

CYPHONAUTES' CILIARY SIEVE BREAKS A BIOLOGICAL RULE OF INFERENCE

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ABSTRACT

Biologists routinely assume that a mechanism demonstrated in one organism will be found in others in which form, function, ancestry, and physical parameters are similar. These criteria failed to predict the feeding mechanism of the cyphonautes larva of bryozoans. Larval and adult bryozoans feed with cilia similar in size and distribution, with similar current velocities, and similar Reynolds numbers. Nevertheless, the larvae filter particles with a sieve of stationary cilia whereas the adults respond to individual particles with an induced local reversal of ciliary beat and concentrate particles without filtration. The larva's ciliary sieve is doubly unexpected because it requires a ciliary stiffness at the maximum recorded for cilia.

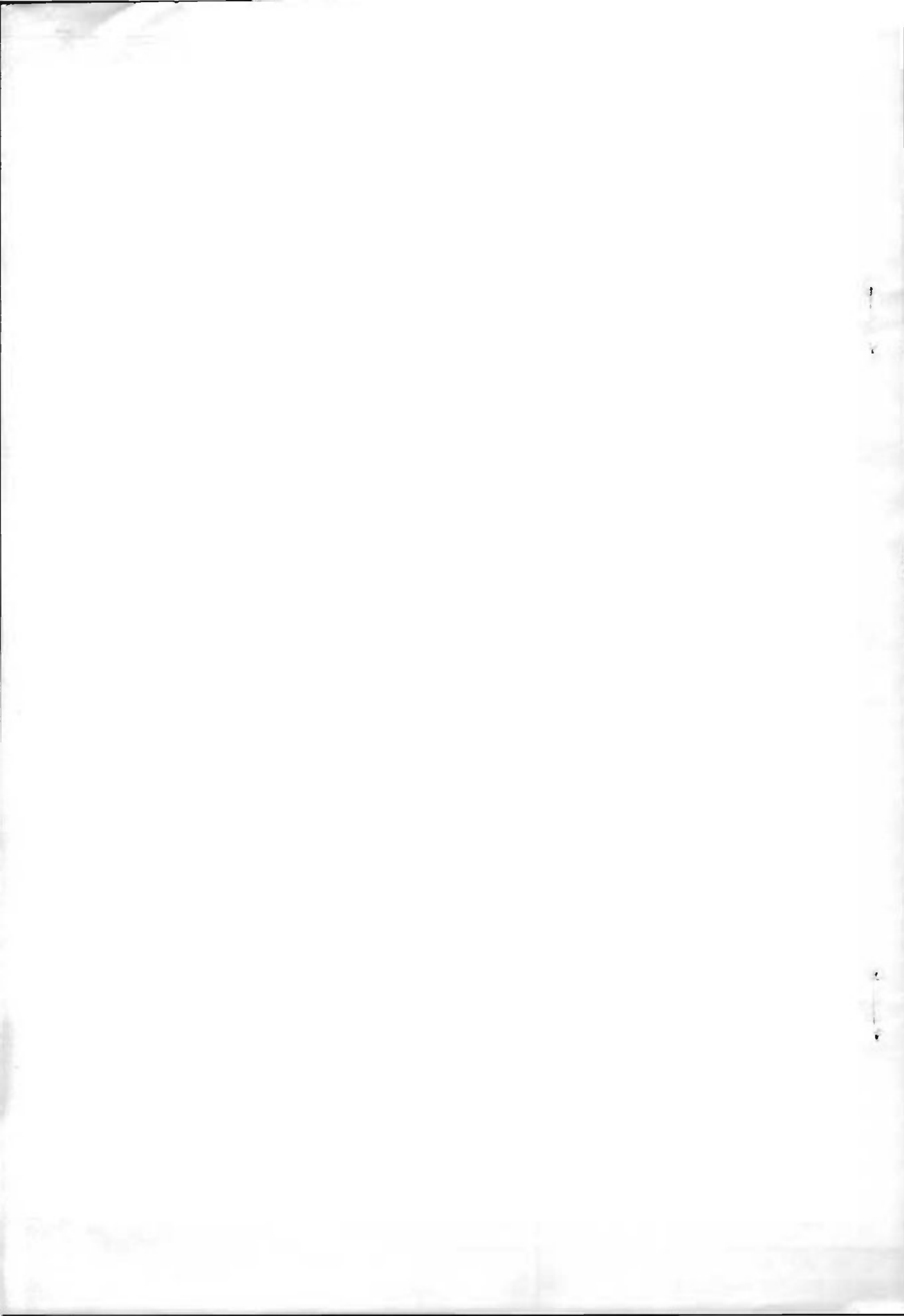
INTRODUCTION

Extrapolation of conclusions from one organism to another is based on morphological and functional similarity, evolutionary relationship, and similarity of size and physical properties. When a mechanism is studied in detail for one species, the same mechanism is routinely assumed to occur in species that meet these criteria of similarity. This basis for generalization about mechanisms rarely fails. These criteria led to the prediction that the cyphonautes larvae of bryozoans would capture particles by the mechanism that has been observed in the adults. The prediction failed.

Larval and adult bryozoans both feed on small suspended particles of food, and both use a similar arrangement of ciliary bands to capture and concentrate the suspended particles. The cilia are arranged in parallel frontal, laterofrontal, and lateral bands (Atkins, 1955; Strathmann, 1973; Winston, 1978) (Fig. 1). The lateral cilia are about 20 μm long and beat with the effective stroke perpendicular to the ciliary bands and away from the frontal surface. The lateral cilia create a feeding current of 1 to 2 mm/s. The laterofrontal cilia are about 20 to 30 μm long and stationary. Observations of particle motion indicate that the frontal cilia beat parallel to the ciliary bands. Food particles are retained upstream from the band of lateral cilia and transported along the frontal surface toward the mouth. This consistency suggests that food particles are concentrated by the same physical mechanism in both larva and adult. Nevertheless, high speed films of particle capture show two different mechanisms.

In the adults the band of lateral cilia concentrates particles by local reversals of beat induced by individual particles (Strathmann, 1982). Because about 50 to 100 μm of ciliary band changes beat to capture a 10 μm particle (Fig. 2), each particle must be captured along with a much larger surrounding parcel of water. The capture and redirection of water along with the particle is inferred from the alteration of ciliary beat because at low Reynolds numbers the alteration of ciliary motion will immediately affect the motion of surrounding water. The adult therefore concen-

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Diagrammatic detail of ciliated ridges

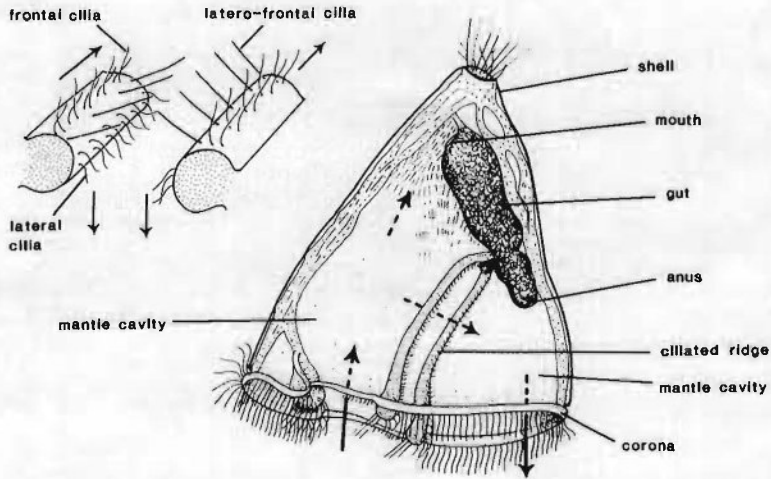


FIGURE 1. Bands of cilia on the two ridges in the mantle cavity of a bryozoan larva are at upper left. Arrows show particle paths near lateral and frontal cilia. Bands of cilia on tentacles of adult zooids are similar. The position of the ciliated ridges and feeding currents in the mantle cavity of a bryozoan larva are at lower right.

trates particles without employing a filter. Instead, particles are redirected to the frontal surface of the tentacle by brief and local alterations of currents by the lateral cilia. The water redirected with a particle is a tiny fraction of the water that passes the tentacles wherever there are no particles to trigger reversals of ciliary beat; therefore the degree of concentration of suspended particles is enormous. For concentration of particles in this system the mechanics of triggering a ciliary reversal is relevant; the mechanics of filtration is not (Strathmann, 1982). The cyphonautes larva was expected to employ the same mechanism (Strathmann, 1973), but high speed films have given no indication of capture by local reversals of ciliary beat and instead demonstrate that the larva uses laterofrontal cilia as a stationary ciliary sieve.

MATERIALS AND METHODS

Larvae were collected from San Juan Channel near the Friday Harbor Laboratories in Washington and from the Gulf Stream off the Smithsonian Marine Station at Link Port, Florida. The larvae from Friday Harbor were filmed at 60 frames per second with a cinecamera equipped with a timing light that marked margins of the 16 mm film. Particles retained on the sieve of laterofrontal cilia were photographed in more transparent bryozoan larvae from the Gulf Stream by electronic flash. The abundance of adults suggests that the larvae from Friday Harbor were *Membranipora membranacea* and the larvae from the Gulf Stream *Membranipora tuberculata*. All observations and photographs were with differential interference contrast optics. The combination of these optics and high speed film results in grainy photos in shades of grey with narrow depth of focus (Figs. 2, 3) but demonstrates events not visible by other means. The films are clearer in motion than in still prints, but even the prints support or reject some hypotheses on particle capture. The cyphonautes larvae in Figure 3 were capturing polystyrene divinylbenzene spheres of 5 μm diameter of the type used to calibrate electronic particle counters. The alga *Dunaliella tertiolecta*

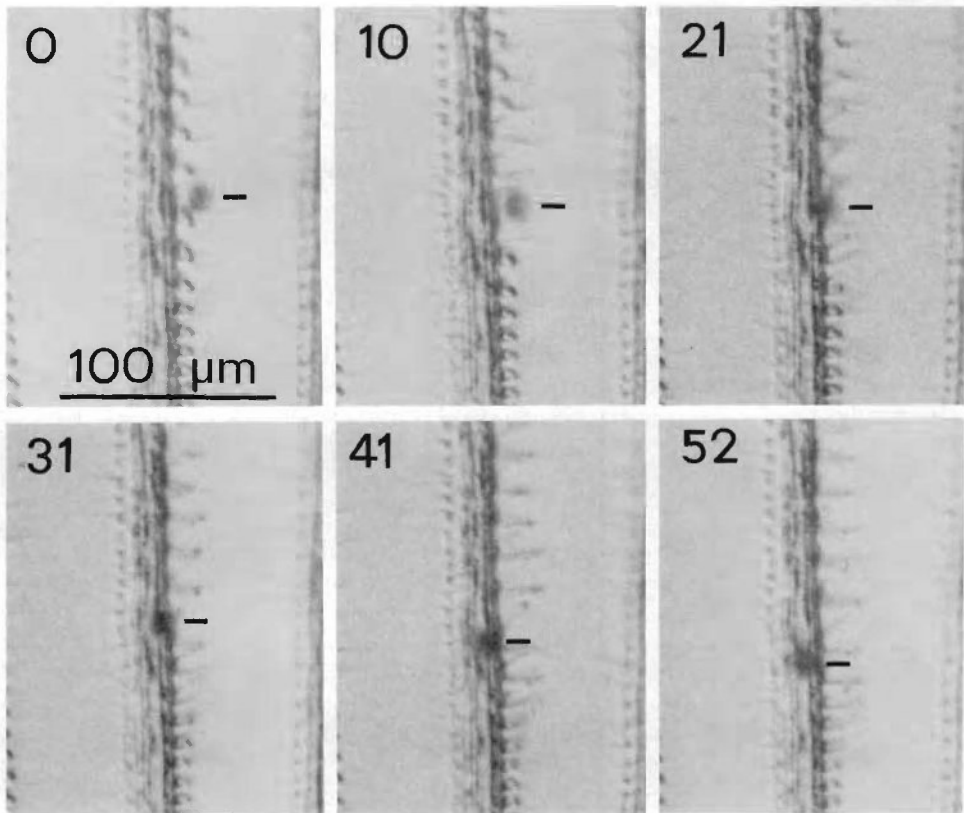


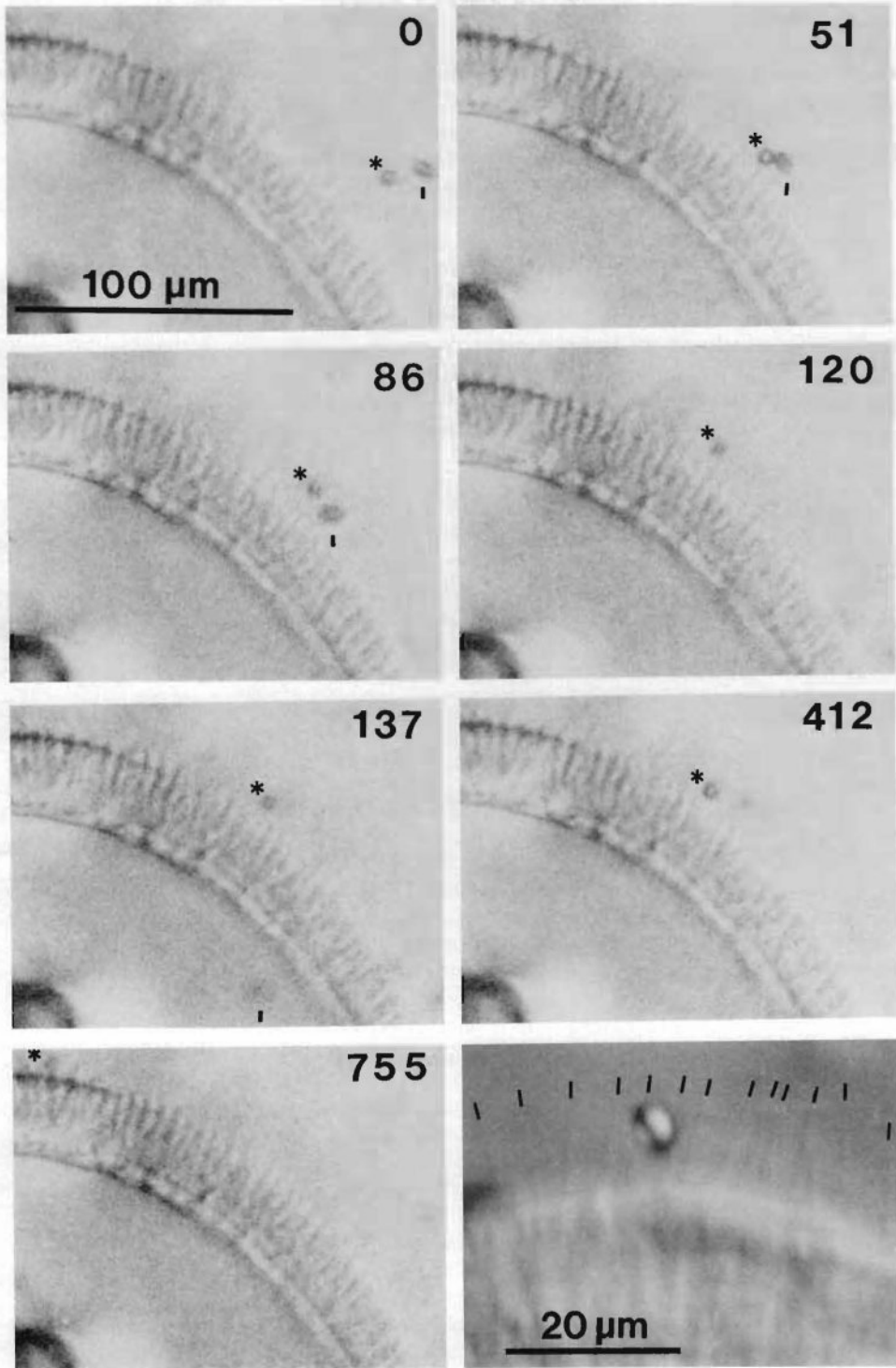
FIGURE 2. Capture of a particle by induced local reversal of lateral cilia on the tentacle of an adult bryozoan. The tentacle is viewed from the frontal surface so that the phased waves of beat of lateral cilia are visible on both sides of the tentacle. The black bar marks the particle's position. Within 10 ms the waves of ciliary beat are disrupted over about $50\ \mu\text{m}$ of the adjacent band as the cilia alter beat in response to the particle. (Note the regularity of metachronal waves and positions of recovery and effective strokes at 0 ms compared to those near the particle at 10 ms.) More cilia alter beat between 21 and 52 ms as the particle is moved toward the frontal surface (center of tentacle) and then proximally along the tentacle (downwards) toward the mouth.

(about $10\ \mu\text{m}$) was also used in observations of the cyphonautes. The adult bryozoan in Figure 2 was capturing an algal cell of about $10\ \mu\text{m}$.

RESULTS

In contrast to the adults, bryozoan larvae captured particles by sieving with their band of stationary laterofrontal cilia: the bands of lateral cilia produced feeding cur-

FIGURE 3. Capture of a particle by a sieve of stationary laterofrontal cilia of a larval bryozoan. The ciliated ridge is viewed from the lateral surface. The asterisk marks the captured particle; the vertical bar marks a particle passing through the sieve. The captured particle is caught at the 51 ms frame and remains nearly stationary for 0.35 s while the other particle passes nearby. At 755 ms the particle is being transported along the frontal surface of the ciliary ridge toward the mouth. There is no discernible disruption of beat of the lateral cilia, whose metachronal waves appear as dark and light bands on the ciliated ridge. Lower right: $5\ \mu\text{m}$ sphere caught by two stationary laterofrontal cilia; black bars mark visible laterofrontal cilia.



rents as in the adult bryozoans, but the cinefilms indicate that larvae concentrated particles by filtration rather than by an induced local reversal of beat. Each particle was retained upstream without the disruption of metachrony that would indicate a reversal of beat of lateral cilia (Fig. 3). Also, particles remained almost stationary in the upstream position while lateral cilia continued to beat and other particles passed nearby (Fig. 3). Particles lodged against the upstream side of the row of laterofrontal cilia and then remained motionless as long as several seconds while the lateral cilia continued beating. Laterofrontal cilia were spaced more closely than $5 \mu\text{m}$ apart and retained plastic spheres of $5 \mu\text{m}$ diameter (Fig. 3). The lack of motion of particles retained by the larva was in striking contrast to the rapid motion of particles retained by the adult (Strathmann, 1973, 1982). Thus particle motion, ciliary motion, and the position of particles on laterofrontal cilia all indicated sieving.

Particles caught on the laterofrontal cilia eventually either moved along the tract of frontal cilia toward the mouth to be eaten or passed the laterofrontal cilia and were lost to the excurrent chamber (Fig. 3). Previously unfed larvae ate almost all particles captured. We could not observe the mechanism by which particles were transferred from the sieving laterofrontal cilia to the upstream cilia that carry food to the mouth.

Could there be a behavioral switch, such that larva or adult could employ both sieving and induced reversal of beat? Comparative observations have failed to support this hypothesis. Motion of captured particles has been observed for at least two species of bryozoan larvae (this study) and four species of bryozoan adults (Strathmann, 1973, 1982), and the observations included both adult and larval stages of a *Membranipora* species from San Juan Channel. R. L. Zimmer (pers. comm.) also observed sieving by the laterofrontal cilia of bryozoan larvae. In all comparisons sieving has been specific to the larval stage, and local reversal of currents has been specific to the adult.

DISCUSSION

Many animals' sieves are composed of parallel cilia or setae, each supported at only one end. If these fibers bend too far under the added drag of a food particle, they will lose the particle. Does the flexural stiffness of cilia place limits on sieving by laterofrontal cilia? Studies of animal filtration have addressed the physics of fluid and particle movement (Strathmann, 1971; Fenchel, 1980; Koehl, 1981; LaBarbera, 1984; Silvester and Sleight, 1984), but not the mechanical properties of filtering fibers. A simple model provides an estimate of the requisite stiffness. Consider a spherical particle of $5 \mu\text{m}$ diameter (D) that encounters a stationary cilium at a distance (L) of $20 \mu\text{m}$ from the base of a cilium with a velocity (U) of 1 mm/s . When the particle is stopped by the stationary laterofrontal cilia, it is exposed to the continuing current from the lateral cilia. The drag force (F) on the particle predicted by Stokes' law, $F = 3\pi \mu UD$, is about $5 \times 10^{-11} \text{ N}$ although accuracy of the estimate is reduced by the proximity of surfaces and by accelerations from nearby lateral cilia. The predicted deflection of the cilium at the point of loading is $x = FL^3/3EI$, with EI the flexural stiffness of the cilium (Gordon, 1976). The highest estimate of flexural stiffness for a cilium (Baba, 1972) is 2 to $3 \times 10^{-19} \text{ N m}^2$. This estimate for flexural stiffness implies deflection of about 1 or $2 \mu\text{m}$. This small deflection should not prevent sieving, but a greater deflection would release particles from the sieve. Thus the laterofrontal ciliary sieve appears to be operating near the mechanical limits for a ciliary sieve. Baba's (1972) estimate is for abfrontal cilia of *Mytilus edulis*. Other published estimates of ciliary flexural stiffness are lower by more than an order of magnitude (Strathmann *et al.*, 1972; Okuno and Hiramoto, 1979). The flexural stiffness of single cilia may be near the limit required for sieving particles.

Ciliary sieves have been reported for other animals, but in these the cilia are either shorter or combined into thicker compound structures (Fenchel, 1980; Dral, 1967). Stationary laterofrontal cilia may occur throughout a related group of animal phyla; they are reported with varying evidence from larval and adult bryozoans (Atkins, 1955; Winston, 1978), larval (Strathmann, 1973) and adult (Gilmour, 1978) phoronids, and adult brachiopods (Gilmour, 1978), but their function is unknown. For the adult bryozoans, phoronids, and brachiopods that have been carefully examined, the motion of captured particles indicates local reversal of currents by the lateral cilia (Strathmann, 1973, 1982). The same is true for larval enteropneusts and larval echinoderms (though these lack laterofrontal cilia) (Strathmann, 1971; Bonar and Strathmann, 1976), and films of feeding echinoderm larvae confirm that induced ciliary reversal is the mechanism of particle capture (Strathmann *et al.*, 1972). All five phyla belong to a larger related group, the oligomera. Because the induced reversal mechanism is characteristic of five related phyla, a different mechanism in the bryozoan larvae is especially surprising. In retrospect one might argue that the close juxtaposition of laterofrontal cilia across a closed chamber is an arrangement more conducive to sieving than the more separated bands on tentacles of adult bryozoans, but brachiopods also have parallel closely arrayed ciliary bands enclosed by a shell and are not known to employ a sieve (Strathmann, 1973). Indeed, the bryozoan larvae are the only animals known to sieve particles by a palisade of long, separate cilia.

Species are too numerous for the underlying mechanisms in a process to be demonstrated on every stage of development of each one. Some basis is required for generalizing from intensive study of a few. The usual guides for extrapolation are homology, analogy, and similarity of physical parameters. These guides have failed in this instance. Generalizations based on homology, analogy, and physical similarity cannot be abandoned (we have used them throughout this paper in generalizing about differences between larval and adult bryozoa), but because these criteria occasionally fail, the generality of mechanisms demonstrated in a few model systems must remain uncertain.

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