long) that orient at an angle to the fused body of the cirrus. Each plate of the cirrus has free ciliary tips; the tips from each plate orient at opposite angles relative to the cirral base giving the appearance of a "V" (arrow) (*see* Silverman et al. 1996). Bar = 10 μm.

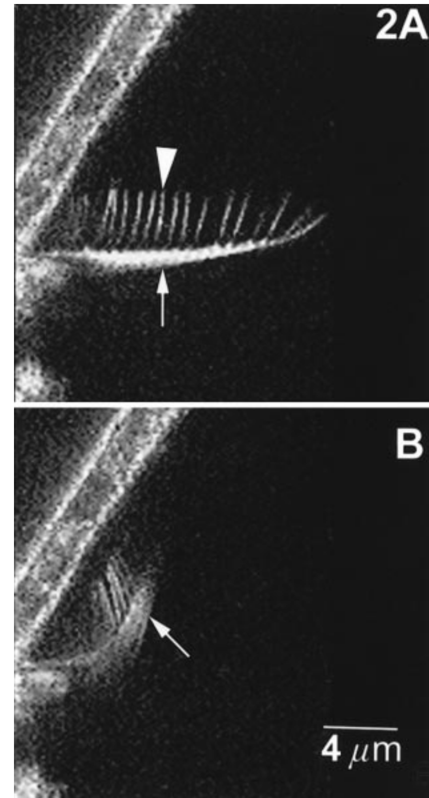

Fig. 2. Laser confocal video images of a cirrus from a living gill strip of *M. edulis.* The focal plane of the video is slightly off the perpendicular to the dorsal-ventral axis of the filament. Thus, the beat of the cirral tip is toward the frontal surface of the filament that lies to the right and into the backgound of the straight edge that represents the lateral surface of the filament. (A) The resolution of individual ciliary tips on one side of the extended cirrus (one cirral plate, arrow-head) is clear. The bending (or power stroke) of the cirrus (B) moves the plane of the ciliary tips toward the frontal surface of the filament. The arrows represent the mid-point of the cirrus. The images indicate a complex cirrus for *M. edulis* as has been reported previously by numerous other investigators. Bar = 4 μm.

Conclusions

These observations suggest that there is little need for "a new explanation of particle capture". Following the presentation of the "new explanation" the following two sentences appear in the discussion (Ward et al. 1998*a*). "Although the ctenidial filaments are the capture units, the latero-frontal tracts play crucial roles in both particle encounter and particle retention. However, the cilia or cirri that form these latero–frontal tracts affect particle capture by altering the pattern of flow around the ordinary filaments and not by physically intercepting particles." Thus, having created a "new explanation" the authors must rely on previous in vitro studies of the latero-frontal cirri. There actually are no original data in the Ward et al. (1998*a*) paper to support any of the comments about latero-frontal cirri function. Because cilia cannot be resolved using endoscopy, ciliary interaction with particles or lack thereof cannot be observed, and the modeling based in part on fluid dynamic considerations and large particle behaviors as viewed grossly add only minimally to understanding. "Bouncing" could derive from cirri-generated currents, but it would also arise if a particle "was thrown at a low angle" onto a beating cirrus. Ward et al. (1998*a*) used millimolar serotonin to reduce particle capture by a resounding 83%. Serotonin at this concentration blocks cirral beating. Yet, these data are used to deny the importance of cirri in particle capture. Rather, they speculate that this is the inefficiency that occurs if particles simply passed over the filaments at a low angle without cirral current. From their modeling it would seem that a reduction of capture might be more on the order of 50% (if interfilament space is equivalent to filament space), and if frontal surface capture at first particle passage is highly efficient. If so, the efficiency and mechanistic dominance of the low angle of approach becomes more questionable. Serotonin-induced cirral-arrest causes cirri to stop in the flexed position, and to occlude the frontal surface of the filaments almost completely, providing

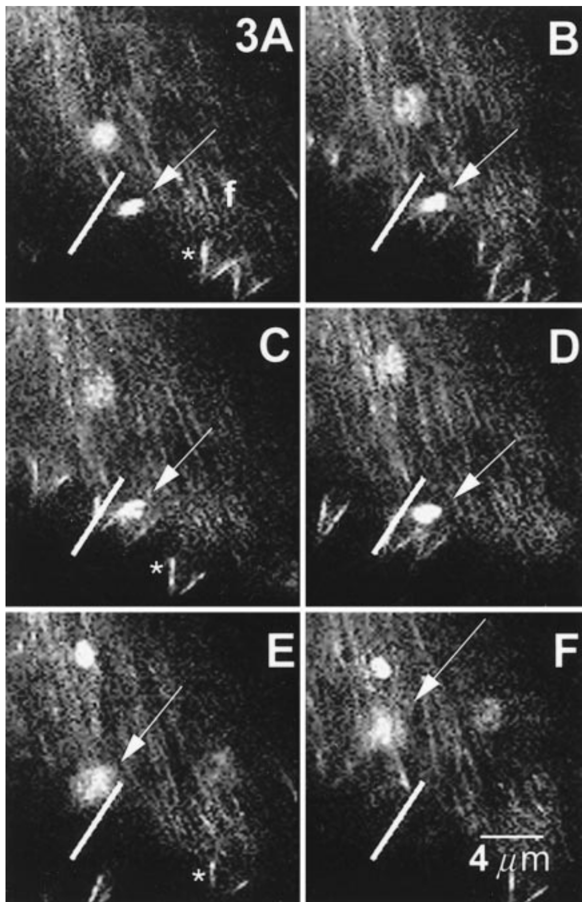

Fig. 3. A series of laser confocal video images (120 frames s^{-1}) of a living gill strip from *M. edulis.* The total time elapsed from the first to last image was 167 ms. The frontal surface of the gill (f), in this focal plane, contains only rows of parallel tips of the frontal cilia, with the vector of motion from the bottom right to the top left of each image. The ciliary tips of latero-frontal cirri are seen as they beat in and out of the plane of focus. The power stroke of the cirrus brings the tip "V" (*) into the plane of focus and over the frontal surface of the filament. The recovery stroke takes the cirri tips into the interfilament space and below the plane of focus (toward the left corner) where they are not visible. The initial location of the 1.0 μm fluorescent particle (arrow) in frame (A) is marked with a diagonal line on the left side of the particle, and the reference line is repeated at the same location in all frames. Once on the frontal surface, the movement (or transport) is away from the reference mark along the frontal surface, and was rapid. In (A), the particle is in the interfilament space (arrow). Particle (arrow) interaction with the cirrus tip during a beat cycle occurs in (B), (C), and (D), followed by movement of the particle in (E) and (F) along the frontal surface. A 4 s video movie (elapsed real-time ~1 s) of this dynamic process is available at ⟨http://www.aslo.org/lo/pdf/vol45/issue5/1199a1.html⟩. The interaction is close as judged by the resolution and magnification associated with these video images, and could be hydromechanical (as might be expected in a low Reynolds number environment) or by direct contact. Bar = 4 μm.

an alternative explanation for the 83% reduction in particle capture.

Hart (1996) has provided a valuable critique of the limitations of video recordings and the problems of interpretation of the images that are captured. There are strengths and weaknesses in every analytical approach to a problem. Making use of and integrating the data obtained from multiple techniques and preparations, both in vivo and in vitro, have allowed significant progress in understanding the mechanisms of particle capture in bivalves.

Harold Silverman, John W. Lynn, and Thomas H. Dietz

Department of Biological Sciences
Louisiana State University
Baton Rouge, Louisiana 70803

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The utility of in vivo observations for describing particle capture processes in suspension-feeding bivalve molluscs

How do suspension-feeding bivalve molluscs capture particles? This deceptively easy question has not proven easy to answer mainly because of the difficulties associated with observing capture processes on the ctendial surface of animals enclosed in opaque shells. A variety of hypotheses have been advanced including particle capture by a mucus sheet covering the ctenidia (MacGinitie 1941), physical entrainment by ''sticky'' laterofrontal cilia or cirri that border the filaments of the ctenidia (so called ''lime twig'' model, Wallengren 1905; Tammes and Dral 1955), mechanical sieving of particles from suspension by these same cilia or cirri (Moore 1971; Owen and McCrae 1976; Silverman et al. 1996), and entrainment of particles by hydrodynamic mechanisms (Jørgensen 1981; 1990). Most of these hypotheses were based on data obtained from surgically altered animals or isolated tissue preparations.

Ward et al. (1998) studied the kinematics of particle capture in bivalves, avoiding surgical manipulation by using a video endoscope (common term to describe a variety of optical instruments inserted through narrow openings for the purpose of viewing concealed structures), and obtained previously unattainable data on the capture process in a number of intact living species. Ward et al. (1998) presented their results from observations of particles approaching, encountering, and being retained by the ctenidium, and synthesized these data into a descriptive model of particle capture by the ctenidial filaments of bivalves. Notably, Ward et al. (1998) reported that none of the previously developed explanations of particle capture in bivalves were fully concordant with their detailed observations of particle encounter and retention. Instead, patterns of particle capture were more consistent with the process of direct particle interception found on the feeding surfaces of many other species of suspension-feeding invertebrates (LaBarbera 1984; Shimeta and Jumars 1991). In the explanation advanced by Ward et al (1998), ciliary processes on the bivalve ctenidia are important in generating the observed patterns of particle motion but were not directly implicated in capturing or filtering particles from the water. Ward et al. (1998) believed that agreement with the process of direct particle interception, which is found in many other phyla, added robustness to their argument in contrast to many previous explanations which suggested that bivalves had evolved unique processes of particle capture (e.g., Jørgensen 1981).

Beninger (2000), Riisgård and Larsen (2000), and Silverman et al. (2000) have commented on various aspects of Ward et al (1998). In this paper, we respond to these comments by grouping our responses under common headings that allow us to deal with similar points raised in all three papers. We believe that some of their confusion stems from the difficulties inherent in trying to discern from our written paper the complex movement of particles during their passage through the pallial cavity and onto the ctendial surface. The best way to convince readers of the validity of our model is by making some of the detailed video images of particle capture available. Short sequences of our video-endoscopy