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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 tentorial 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 tentorial 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 tentorial 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

observation can be downloaded from the L&O web page (<http://www.aslo.org/lo/pdf/vol45/issue5/1203a1.html>). Representative images are presented that allow the viewer to assess essentially subjective factors, such as image quality, and also the more crucial images of particle movement upon which the model of Ward et al. (1998) was based. Additional images can be obtained from the senior author.

Image quality and resolution of the video endoscope system—Beninger (2000), Riisgård and Larsen (2000), and Silverman et al. (2000) question the image quality and resolution of the endoscope system used by Ward et al. (1998); describing our system in vague terms such as having low magnification and limited resolution. In particular, Beninger discusses his experience with video endoscopy and suggests that Ward et al. (1998) overstate the ability of their video endoscopy system to visualize fine detail such as small particles and even individual ctenidial filaments that measure tens of μm in width. We readily admit that many of the early endoscopic studies that Beninger (2000) is referring to (e.g., Ward et al. 1991; 1994; Beninger et al. 1992) employed a system with limited resolution, because the first system included a monochrome CCD camera with a low intensity light source (150 W Halogen; *see* Web video, “Quality 1”). Since that time, improvements in low lux CCD color cameras, including digital acquisition of images (e.g., COHU), and the use of high-intensity, high-quality light sources (e.g., 250 W Xenon) have greatly improved the clarity and resolution of the images obtained by video endoscopy (*see* Web video, “Quality 2”).

Beninger (2000) suggests that Abbe’s equation would be a more accurate means of calculating the resolution of our endoscope system than the empirical approach used by Ward et al. (1998). The form of Abbe’s equation presented by Beninger assumes that the specimen is illuminated by a condenser whose numerical aperture is identical to that of the objective (Inoué 1986). This is the arrangement on compound and stereo-microscopes, but it is not found on endoscopy systems used by Ward and co-workers. The rod lens of the rigid endoscope (not a fiber optic bundle as indicated by Beninger) has a wide focal length (ca. 1 mm to infinity), has no condenser to focus the reflected light, and produces increasing magnification with decreasing distance to the subject. In addition, the resolution of the endoscope is affected by the field of view, direction/angle of view, depth of field, and the particular light source and light guide cable used to illuminate the objects of interest (pers. comm., J. Leo, Sr. Product Manager, Olympus America). Because of these variables, resolution is not calculated or given by the manufacturer. The method recommended by the manufacturer (Olympus America) to determine optical resolution is to empirically derive this number using a complete system that includes the scope, light source, light guide cable, and specific imaging conditions. Therefore, the equation one would use to calculate the theoretical resolution for any given endoscope is not relevant.

In optics, spatial resolution is determined by measuring the distance between line pairs (Inoué 1986). Ward et al. (1998) calibrated their system by observing reflective and nonreflective particles (polystyrene, alumina, and silica) of

known size on the ctenidium. Results of the calibration trials indicated that their system had approximately 3 μm resolution. More recently, we verified the estimated 3 μm resolution by placing an objective calibration-micrometer in water and focusing on the graduated scale with the video-endoscope system used by Ward et al. (1998) (*see* Web video, “Resolution 1”). It should be noted that although approximately 3 μm was the limit of resolution of the system, Ward et al. (1998) generally used larger particles (4 to 95 μm diameter; *see* p. 742) to provide information on the movement of particles, and hence water flow (*see below*), at the level of the ctenidial filaments (*see* Web video, “Resolution 2”).

Determining magnification is more straightforward. Magnification of the endoscopes used by Ward et al. (1998) increase exponentially with decreasing distance from the subject (Olympus tech. notes). The endoscope system used by Ward et al. (1998), therefore, afforded a magnification that ranged from about 150 \times to 20 \times at distances which were typically used (approximately 3–20 mm).

The endoscope technique cannot visualize small and rapidly beating cilia, even though they are in a similar size range as the small particles we used, simply because the cilia are translucent and lack the contrast of solid particles. Nevertheless, the gross activity of cilia can be observed using the endoscope. For example, the activity of cilia involved in transporting particles on the surfaces of pallial organs can be discerned from the motion of the particles being transported. This indirect observation of ciliary activity has been used previously to describe particle transport in a variety of intact bivalves (e.g., Ward et al. 1994; Beninger and St-Jean 1997). Ward et al. (1998) used such observations combined with results from experimental manipulations (e.g., serotonin additions to inactivate laterofrontal cirri) as surrogates for visualizing the cilia directly, enabling them to study indirectly the net effects of cilia and cirri on particle capture. This approach of using particle motion to examine the interactions between particles and ciliated surfaces is widely used (Hart 1991; Nielsen et al. 1993; Mayer 1994; Riisgård et al. 1996) and, indeed, was used by Riisgård and Larsen (2000). Studying ciliary processes indirectly differs greatly from direct observations of the action of cilia on tissues that have been dissected from the animal using higher magnification microscopes (e.g., Riisgård et al. 1996; Silverman et al. 2000). This latter approach certainly is the only possible way at this time to visualize the activity of individual cilia and cirri, as it relates to particle kinematics. But as we contend in the following section, the advantages of such higher magnification observations have to be offset against the likely artifacts associated with studying isolated preparations. Ultimately, a complete understanding of the entire feeding process in suspension feeding bivalves will only be obtained by integrating data obtained at a number of different scales using the most appropriate observational techniques.

In vitro vs. in vivo observations—Beninger (2000), Riisgård and Larsen (2000), and Silverman et al. (2000) all comment on the reservations expressed by Ward et al. (1998) concerning the validity of published data obtained from studies of isolated tissues and surgically altered preparations.

The reasons behind our concerns about possible inaccurate information from isolated ctenidial preparations are detailed in Ward et al. (1998) and will not be reiterated here. This is a fundamental issue because the extrapolation of observations of particle movement made at high magnification on isolated tissues, which in some instances have been treated with chemicals to maintain ciliary function, to whole animal/environment interactions may or may not be valid. For example, Riisgård et al. (1996) stretched ctenidial sections of *Mytilus edulis* between two rods to re-create the correct width of the interfilament canals and had to reactivate the lateral cilia by adding 10^{-6} M serotonin to the medium. In contrast, Silverman et al. (2000) state that the lateral cilia in isolated gill fragments of *M. edulis* exhibited a normal beat frequency of ~ 20 Hz, even though no pharmacological agents were used and the ctenidial fragments were not stretched. It is unclear why there should be such a divergence in these fundamental methods, but it raises the question of whether the rate of water flow through the interfilamentary spaces in at least one of these studies was abnormal. We believe that it is incumbent on those making such observations on isolated tissues to demonstrate that artifacts are not being introduced into their data. The literature is replete with such artifacts giving rise to incorrect explanations of bivalve feeding mechanism. For example, concerns about using dissected animals to study particle capture were first raised by Tammes and Dral (1955), and later by Dral (1967) who was one of the first to observe aspects of particle capture in nonsurgically altered bivalves by looking through the thin, almost transparent valves of recently metamorphosed juvenile mussels. Similarly, Jørgensen (1976) also contends that, "The mechanisms of feeding, therefore, cannot be finally understood from studies on exposed gills or gill fragments" (p. 342). More recently, based on a detailed review of these problems, Jørgensen (1990) cautions that excised ctenidium tissue produce an abnormal amount of mucus that interferes with normal processes of particle movement.

Other artifacts associated with observing isolated tissues may be more difficult to discern. For example, Beninger et al. (1990) reported the presence of compact mucus cords in the dorsal tracts of the ctenidia of scallops, *Placopecten magellanicus*, that had been dissected open. Using video endoscopic techniques Ward and co-workers (Ward et al. 1991; Beninger et al. 1992) found that these cords were in fact artifacts caused by the collapse of the mucus slurry when the hydrodynamics of the ctenidia were destroyed during dissection. Consequently, even though the dorsal tract cilia may have been operating normally, dissection perturbed the hydrodynamics of the intact system thereby producing artifacts.

The concern expressed by Jørgensen (1976; 1990), and which Ward et al. (1993; 1998) echoed, about interpreting observations on surgically altered preparations does not mean that the study of dissected specimens and isolated structures is never a valid technique. Much valuable information on feeding structures is based on histological techniques and this information can provide insight into feeding processes, abnormalities of the ctenidia, and first-order approximations of particle capture mechanisms (Ward et al.

1994; MacDonald et al. 1995; Potter et al. 1997). In fact, Ward (1996) and Ward et al. (1998) also used results obtained from isolated structures to provide important pieces of information used to develop their model of particle capture. We do contend, however, that studies of the mechanisms of particle capture based solely on isolated structures or dissected animals cannot ensure that results are typical of the process in living animals. Such observations can be a useful adjunct to in vivo studies, provided that the limitations on interpreting the results are clearly understood (see p. 742, Ward et al. 1998).

Beninger (2000), Riisgård and Larsen (2000), and Silverman et al. (2000) argue that their invasive techniques are as useful, if not more so, than in vivo observations for elucidating the mechanisms of particle capture. Although the observations of Silverman et al. (1996; 2000) provide valuable data concerning the movement of cilia and cirri, it is still not clear if they advance understanding of particle capture dynamics in living bivalves. For example, the conclusions of Silverman et al. (2000) concerning the ctenidia of *M. edulis* are that (1) the laterofrontal cirrus interacts in some way with the particle and directs it onto the frontal surface, and (2) the particle is then transported toward the ventral groove. Almost identical conclusions were reached over 100 years ago by Alder and Hancock in 1851, (cited by Moore 1971), and are no different than those published by Atkins (1938), Tammes and Dral (1955), and Owen (1974).

We contend that water flow past ctenidial filaments isolated from adjoining pallial organs by dissection will be different from flow in intact animals where the close proximity of four demibranchs, enclosed by the mantle inside a rigid shell, imposes constraints on the pattern of water movement. Consequently, processes observed on small pieces of tissue suspended in bowls of water may be anomalous, and at the very least the results obtained should be interpreted with caution. In particular, Silverman et al. (2000) argue that the confocal microscope provides a more detailed observation (higher magnification and resolution) of the movement of the laterofrontal cirri than endoscopy. That is correct. However, in the case of particle capture dynamics, the detailed study of an anomalous process would be pointless. Yet Silverman et al. (1996; 2000) do not acknowledge that placing a section of ctenidium between a microscope slide and cover slip destroys the subtle interactions between adjacent feeding structures and could alter the hydrodynamics of flow across these feeding organs. The data presented by Silverman et al. (2000) obtained using the confocal microscope is certainly interesting and at this time cannot be obtained in any other manner. Nevertheless, further work is required to demonstrate that this technique is providing valid data on the mechanisms of particle capture in bivalves.

Riisgård and Larsen (2000) also make the case that isolated ctenidial filaments allow for the use of higher power microscopy and much better resolution of ciliary action. While this is obviously true we are still concerned that the resulting observations may be of phenomena that do not reflect the actual situation in an intact bivalve. For example, preparations such as those used by Riisgård et al. (1996) eliminate any coordinated action of the abfrontal cilia of the filaments. This restriction might reduce flow velocities

through the interfilamentar spaces (Jones et al. 1990; 1992; *see* additional arguments under “Particle paths and flow”). In contrast, video endoscopy can be used to view processes at a wide range of scales including large-scale whole-animal processes that are most likely to be influenced by the overall geometry of the organism.

In future studies, we believe that it is incumbent on researchers who study aspects of particle capture on exposed ctenidia or isolated structures to demonstrate that their preparations are functioning in a manner identical to ctenidia *in vivo*.

Misinterpretations of methods—Certain of the comments expressed in Beninger (2000) suggest that he may have misinterpreted some of the methods and results presented by Ward et al. (1998).

(1) Under “Basic suppositions” (comment 2), Beninger suggests that particles approaching the frontal surface of the ctenidia should curve towards the epithelium as they progressively encounter the higher current velocity associated with the action of the frontal cilia closer to the ctenidial surface. We assume that he is referring to a dorsal-ventral curvature, because this is the direction of the active stroke of the frontal cilia of most bivalves (note that such particle movement differs from curvature perpendicular to the ctenidial plane along the anterior-posterior axis, which is discussed below in “Particle paths and flow”). We did not observe dorsally or ventrally curved trajectories of particles just prior to encounter with ordinary filaments. Perhaps this is because the frontal cilia of bivalves such as *M. edulis* have been shown by Beninger et al. (1997) to be primarily moving rafts of cohesive mucus that serve to transport particles along the frontal surface of the ctenidia. Consequently, it is unlikely that these frontal cilia would have a major influence on water currents and hence on particle motion prior to encounter with the mucus layer. In addition, slight curvatures along a dorsal-ventral axis would have little effect on the particle approach angle estimated by Ward et al. (1998), which is largely along the posterior-anterior axis. The idea that the frontal cilia of ordinary filaments transport mucus is consistent with observations described in Ward et al. (1998) (*see* Web video “Particle tracking”); during encounter with a ctenidium, particles are directed in four main ways until they are retained by mucus on the frontal surfaces and transport either dorsally or ventrally toward the ctenidial margins. Ward et al. (1998) did observe a dorsally directed component of flow in the pallial cavity of bivalves which was most evident in species with plicate, heterorhabdic ctenidia (e.g., oysters). In these species, the dorsally directed component may have been produced, in part, by the frontal cilia of the principal filaments which probably transport a mucus-water slurry rather than cohesive mucus (Beninger et al. 1992; Ward et al. 1994).

(2) Under comment 3 of “Basic suppositions,” Beninger discusses the vector information presented in fig. 1 by Ward et al. (1998). We did not present the magnitude of any of these vector components because, unfortunately, the endoscope only allows two-dimensional observations and a stereoscopic system is required to measure the magnitude of these vectors reliably. What we did state, however, was that

the sum of these flows resulted in particles approaching the ctenidial plane at an angle of approximately 30° in actively feeding animals, and that this was similar in all bivalve species studied, including those with plicate ctenidia (see Web video, “Dye study,” and “Particle tracking”).

(3) Under “Basic suppositions” (comment 4), Beninger suggests that fig. 2 in Ward et al. (1998) does not really depict particle capture kinematics on a “typical” bivalve ctenidium. His statement is simply not correct. This schematic figure illustrates the four patterns of particle movement that Ward et al. (1998) observed *in vivo* when particles encounter **any** ordinary filament (*see* Web video, “Particle tracking”). The schematic not only demonstrates what was found for the filaments of a homorhabdic, filibranch ctenidium, but also is entirely consistent with what can be found on three ordinary filaments located on the plical crest of a heterorhabdic, pseudolamellibranch ctenidium. We note that although the filibranch condition is not the most common ctenidium type found among members of the Bivalvia, it is found in members of the most commonly studied family of bivalves (i.e., Mytilidae, e.g., the blue mussel *M. edulis*).

(4) Finally, under “Limitations of endoscopy” (comment 3), Beninger expresses concern about the degree of subjectivity associated with quantifying endoscopic visual information. Although video-endoscopy obviously requires the investigator to interpret images, it is no different in this respect from other techniques such as the compound optical microscope used extensively by Beninger (e.g., 1990), the dissecting microscope linked to a video recorder used by Riisgård and Larsen (2000), and the confocal microscope linked to a video recorder used by Silverman et al. (1996; 2000). In addition, video endoscopy is much more than simply “seeing what the animal is doing,” it is a tool that can be used to collect quantitative data needed to test specific hypotheses concerning particle encounter (e.g., particle approach velocities and angles), retention efficiency, transport rates, and selection efficiency (Ward 1996; Ward et al. 1997; 1998). One way to allay concerns about subjectivity is to allow people to see parts of the raw video footage. We encourage readers to do this by downloading our images from the L&O website, and hope that other groups will use video endoscopy to further study and test our model of the process of particle capture in suspension feeding bivalves.

Beninger also suggests that fig. 3 of Ward et al. (1998) does not show actual particle paths, but rather is a schematic of perceived paths. Again, this assertion is simply incorrect. Individual particles can easily be tracked using endoscopy by reducing the particle concentration delivered to the bivalves, then using one of a number of image analysis techniques to digitize the individual frames and transfer the exact position of a particle to the corresponding ctenidial location on a median frame. As long as the time frame is short, there is very little spatial error with this technique (time frame of fig. 3 of Ward et al. (1998) is less than 1 sec). Because digitization reduces resolution and contrast, particles need to be highlighted as white dots for presentation. Data, such as those presented in fig. 3 of Ward et al. (1998), were obtained by careful frame by frame analysis of the movement of a single particle above and along the ctenidial segments shown. Consequently fig. 3 is not a “schematic,” but represents ob-

servations from several consecutive video images which allowed us to discern four basic patterns of particle encounters with the ctenidial filament (Ward et al. 1998; see Web video "Particle tracking"). This approach of overlaying consecutive video frames to make a composite for presentation on the printed page is a standard technique applied by researchers who use video data (e.g., Hart 1991; Nielsen et al. 1993; Mayer 1994).

Particle paths and flow—Two fundamental assumptions implicit in the work of Ward et al. (1998) were (1) that the motion of particles observed through the endoscope accurately indicated water flow even when particles began to be influenced by the laterofrontal cilia/cirri and ctenidium, and (2) particles departed from flow streamlines only when they encountered the ctenidial filaments. The assumption that the motion of very small, low density particles is equivalent to water flow is a basic tenet of experimental fluid mechanics, since most techniques for measuring small scale flow (e.g., laser doppler velocimetry, acoustic doppler velocimetry, and particle image velocimetry) actually measure the motion of very small seed particles. However, the degree to which this assumption remains valid in the very near field of bivalve ctenidia and cilia was not addressed in Ward et al. (1998). The inertial impaction index of Rubenstein and Koehl (1977), developed to assess the degree to which a particle approaching an obstacle will tend to leave flow streamlines and contact the obstacle due to inertia, may be taken as a reasonable indication of the tendency of particles to follow flow. It is defined by the relationship $N_I = (s - 1)d_p^2V/18\nu d_o$, where s is the specific density of the particle, d_p is the diameter of the particle, V is the particle approach velocity, ν is the kinematic viscosity of the water, and d_o is the diameter of the obstacle. If $N_I \sim 1$ particle inertia is important and the particles do not always follow the flow, but if $N_I \ll 1$ particle inertia is negligible (Rubenstein and Koehl 1977) and particle motion is a very good indicator of flow. Using $s = 1.05$ (polystyrene spheres), $d_p = 4\text{--}95 \mu\text{m}$, $V = 2,000 \mu\text{m sec}^{-1}$ from Ward et al. (1998), $\nu = 1.2 \times 10^6 \mu\text{m}^2 \text{sec}^{-1}$, and $d_o = 10\text{--}60 \mu\text{m}$ to represent obstacles ranging from the height of a row of laterofrontal cilia to the diameter of a ctenidial filament, we obtain $N_I = 0.000001\text{--}0.004$. In other words, over the range of particle sizes, particle densities and flow speeds measured by Ward et al. (1998), particle motion was a very good indicator of flow. We do not consider obstacles of the diameter of a single cilium because of arguments presented by Ward (1996), and elsewhere in this paper showing that, at the very low Reynolds numbers of flow near bivalve ctenidia, rows of cilia (or cirri) should act as solid paddles and not as individual sieving elements.

The equivalence of particle motion and water flow has important implications. For example, Ward et al. (1998) observed that particles were excluded from direct passage through interfilamentary spaces in *M. edulis*, which we now can state with more confidence indicates exclusion of direct water flow as well. Again, the rapid deceleration and reversal of particle motion termed "trap-and-flip" capture by Ward et al. (1998) most likely indicates rapid deceleration and reversal of the flow as well. Though counter-intuitive from a large-scale perspective, abrupt changes in flow at these small

scales are possible because fluid inertia is negligible compared to viscosity; i.e., the Reynolds numbers are extremely small. Ward et al. (1998) only presented schematic diagrams of typical time-averaged flow patterns observed in the immediate vicinity of bivalve ctenidia, but the individual observations underlying those diagrams gave tantalizing evidence of complex spatially and temporally varying flow patterns that remain to be explored.

One of the fundamental points of Ward et al. (1998) was that these flow patterns must be explored in the context of intact, active feeding structures. The large scale geometry and dynamics of the whole ctenidial system constrain the small-scale flow near the ctenidia, at the same time as ciliary and cirral pumping activity at the ctenidia control the intensity of the large scale flow. In other words, flow through the bivalve ctenidium represents a balance between small scale pumping action and larger scale constraints. A partial approximation of this balance is illustrated by Riisgård and Larsen (2000). They present model results for flow between two geometrically correct ctenidial planes with appropriate overall flow rates achieved by uniform suction through the ctenidial planes. At relatively large scales (away from the ctenidial plane), the predicted flow patterns closely match what Ward et al. (1998) observed; i.e., an approximately 30° angle of approach to the ctenidial plane. At small scales (near the ctenidia), however, we argue that their model is a poor representation of flow in bivalves. Riisgård and Larsen (2000) represented uniform suction through the ctenidial plane by imposing a uniform perpendicular velocity of 0.85 mm s⁻¹. The fact that their modeled flow approaches the ctenidial plane at a perpendicular angle is therefore due to this imposed boundary condition, which may or may not represent reality. Furthermore, it should be apparent that uniform suction is not a good representation of flow through the ctenidial plane in the very near field. Rather, we believe that low Reynolds number flow forced through rows of parallel cylinders at an oblique angle is a better model. The problem of perpendicular flow forced through rows of parallel cylinders at low Reynolds numbers was solved by Kirsch and Fuchs (1967) and cited by Ward et al. (1998). The problem of oblique flow through rows of parallel cylinders is considerably more complex and, to our knowledge, it has not been solved. The solution of this problem was beyond the scope of Ward et al. (1998), but it would address the actual nature of flow through the ctenidial plane better than either the solution presented by Riisgård and Larsen (2000) or the schematic diagrams presented by Ward et al. (1998).

Riisgård and Larsen (2000) also present microscopic observations through the gape of an intact bivalve that show particles curving in towards a perpendicular approach to the ctenidia. They use these observations and the results of their model (discussed above) to imply that the particle paths and schematic diagrams of Ward et al. (1998) showing a low angle of approach very close to the ctenidial plane must be wrong. We have no quarrel with their observations, though we do not agree with their model. We believe that their observations and those presented by Ward et al. (1998) are actually both correct, and are not inconsistent. We speculate that the steeper approach angles that they observed may have

been due to the proximity of their observations to the posterior margin of a ctenidium, where demibranchs of the ctenidia are spaced farther apart and the constraint of an opposing ctenidial plane is not as strong. Furthermore, in their fig. 2, particle paths begin to curve toward the ctenidia 100–150 μ away but do not become normal to the surface until they are much closer, and then in less than half of the cases presented. In the majority of instances shown there is still some component of parallel flow within a few tens of μm above the surface.

The observations presented in Ward et al. (1998) were made closer to the middle of ctenidia between essentially parallel planes, representing the majority of the bivalve ctenidium for which there are strong constraints on the angle of particle approach. With regard to the data presented and its interpretation, Ward et al. (1998) stated as fact only what they observed directly: “Even within 10–20 μm of the filaments there was a component of the flow parallel to the ctenidial surface” (p. 744). Note the clear appearance of only slight downward curvature in the video micrographs presented in fig. 3 of Ward et al. (1998), suggested by the slightly closer spacing in particle position closer to the ctenidium. This interpretation of only slight curvature in particle paths is supported by dye studies which demonstrated that even within tens of microns of the ctenidial surface there was a component of flow directed anteriorly along the ctenidium (Ward 1996; Ward et al. 1998; see Web video, “Dye study”). These observations were incorporated directly into the schematic diagrams and the kinematic model presented by Ward et al. (1998), which were intended as a summary of many more observations than could be presented in one paper and as a working hypothesis to explain observed phenomena. The explanation proposed by Ward et al. (1998) challenges previous points of view and it proposes flow kinematics that are not obvious until one views the endoscopic video recordings. However, this explanation is not arbitrary. It is based on fluid dynamical thought, if not fluid dynamical modeling, and most importantly is based on high quality observations in intact, actively feeding bivalves.

One of the primary fluid dynamical considerations underlying the flow patterns proposed by Ward et al. (1998) is that low Reynolds number flow should adjust to minimize spatial gradients in streamline spacing if at all possible. Spatial gradients in streamline spacing indicate shear, which is directly proportional to viscous drag at low Reynolds numbers. This is one way of explaining the far-reaching influence of obstacles at low Reynolds numbers—spatial gradients in streamline compression around the obstacle are minimized by spreading the compression out over relatively large distances. Another fluid dynamical consideration is that accelerations and decelerations due to streamline turning are relatively unimportant because fluid inertia is relatively unimportant at low Reynolds numbers. These considerations, constrained and informed by direct observations, lead to the streamline diagrams proposed by Ward et al. (1998). They also highlighted the observation that streamline compression in the interfilamentar gap is minimal at a 30° angle of approach (for equal widths of filaments and interfilamentar spaces) and maximal at a perpendicular angle of approach. It is rather remarkable that the approach angles observed by

Ward et al. (1998) were almost exactly those required to minimize streamline compression, especially because, as Riisgård and Larsen (2000) state, “it is obvious that a . . . low angle with respect to the frontal surface plane is . . . a result of flow fields set up by the parallel ctenidial filaments” (p. 1194). In other words, the large scale geometry of the bivalve appears to set up flow conditions that may in some way be optimal for the small scale geometry of the ctenidium. We emphasize, that the flow patterns proposed by Ward et al. (1998) are hypothetical. We welcome further investigations using more advanced methods than were available to us at the time, but we resist offhand dismissal of our ideas because of preconceived biases.

Conclusions

The critical comments of Beninger (2000), Riisgård and Larsen (2000), and Silverman et al. (2000) do not undermine the main points of Ward et al. (1998). Comments such as those by Silverman et al. (2000) that, “there is little need for ‘a new explanation of particle capture’ ” (p. 1202), and “there . . . are no original data in the Ward et al. (1998) paper to support any of the comments about laterofrontal cirri function” (p. 1202), suggest that Silverman and co-workers have overlooked the most important findings of Ward et al. (1998), including observations that particles can be intercepted directly by ordinary filaments, particles can traverse several interfilamentary spaces without being swept through these spaces (i.e., lost by the ctenidium), and particles can be captured by the ctenidial filaments in the absence of laterofrontal cirral activity. These observations are consistent with the hypothesis that particle capture in bivalves is a hydrosol filtration process similar to that employed by other aquatic suspension feeders; evolutionarily, this is a reasonable starting point. Some of the points published in Ward et al. (1998) are at odds with previously published concepts, but they do provide an explanation that is consistent with many previous observations concerning particle capture.

For example, Ward et al. (1998) suggest that bivalves do not necessarily need laterofrontal cilia or cirri for particle capture; a concept that is supported by theoretical arguments of particle encounter mechanisms at low Reynolds number (Shimeta and Jumars 1991). In addition to data published by Ward et al. (1998) demonstrating that direct interception of particles can occur on the ctenidial filaments even when the laterofrontal cirri are inactivated, at least one other researcher has reported a similar observation. Foster-Smith (1975) removed a 1.5 cm square portion of shell and mantle of three bivalve species (*M. edulis*, *Cerastoderma edule*, and *Venerupis pullastra*), and replaced it with a glass window. He then made extensive, in vivo microscopic observations of particle capture and transport. For all three species he reported that, “particles may be drawn directly on to the gill filaments without first being caught by the laterofrontal cilia” (p. 574).

The concept that the filaments of the ctenidia are the capture unit is a fundamentally different way of examining particle capture in bivalves. Ward et al. (1998) proposed that the laterofrontal tracts are subsidiary to the filaments, and in

the absence of these tracts bivalves can still capture particles by direct interception on the filaments, albeit at a greatly reduced efficiency. The laterofrontal tracts serve a crucial function by redirecting water flow around the filament thereby increasing the efficiency of particle encounter (an important distinction from retention). We suggest that the laterofrontal cilia or cirri of bivalves functions in a manner similar to other suspension feeders possessing fine feeding structures (Cheer and Koehl 1987), and act more like solid paddles than like sieves. This contention is supported by a number of arguments including the vortical movements of small particles on the lateral edges of filaments observed by Ward et al. (1998), and several theoretical considerations which indicate that little water flow leaks through the branching cilia of the laterofrontal cirri, and that these cirri function to move water not filter it (Jørgensen 1981; Nielsen et al. 1993; Riisgård et al. 1996). If we assume that the physics of bivalve feeding is similar to other suspension feeding organisms, and consider the data and theoretical arguments posed then the most parsimonious overall explanation of our observations is that suspension-feeding bivalves employ aspects of hydrosol filtration for particle capture.

We are dismayed at the vehemence of some statements by Silverman et al. (2000) and Riisgård and Larsen (2000), who say, for example, that our work “is unfounded, [with] no reasonable arguments for refuting earlier observations on isolated ctenidial filaments” (p. xx). Our research is part of the continuing process of understanding the complex process of particle capture in bivalve molluscs, and we believe that our work complements many of their recent observations and models examining overall geometries. For example, the observation presented in Riisgård et al. (1996), which was not available to us at the time we wrote our paper, that particles are stopped over the interfilamentar gap and transferred to the frontal surface of the filament by the action of the laterofrontal cirri is very similar to our observations. Their paper also shows that the cirri most probably act as a paddle, not as a sieve, again in agreement with Ward et al. (1998). The calculations presented in Riisgård et al. (1996) show the sensitivity of viscous flow to spacing between cilia. This is the same argument advanced by Ward et al. (1998; fig. 6) to explain why water flow through the interfilamentary gap of bivalves with laterofrontal cirri is blocked by the branching ciliary subunits and instead passes through the larger gap at the base of the cirri. The observations presented in Riisgård et al. (1996) are consistent with the most important point of Ward et al. (1998), which is that particle capture in bivalves occurs by direct interception with the frontal surface of ctenidial filaments, aided by flow patterns around the filaments.

As we readily admit in the final paragraph of Ward et al. (1998), the model we proposed only provides a starting point and needs to be tested and refined with new data from a wider range of bivalve species with different types of ctenidia, especially the eulamellibranchiate species. As Hilborn and Mangel (1997) so succinctly state, “Models are metaphorical descriptions of nature, and there can never be a ‘correct’ model. There may be a ‘best’ model which is more consistent with the data than any of its competitors” (p. xii).

We encourage others to test our model using their own specialized techniques, and hope to see published, ever more comprehensive and detailed descriptions of the feeding process in bivalve molluscs.

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