

COMMENT

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A comment on experimental techniques for studying particle capture in filter-feeding bivalves

During the last decade a growing interest in understanding and quantifying the process of filter-feeding by bivalves has led to new experimental techniques employing video recording of magnified views of water motion and particle capture. A key issue in such studies is gaining optical access while maintaining undisturbed, natural feeding. Techniques include isolated gill-filament preparations observed through high-powered microscopes (Nielsen et al. 1993; Riisgård et al. 1996; Silverman et al. 1996*a,b*), and intact bivalves observed by means of endoscopy, using an optical insertion tube introduced into the mantle cavity (Beninger et al. 1992; Ward et al. 1993; 1996). The purpose of the present comment is to clarify the relative merits of the above-mentioned techniques and to rule out misconceptions recently introduced by Ward et al. (1998). The present views are supplemented by new video observations, using a dissecting microscope having sufficient magnifying power and working distance to allow outside observation of the gills of an intact and actively feeding mussel. Further, paths of particles approaching the gill surface are computed for a model problem simulating the space between two demibranchs.

Current controversy—Ward et al. (1998) claim that their use of video endoscopy has led to a new integrative explanation of particle capture in suspension feeding bivalves. They refute previous interpretations of the particle capture process based on observations of isolated gill filaments. Thus, Ward et al. (1998) assert that the “current controversy” is attributable to artifacts that have arisen when specimens were prepared for observation because dissection destroys subtle hydrodynamic interactions between feeding structures and particles, and alters the normal flow of water through the pallial cavity. A low angle of particles approaching the gill filaments is claimed to be crucial and disturbance of the angle ($\approx 30^\circ$) is assumed to change the way in which particles interact with the gill filaments. This in turn has led to conclusions concerning particle capture that are speculative and unsubstantiated. By making these statements Ward et al. allow themselves to neglect observations made on isolated gill-filament preparations reported in a number of papers on mussels dealing with the mussel pump and the particle capture mechanism (Silverman et al. 1996*b*; Nielsen et al. 1993; Riisgård et al. 1996) where considerably higher magnification and more controlled positioning were used than is possible with video endoscopy.

Advantages of isolated gill-filament preparations—In physiological studies, it is an accepted procedure to separate

certain organs for special studies and treatments to obtain a deeper insight into details about the normal function of the organ. By removing bivalve gill filaments for studies at high magnification important and detailed information about ciliary structures, water flows, and particle capture may be obtained as long as the observer is aware of possible changes in the normal function. To allow a blue mussel (*Mytilus edulis*) gill-filament preparation to both pump water and capture suspended particles, a nerve transmitter (10^{-6} M serotonin) is added to restore the ciliary activity. By checking that ciliary beat frequencies, water flow velocities, and particle movement patterns are comparable to the normal undisturbed situation, one may obtain information from gill-filament studies otherwise not available with the video endoscope technique, which has a relatively low magnification (Silverman et al. 1996*a,b*; Nielsen et al. 1993; Riisgård et al. 1996).

Particle retention and water motion by *M. edulis* gill-filaments stimulated with serotonin were studied by Nielsen et al. (1993). Video observations and numerical modeling showed that the mean velocity of the volume flow through the interfilament canals was about $V_{if} = 1.7 \text{ mm s}^{-1}$. This value corresponds to velocity calculations based on laboratory measurements of filtration rate (F) and gill area (G) using the ratio $V_{if} = 2F/G$, because the interfilament gap occupies about 50% of the gill area. Estimated velocity values were 1.68 mm s^{-1} (Møhlenberg and Riisgård 1979), 1.73 mm s^{-1} (Jørgensen et al. 1990), 1.41 mm s^{-1} (Riisgård 1990), and 1.68 mm s^{-1} (Meyhöfer 1985). From this it appears that the approach velocity of particles in the direction normal to the gill surface, i.e., upstream of the interfilament canals, must be about $V_0 = F/G = 0.85 \text{ mm s}^{-1}$. Nielsen et al. (1993) found that the maximum velocity of particles near the tip of the water-pumping lateral cilia (lc) was about 3 mm s^{-1} which is 70–80% of the mean tip speed of the 15- μm long lateral cilia in their effective stroke, estimated to be 3.7 mm s^{-1} at 19 Hz beat frequency.

The role of laterofrontal cirri (lfc) in particle capture by *M. edulis* was studied by Riisgård et al. (1996) using gill-filaments mechanically adjusted by “stretching” in a microscope observation chamber to avoid wall effects and to restore the normal interfilament gap of about $40 \mu\text{m}$ (i.e., “undisturbed geometry”) of the intact mussel. When stimulated with serotonin both the beat frequency of the lc (about 20 Hz) and lfc (about 12 Hz) were similar to values measured in intact, small, transparent mussels (Dral 1967; Jørgensen and Ockelmann 1991). Therefore, the through current was assumed to be $V_{if} = 1.7 \text{ mm s}^{-1}$, and the video

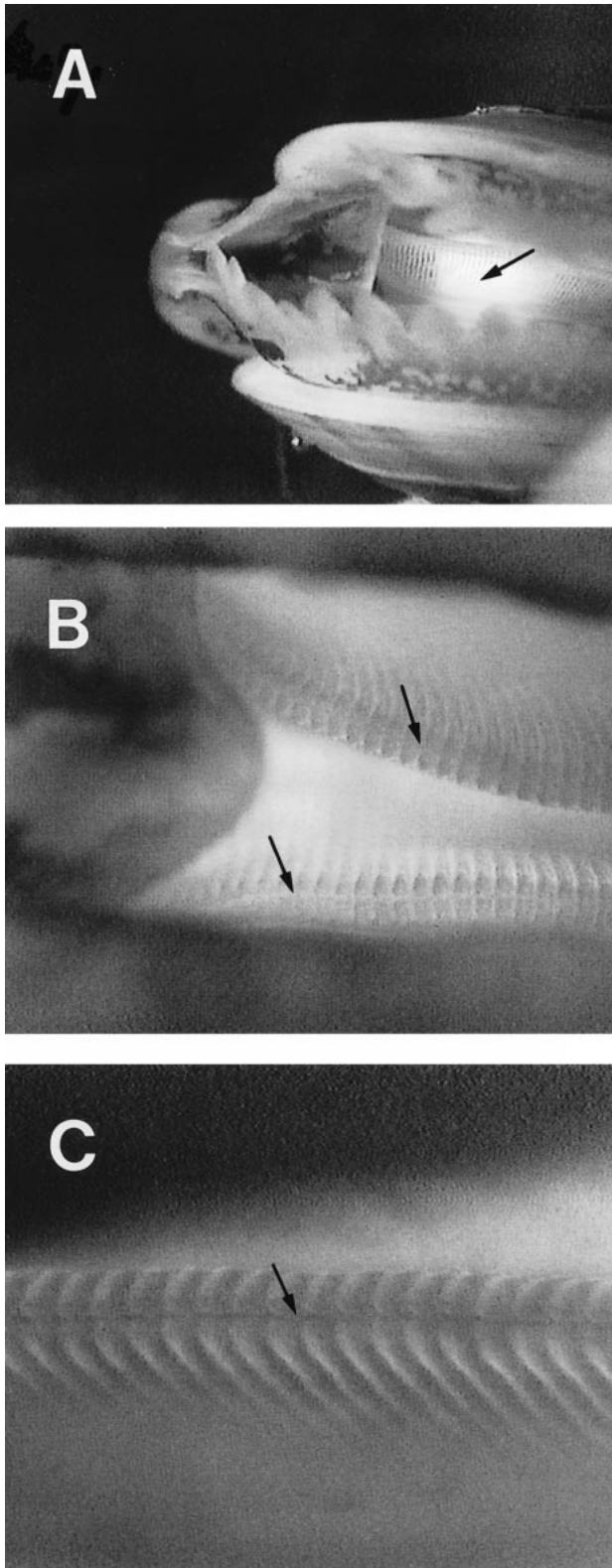


Fig. 1. Videographic prints of an intact *M. edulis* lying on its side and illuminated by means of a cold lamp from behind through the exhalant opening. (A) View of gills (arrow) behind the open valves and mantle edges. (B) Two inner demibranchs with marginal food grooves (arrows). (C) Demibranch built up of 40 μm wide gill filaments, with ventral marginal food groove (arrow).

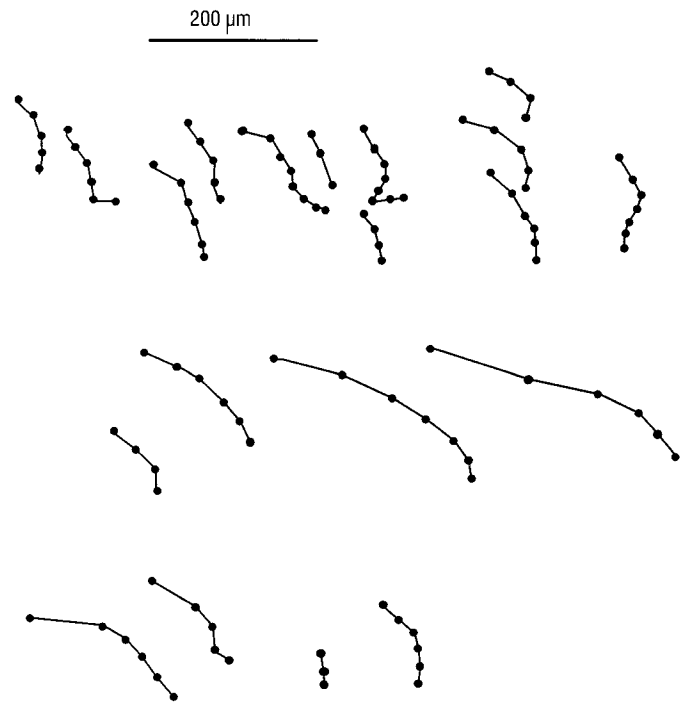


Fig. 2. Paths of algal cells (11 μm) approaching the gill surface of *M. edulis* obtained by means of video recording at three different locations (I, II, III). Particles are coming from the left down towards the gill surface which is approximately horizontal and located just below lowest dot of each particle path. The time interval between each dot symbolizing an algal cell is 0.02 s. Because the particles come from different angles and heights above the somewhat inclined gill surface (see Fig. 1B,C) no exact vertical scale bar can be shown.

observations made by Riisgård et al. (1998) showed that 6- μm diameter suspended algal cells carried with the through current were stopped for a while at the entrance to the interfilament gap. Then the path was reversed 180°, and the particles (during 0.06 s) were transferred to the frontal side of the gill-filament to be transported towards the marginal food groove. Transfer of particles from the through current to the frontal current can be explained only by intervention of the lfc which beat against the current at an angle of 90°. Based on video observations and theoretical estimates Riisgård et al. concluded that particles larger than 4 μm are stopped and transferred to the frontal side of the gill filament, whereas smaller particles either follow the flow around the lfc and escape or they are stopped by the lfc's branching cilia (see also Silverman et al. 1996a,b). This explanation is in agreement with experimentally measured particle retention efficiency in mussels (Møhlenberg and Riisgård 1978; Riisgård 1988; Jørgensen 1996).

New observations and model simulation—New supplemental video observations have recently been made on intact *M. edulis* by Riisgård and Larsen (unpubl.). Using a horizontal dissecting microscope, the gills of an undisturbed, actively feeding mussel (15 mm shell length, 19° C) could be clearly seen behind the gaping valves when the animal was fixed on its side in an observation chamber. By manipulating

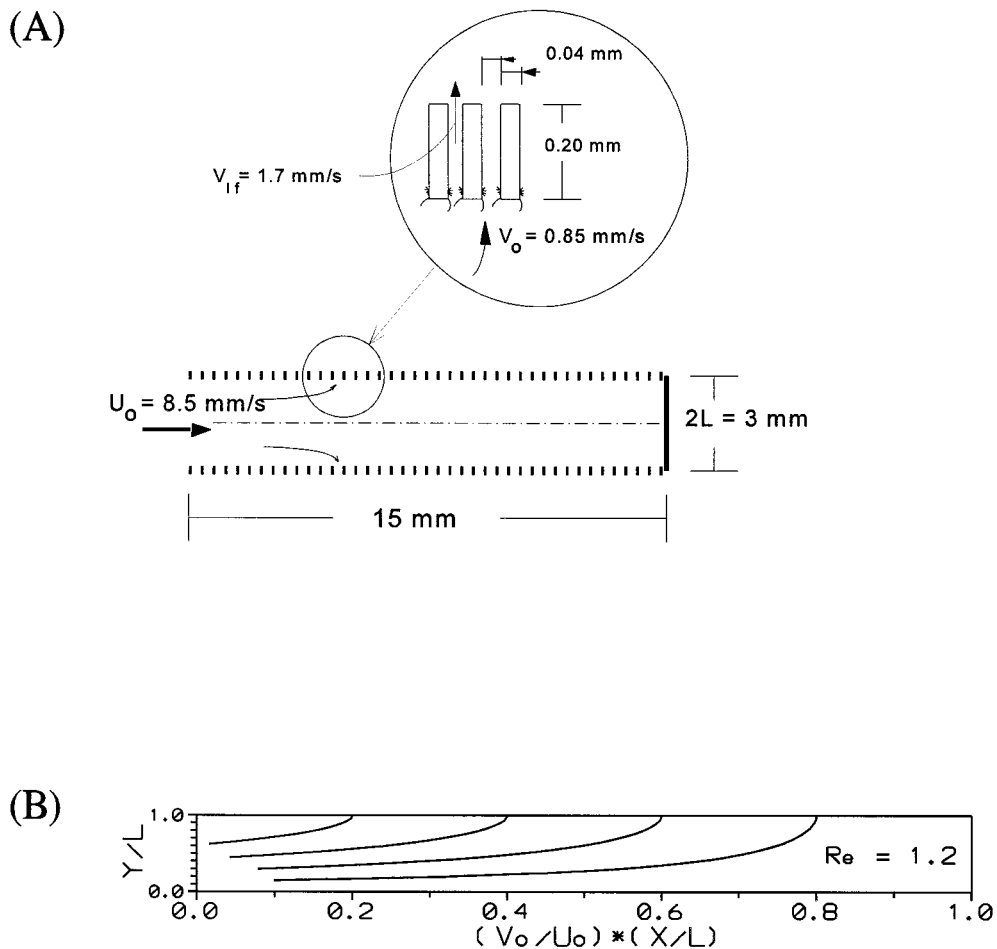


Fig. 3. (A) Model simulating the space between two demibranchs as a channel of length $(U_0/V_o)L$ and width $2L$ with uniform suction at walls representing entrances to gill interfilament canals (insert). (B) Computed streamlines in channel (centerline at $y = 0$, porous walls at $y = \pm L$) with inflow from the left and an impermeable wall at the right. Suction Reynolds number, $Re = 1.2$.

the light from a cold fiber-optic lamp through the exhalant opening we obtained video recordings through the inhalant opening, of quality corresponding to those obtained by means of the video endoscope (Fig. 1). In spite of the poor depth of the microscope focus (about $100 \mu\text{m}$) it was possible to trace *Thalassiosira weissflogii* ($11 \mu\text{m}$) algal cells approaching the gill surface during the last ca. 0.14 s (or 6–7 video frames). Approach speed was measured to be about 1 mm s^{-1} , in agreement with the speed V_0 predicted by Riisgård et al. (1996) (see above), and the particle paths were always curved in the direction normal to the gill frontal surface previous to capture, (see Fig. 2). Most particle paths appear to approach the gill surface at angles between 70° and 90° degrees, and none were observed below 45° immediately above the gill surface. Such curved particle paths were to be expected because of the parallel arrangement of the lc pumps; it is obvious that a curved particle path (in particular a low angle with respect to the frontal surface plane) is not a prerequisite for particle capture, but a result of flow fields set up by the parallel gill filaments with the water-pumping bands of lc.

As pointed out by Ward et al. (1993), it is true that the

far-field velocity distribution for isolated gill filaments will be different from that prevailing in the spaces between mantle cavity and gill and between gills, all depending on the position relative to the inhalant valve through which all fluid must enter. Thus, water drawn through filamental canals along the dorsal end contributes to the global mantle- and gill-space flow that may involve a high component of velocity past filaments. However, high values of this velocity component will prevail away from the gill surface, not close to it, as it must vanish at the surface whereas, on the other hand, the velocity component normal to the gill surface is maximal. The expected streamline pattern will resemble closely, that observed in a channel with porous walls subject to suction, where streamlines curve to become normal to the surface as it is approached (see Fig. 3), exactly as shown by particle paths in Fig. 2. This figure also shows that the axial velocity component may be several times the normal component at the approach to interfilamental canals.

The streamlines in Fig. 3 were computed for the classical model problem of quasideveloped laminar flow in a parallel-wall channel subject to uniform suction at walls (see Berman 1953, for details). Specifically, the model problem simulates

the space between two demibranchs as a channel of length $(U_0/V_0)L = 15$ mm and width $2L = 3$ mm with uniform suction at the walls (mean velocity $V_0 = 0.85$ mm s⁻¹). The figure shows the channel (centerline at $y = 0$, porous wall at $y = \pm L$) with inflow from left (mean velocity $U_0 = 8.5$ mm s⁻¹) and an impermeable wall at the right so that all water leaves through porous walls representing entrances to interfilamental canals. The suction Reynolds number is $Re = V_0L/\nu = 1.2$ ($\nu =$ kinematic viscosity of seawater $= 1.05 \times 10^{-6}$ m² s⁻¹), but streamline shape is insensitive to this parameter (see, e.g., results by Berman (1953) calculated for $Re = 1.0$). Examination of the results in Fig. 3 indicates that the streamlines are essentially normal to the wall within a distance of about 0.10–0.15 mm or more ($y/L = 0.1$) while a 30° inclination may be found at a distance of about 0.4–0.5 mm from the wall. Thus, established fluid mechanics considerations suggest that there should be no significant differences in approaching velocity distributions to isolated gill-filaments and intact gill-filaments within an upstream distance 3–4 times the width of interfilament canals. Also, it is evident that the shape of an observed particle path is very sensitive to the angle at which this spatial curve is viewed.

Unfounded “new explanation”—The above considerations contradict the view advanced by Ward et al. (1998) who assumed straight particle paths and a fixed approaching angle of about 30°. Further, these authors estimated that the mean water flow through the interfilament gap was 2.65 mm s⁻¹ which implies that the pumping rate should be about 60% higher than ever measured in intact mussels under optimal laboratory conditions. The “new explanation” of particle capture in bivalves made by Ward et al. (1998)—and Ward (1996) who previously reported very similar data and viewpoints in another journal—is unfounded, and there are no reasonable arguments for refuting earlier observations on isolated gill preparations. Neither is there any justification for the claim that inclined flow towards an array of cylinders should entail reduced drag for the through flow.

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