Motile cilia create fluid-mechanical microhabitats for the active recruitment of the host microbiome


We show that mucociliary membranes of animal epithelia can create fluid-mechanical microenvironments for the active recruitment of the specific microbiome of the host. In terrestrial vertebrates, these tissues are typically colonized by complex consortia and are inaccessible to observation. Such tissues can be directly examined in aquatic animals, providing valuable opportunities for the analysis of mucociliary activity in relation to bacteria recruitment. Using the squid–vibrio model system, we provide a characterization of the initial engagement of microbial symbionts along ciliated tissues. Specifically, we developed an empirical and theoretical framework to conduct a census of ciliated cell types, create structural maps, and resolve the spatiotemporal flow dynamics. Our multiscale analyses revealed two distinct, highly organized populations of cilia on the host tissues. An array of long cilia (~25 µm) with metachronal beat creates a flow that focuses bacteria-sized particles, at the exclusion of larger particles, into sheltered zones; there, a field of randomly beating short cilia (~10 µm) mixes the local fluid environment, which contains host biochemical signals known to prime symbionts for colonization. This cilia-mediated process represents a previously unrecognized mechanism for symbiont recruitment. Each mucociliary surface that recruits a microbiome such as the case described here is likely to have system-specific features. However, all mucociliary surfaces are subject to the same physical and biological constraints that are imposed by the fluid environment and the evolutionary conserved structure of cilia. As such, our study promises to provide insight into universal mechanisms that drive the recruitment of symbiotic partners.

Significance

Recent findings demonstrate that microbiome communities often reside on mucociliated surfaces. While mucociliary clearance of bacteria from such surfaces has been extensively studied, the process of bacterial recruitment has remained unexplored. Here, using a simple model system, we show that ciliated surfaces, in addition to their clearance function, can create fluid-mechanical microhabitats with distinct transport and mixing properties that facilitate the active recruitment of symbiotic candidates from a background of suspended particles. Although each specific system will have unique properties, because ciliary structure and function are highly conserved, studies of models will contribute to an understanding of rules governing the selective behavior of ciliated surfaces.


Conflict of interest statement: Coauthor E.A.C.H.-H. and reviewer M.A.R.K. are both affiliated with the University of California, Berkeley, but in different departments. See Profile on page 9494.

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We conducted our case study using the symbiosis between the squid Euprymna scolopes and the bioluminescent bacterium Vibrio fischeri, which is an established invertebrate model for investigating interactions between bacterial partners and their host epithelia (for review see ref. 16). Newly hatched E. scolopes recruit V. fischeri to the surface of their nascent light-emitting organ from inhaled seawater. The seawater, which contains a diverse background of other bacterial species and suspended abiotic particles, is drawn into the mantle cavity for the animal’s respiratory flow and passes across the light organ. During embryogenesis, the light organ surface develops two complex, juvenile-specific ciliated fields—each featuring two appendages—that are lost after colonization by symbiotic bacteria, which indicates a possible role of the cilia in promoting symbiont recruitment (Fig. 1B).

During initiation of symbiosis, V. fischeri cells become competitively dominant in bacterial aggregates located above the entry pores on the ciliated surface. They reside in these aggregates for a few hours, a time during which they become physiologically prepared or “primed” for their eventual migration through the pores and into the light organ (16). The strict timeline, well-defined localization, and exclusiveness of the squid–vibrio partnership, which occurs in an experimentally accessible, yet intact, internal organ, have revealed highly conserved biochemical mechanisms that also govern specific bacteria–host associations in mammals (16). Here, we used the squid–vibrio symbiosis to investigate the general question of how ciliary activity aids in the transport and localization, and exclusiveness of the squid–vibrio partnership, and into the light organ (16). The strict timeline, well-defined

Results

Evidence for a Cilia-Mediated Bacteria–Host Association. Like the respiratory airways, the light organ is continuously exposed to inhaled particulate matter of a wide range of composition and size (20). In the early stages of symbiosis, wild-type V. fischeri, as well as nonmotile mutants of V. fischeri, nonsymbiont bacteria, and bacteria-sized synthetic particles, all accumulate at the light-organ surface (Fig. 1E), after which further selection takes place (17). These findings indicate that neither a specific bacterium shape nor a specific behavior is necessary for the first stage of association. This result poses two questions: (i) Does the respiratory flow directly deliver bacteria-sized particles to the ciliated surface? And (ii) how is larger particulate matter excluded from this surface?

Using video microscopy and particle tracking, we observed two counterrotating fluid vortices near the appendages of the light organ (Fig. 1E). These vortices occur in vivo as well as in excited
light organs, indicating little contribution of the mantle geometry in directing particle trajectories at this scale. Further, during respiration at rest, the mantle pulsates at a much lower frequency (2 Hz) than the ciliary beat frequency (10 Hz), producing a creeping flow that incrementally refreshes the fluid volume in the mantle but contributes negligible inertial mixing and is at least six times slower near the light organ than the cilia-generated surface flow (Fig. S1 and Movie S1). These observations raise the question of whether the mantle-driven flow could play a direct role in delivering suspended particles to the ciliated surface. Such particle capture from oncoming flow has been rigorously studied in aquatic suspension filter feeders (21–24). Mechanisms for particle capture include direct interception, inertial impaction, gravitational deposition, diffusion, and motile-particle deposition (5).

We can rule out a dominant role of motile-particle deposition given the particle and flow properties observed in our system (Model of Particle Capture by Direct Interception) (25, 26).

To test experimentally whether DI could be a major mode of particle capture, we exposed the animals to a suspension containing both V. fischeri-sized (1 μm diameter) and larger (4 μm diameter) particles. We observed that 1-μm particles accumulated on the side of the appendages facing the pores, as previously reported (17), whereas the 4-μm particles tended to adhere to the outside of the appendages (Fig. 2A and 2B). This size bias is consistent with a role of the light organ in retaining V. fischeri and rejecting larger suspended particles. The DI model, however, predicts the opposite trend, i.e., a lower capture rate of V. fischeri-sized particles compared with larger particles (Fig. 2C) (27).

Interestingly, impaired ciliary activity results in indiscriminate particle adhesion throughout the light-organ surface (Fig. S2). Together, these results suggest that particles are not captured by a passive, direct interception mechanism, but instead by an active, cilia-driven filtering mechanism that enables both clearance and selective, localized, aggregation of bacterial candidates at the ciliated surface. We next examined the structure, kinematics, and spatial organization of the cilia covering the surface and then quantitatively related these properties to the ciliary flows and filtering functions that emerged.

Structural and Kinematic Characterization of the Ciliated Epithelium. We used a rapid-fixation protocol, which “freezes” ciliary motion, followed by scanning electron microscopy (Fig. S3) to generate a snapshot image of ciliary activity on the light organ (28). We identified two distinct populations of cilia: long cilia beating in a metachronal wave along the outside of the appendages and short cilia with no detectable coordination covering the region around the pores and along the medial side of the appendages. High-speed video recordings (Movie S2) and confocal imaging, followed by kymograph analyses, ciliary beat frequency analysis, and kinematic analyses (Fig. 3 A–J), confirmed structural and kinematic differences between the two ciliary populations and enabled us to derive a tissue-wide map of spatiotemporal cilia organization and activity (Fig. 3K). The long cilia are 25 μm in length and exhibit a stereotypic movement
commonly seen in motile cilia, i.e., an asymmetric stroke pattern and metachronal coordination across neighboring cilia. In contrast, the short cilia are only 10 μm in length and display a temporally and spatially symmetric beat pattern that had no discernible coordination across neighboring cilia under any experimental condition. This unusual behavior is intriguing because, to our knowledge, symmetric and uncoordinated kinematics have been associated only with perturbed or pathological systems because of the focus on stereotypic structures and behaviors of motile cilia.

**Long Cilia Help Select and Focus Bacteria-Sized Particles.** Particle tracking and velocimetry in excised light organs revealed that the two populations of cilia generate two distinct flow compartments (Fig. 4A and B, Fig. S4, and Movie S3): a vortical flow region consisting of two counterrotating vortices above the long cilia of the appendages and a sheltered zone near the pores above the short cilia. Both passive particles and motile *V. fischeri* cells that were caught in the vortices followed curved trajectories converging near the ciliated surface, where flow velocities reached up to 600 μm/s. Most of this entrained material was diverted into the central outward jet between the two vortices and was deflected away from the sheltered zone; however, a small fraction of particles entered and remained in the sheltered zone, often together with host-shed mucus (Movie S4). Moreover, the fast, near-surface flow generated by the long cilia prevented particle adhesion to the outer side of the appendages, while wrapping around and sheltering the zone lined by short cilia (Fig. 4C and D), where particles were found to directly bind to the surface. We specifically verified that the vortical flow and deposition of *V. fischeri* and other small particles in the sheltered zone were not an artifact of removing the organ from the squid’s mantle cavity, but also take place inside the intact, living animal (Fig. S5 and Movie S5). Further, captured bacteria that actively migrate and other small particles in the sheltered zone were not an artifact of removing the organ from the squid’s mantle cavity, but also take place inside the intact, living animal (Fig. S5 and Movie S5).

We used a computational model to probe the role of the long cilia in creating the two flow compartments. Namely, we reconstructed the cross-section of the organ’s appendages by circumscribing the cilia tips, thereby producing two circles. We modeled the collective activity of the cilia by prescribing a tangential velocity around these circles that reflects the observed direction of the ciliary beat (Fig. 4C, Fig. S6 A–F, and Movie S6). This model does not take into account the beat pattern of the individual cilium; it rather accounts for the effective slip velocity caused by the ciliated surface. The resulting flow was obtained by solving Stokes equations for low-Reynolds numbers subject to the prescribed tangential velocities. This flow pattern recapitulates the cilia-driven flow observed empirically, i.e., a pair of
counterrotating vortices and a central sheltered zone (Fig. 4E).
Although this flow pattern is robust to small perturbations in the
tangential velocity profile, it is not necessarily reproducible by
profiles corresponding to arbitrary ciliary beat patterns and spa-
tial distributions (e.g., Fig. S6 K–M). This finding indicates that
formation of the two distinct flow zones is sensitive to the spa-
tiotemporal organization of ciliary beat.
Next, to explore whether the flow field constitutes a hydro-
dynamic sieve, selectively barring entrance of suspended mate-
rial into the sheltered zone, we seeded the computed flow field
with particles of finite diameter \( d_c \) that faithfully follow the
flow streamlines (Fig. S6G). These particles model the transport
of bacteria, which, under the flow condition given here, cross
streamlines neither by diffusion nor by gravity or inertia because
the bacteria are near neutrally buoyant (see Flow Visualization
and Analysis and Table S1 for details). We observed size-selective
streaming, wherein only particles up to a critical diameter \( d_c \)
entered the sheltered zones (Fig. 4F). Particles larger than \( d_c \)
span so many streamlines that they are diverted into the cen-
tral outward jet, whereas smaller particles can follow the com-
presed near-surface streamlines into the sheltered zone.
Interestingly, this mechanism has also been exploited in microfluidic
devices for particle sorting (32, 33). We found that the value of
\( d_c \) increases monotonically with the ratio of appendage spacing
\( \Delta \) and diameter \( D \) (Fig. 4G) because, for smaller ratios of \( \Delta/D \),
there is greater convergence of outward-bound streamlines near
the appendages, preventing any particles spanning these stream-
lines from entering the sheltered zone. Hence, for increasing
ratios of \( \Delta/D \), particles of increasing diameter are captured, and
the capture rate of all particles uniformly seeded across the span
of the two appendages also increases (Fig. 4H and Fig. S6 I–K).
For empirical ranges of \( \Delta \) and \( D \) (Fig. S3), the model predicts
a median value of \( d_c = 4 \, \mu \text{m} \) (Fig. 4I), which is congruent with
the empirical outcomes. These results support our hypothesis of
a cilia-driven selective mechanism that biases particle capture
rates in favor of particles the size of bacterial symbionts.

**Short Cilia Enhance Molecular Mixing.** We next investigated the
role of the short cilia lining the sheltered zones. Tracer trajec-
tories suggested a mix of diffusive transport with crisscrossing
directional flow (Fig. 5 A–C and Fig. S7); therefore, we specu-
lated that the combination of symmetric beat kinematics in
individual cilia and random stroke phase between neighboring
cilia may result in enhanced fluid mixing, but no net transport.
To test this hypothesis computationally, we developed a carpet
model consisting of discrete cilia, where the beat kinematics
of each cilium are adapted from empirical measurements (Fig. 2G
and Fig. S8). We considered three modes of phase coordina-
tion: synchronous activity with no phase differences (SYNC),
metachronal beating with a phase difference of \( 45^\circ \) between
neighboring cilia (META) (34), and random phase coordina-
tion, where each cilium is randomly assigned a phase between
0° and 360° (RAND) (Fig. 5D). Solving for the resulting flow
fields, we found three distinct average patterns: specifically, zero
net velocity in the SYNC mode, laminar flow in META mode,
and vortical flow in RAND mode (Fig. 5E). To investigate the
mixing performance of each flow field, we seeded horizontal or
vertical strips of nondiffusing particle tracers (Fig. 5F) and let
each ciliary beating mode. Axes are normalized to cilia length. (E–H) For
each mode, (E) average flow field over one beat cycle; (F) mixing of hori-
zontally (Top) and vertically (Bottom) stratified particles after 16 beat cycles;
(G) mixing efficiencies in the horizontal and vertical dimensions of different
beat modes (box plots: results of 10 Monte Carlo simulations); and (H) aver-
age flow rates generated per cilium per beat cycle (box plots: results of 10
Monte Carlo simulations). In G and H, central rectangle spans first to third
quartile of data, red line denotes median, “whiskers” show minimum and
maximum, and red cross denotes outlier.
this distribution of particles evolve during multiple cycles of ciliary beat. After 16 cycles, tracer distributions were strikingly different among the three cases: While there is no obvious pattern change in SYNC mode, and limited distortion in META mode, the RAND mode disrupts much of the initial stratification by stretching and folding fluid filaments, a hallmark of so-called chaotic mixing (35). To quantify mixing, we defined a mixing efficiency of \( \eta = -\ln(m/m_0)/N \), where \( m_0 \) and \( m \) are the mixing numbers equivalent to the average minimum distance between tracer particles of different colors after 0 and \( N \) cycles, respectively (36, 37). Concordant with the qualitative results, the RAND mode outperforms the other modes in both horizontal and vertical mixing, with mixing efficiency depending on the particular phase distribution used in the simulation (Fig. 5G). Importantly, mixing in RAND mode predicts that initially neighboring particles quickly diverge on separate trajectories (Fig. S8), an expectation that matches the empirical data (Fig. 5C).

We then measured fluid transport in terms of volumetric flow rate \( Q \), defined as the average volume of fluid moved per cilium per beat cycle (Fig. 5H). SYNC mode created zero net fluid transport, as expected from the Scallopl theorem at low Reynolds numbers (38), while the META mode generated a small forward flow, and the RAND mode was equally likely to result in forward or backward flow, equivalent to a net flow rate near zero over time. It should be noted that the asymmetric beat generally associated with motile cilia will result in directional fluid transport in both the SYNC and RAND modes (39).

Taken together, these results suggest that a symmetric ciliary beat with a randomized phase achieves chaotic mixing that accelerates molecular transport without generating net fluid transport and effectively doubles the rate of diffusion of biochemical molecules in the kilodalton mass range (37). Such “enhanced” diffusion accelerates the formation of concentration gradients emanating from chemical signal sources, a mechanism exploited in microfluidic devices (40). Specifically, the characteristic time \( T \) to develop a steady-state gradient across a distance \( L \) is determined by diffusion rate \( D \), with \( T \sim L^2/(2D) \). (41). The faster spread and gradient formation of effector molecules would foster the biochemical dialogue among captured \( V.\ fischeri \) and between bacterial and host cells, including nitric oxide-mediated bacterial selection, chitobiase-dependent bacterial priming and chemotaxis, and the interaction with host-released antimicrobial molecules, including BPIs, lysozyme, PGRP2, and galaxin (16, 18). Indeed, a bacterial population in a well-mixed environment, i.e., with fully developed concentration gradients, can better initiate group behaviors by deducing bacterial cell density from the local concentration of quorum signals (42, 43).

Mixing without transport has not been previously described in common ciliary arrangements (34), demonstrating the importance of considering individual ciliary beat together with collective organization. In our analyses, it was first important to implement the symmetric stroke cycle, which by itself does not create any net flow because of the time reversibility in low-Reynolds number regimes (38) (Fig. S8). Second, because any flow-generating asymmetry must therefore arise from the activity of multiple cilia, it was necessary to recapitulate random-phase coordination among neighboring cilia. For the squid-vibrio system specifically, these findings add a fluid-mechanical dimension to the symbiont–host dialogue (Fig. S9), and they refute the longstanding assumption that flow generated by ciliary beat would necessarily compromise the formation of biochemical gradients for bacteria–host signaling (31). Taken together, our study has revealed a class of motile cilia with structural and kinematic adaptations that support fluid mixing in a stagnant zone and, hence, extend the known spectrum of ciliary functions.

**Discussion**

Our finding of different functional modalities of distinct cilia populations on mucus epithelia opens vistas for the understanding of these important subcellular structures in animal biology. It showcases how the impact of ciliated-tissue patterning extends well beyond the tissue surface, where it controls the formation of distinct fluid-mechanical environments. The combinatorial powers of ciliary parameters described in our study, such as beat direction, kinematics, and coordination, suggest a richness of potential scenarios for shaping the extracellular fluid environment at multiple scales to drive tissue homeostasis and remodeling, much as described for other tissue-organizing mechanical forces (44). Indeed, new imaging techniques have recently mapped both structurally distinct populations of cilia and spatiotemporally varying ciliary flow dynamics, in the ventricles of the mouse brain (45, 46). Multiscale analyses of ciliated tissues not only reveal new tissue-level phenomena, but also enable a quantification of their functional roles in different tissue environments. Such analyses require the integration of empirical and computational approaches for studying cilia function, like those developed in this study, as well as for investigating the particular fluid environment, including air and mucus (47).

In addition, our findings provide a mechanism by which ciliated epithelia generate a landscape of different fluid-mechanical microenvironments that support the formation of distinct “bio-geographic” sites for the microbiota. Such a spatial series of ecological niches has previously been demonstrated along other epithelia, such as the mammalian gut lining, where tissue morphology and mucus interact to shape discrete microenvironments, each selecting for characteristic microbial communities important for gut function (48, 49). Furthermore, this study suggests how a microbial pathogen might alter ciliary movement to foster tissue colonization. These pathogens often misappropriate the mechanisms by which a host interacts with its beneficial bacteria, such as using the bacterial surface molecules lipopolysaccharide and peptidoglycan to signal the host (50). Here we have identified a distinct cilia-generated flow that creates a highly localized sheltered zone within which the functional properties differ markedly from adjacent regions and in which bacterial cells accumulate. While the features of such biomechanical environments may have evolved to foster interaction with the beneficial microbiota, they may also be conscripted, or created de novo, by pathogens. For example, the human airway pathogen *Borde-tella* spp. releases ciliostatic compounds, locally reducing ciliary beat and creating a micromechanical niche that favors pathogen attachment (51).

In conclusion, this study demonstrates that internalized ciliated epithelia can perform diverse and intricate fluid-transport tasks rivaling those of the externally ciliated surfaces of aquatic animals (7, 22, 52). Importantly, we have developed a theoretical and empirical framework for investigating the functional complexity of mucociliary epithelia. This framework will inform efforts to identify novel roles for cilia-generated flow and mechanical landscapes in human tissues and increase our appreciation of the functional scope of these important subcellular structures in animal biology.

Detailed methods and raw video recordings are available in Supporting Information.

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Juvenile E. scolopes were obtained immediately after hatching from breeding colonies maintained in artificial seawater (ASW), as described previously (53). Between hatching and the start of experimental procedures, the hatching squid were kept individually in 5 mL of filter-sterilized ASW.

**Bacterial Culture.** GFP or RFP plasmid carrying V. fischeri wild-type strain ESi14 was grown at 25 °C in tryptone-enriched ASW medium to the midlogarithmic phase to ensure bacterial motility. Cell density was estimated using optical density (OD) at 600 nm.

**Particle and Bacteria Capture at the Ciliated Surface.** Squid were placed individually in 5 mL of filter-sterilized ASW previously exposed to adult, colonized animals and hence containing mucus-release–stimulating bacterial peptidoglycan (PGN) (27) but no bacteria. V. fischeri and fluorescent polystyrene beads with 1-µm or 4-µm diameter (FluoSpheres Polystyrene Microspheres; Invitrogen) were added at equal concentrations and at a total concentration of 10^7 units/mL. After 100 min of exposure, the animals were imaged using confocal microscopy to determine the number of bacteria or beads attached to the ciliated surfaces of the light organ. Only intact and beating ciliated fields were included in the analysis.

**Statistical Analysis of Particle Capture.** Capture data of particles were tested for the null hypothesis that the datasets were drawn from continuous distributions with equal medians, against the alternative hypothesis that there was an increase in the median capture rate for smaller particles. We conducted a one-sided, non-parametric statistical test (Wilcoxon rank sum test) at a 5% significance level, using Matlab (MATLAB, The MathWorks, Inc.).

**Confocal Imaging.** For preparation, the animals were rinsed in ASW and incubated for 10 min with 10 µg/mL fluorescently labeled wheat-germ agglutinin (WGA AlexaFlour 633; Molecular Probes). This lectin specifically binds to N-acetylgalactosaminic acid (sialic acid) residues present in the mucus lining of mucociliary surfaces (27). After staining, the squid were rinsed in ASW and anesthetized in 2% (vol/vol) ethanol in ASW. The animals were placed into a depression well slide with their abdominal side upward, and a window was cut into the mantle and funnel to expose the underlying ciliated surface. The preparation was viewed by a Zeiss LSM 510 or 710 confocal microscope.

**Flow Visualization and Analysis.** Unless indicated otherwise, for quantitative flow analysis, yellow-green fluorescent polystyrene microbeads (ex/em 505/515 nm; 1 µm and 4 µm diameter; Invitrogen) or GFP-expressing V. fischeri were added to the bath to serve as flow tracers. We verified that the particles are faithful tracers of the flow field and do not themselves alter the flow field by computing the particle Stokes number St. If St ≪ 1, tracing accuracy errors are below 1% (54). Here, the Stokes number is defined as $St = \frac{f_t}{f_d}$, where $f_t$ is the characteristic lag time of the particle’s response to a change in fluid velocity (relaxation time), and $f_d$ is the time of the fastest fluctuation of the flow field. For a spherical particle, $f_t$ is given by $f_t = \frac{R_p}{D_t} (2/18).$ where $R_p = 1.05$ g/cm$^3$ is the density of the polystyrene particles, $\mu = 1.08 \times 10^{-3}$ Pa·s is the dynamic viscosity of seawater at 20 °C, and $D$ is the diameter of the particle. For the cilia-generated flow field, we define $f_t$ as the duration of one ciliary beat cycle; i.e., $f_t = 0.1$ s at the 10-Hz beat rate observed in the squid cilia system. For the two particle diameters $d = 1$ µm and $d = 4$ µm, we get $St = 0.54 \times 10^{-6}$ and $St = 8.6 \times 10^{-6}$, respectively, indicating that the particles faithfully trace the flow as their response time is much faster than the fastest changes in the flow field.

For flow visualization, we used a portable laser with 473-nm wavelength (Aquarius Pro; Laserglow Technologies) diverged into a plane through a plano-concave cylindrical lens (focal length $f = -4$; Thorlabs). The laser was used to illuminate an ~0.5-mm-thick 2D cross-section of the flow. The preparation was imaged under a dissection scope equipped with a high-definition camcorder (Sony HDR-CX560) and an optical 515-nm long-pass filter (Thorlabs). Particle traces were visualized by computing the SD of each pixel’s brightness over all movie frames, using ImageJ (55). Individual particles were tracked using the ImageJ-Fiji plugin TrackMate (56) to measure velocities. Digital particle image velocimetry (DPIV) was performed using the Matlab-based software package PIVlab to compute average flow fields and streamlines from the particle displacements (57).

For ex vivo visualization and analysis of the ciliary currents, the light organ was excised and placed in ASW with suspended bacteria or microbeads. Video recordings of V. fischeri were taken at lower magnification than for particles because the GFP-fluorescent bacteria emit less light than light-scattering particles, hence limiting spatial resolution and accuracy of the tracked trajectories. For qualitative analysis of the flow, the ink sac embedded in the light organ was pierced using a microinjection needle, allowing the escaped ink to form a streakline that visualizes the oscillating nature of the flow field generated by metachronal ciliary beat (34) (Fig. 4 A). Particle motions in the sheltered compartment were analyzed using mean-square displacement (MSD) analysis of the particle trajectories in 2D with the Matlab-class msdanalyzer (58).

For in vivo visualization and analysis of the ciliary currents, the animals were prepared and imaged with confocal microscopy as described above. Fluorescent particles or bacteria were added to the preparation at a final concentration of 10^7–10^8 units/mL. Only intact and fully beating ciliated fields were included in the analysis. Particle or V. fischeri transport was imaged using time-lapse recording to reveal flow fields and particle accumulation.

For measuring in vivo inhalation flow, the intact and nonanesthetized animals were placed ventral side up in a plastic dish containing ASW with 10^7 fluorescent particles/mL with 1 µm and 4 µm diameter. We confirmed that the animals in- and exhaled particles of both sizes indiscriminately (Movie S1). Data from individually tracked particles and the DPIV-derived flow field of the funnel were used to estimate the Reynolds number, i.e., $Re = \frac{UD}{\nu} < 1$, where maximal flow velocity $U = 0.5$ mm/s, exit nozzle diameter $D = 0.35$ mm, and laminar viscosity $\nu = 1$ mm²/s.

**Model of Particle Capture by Direct Interception.** We first assessed which of the common mechanisms underlying the capture of nonmoving particles at filter feeding structures would be most effective for the capture of microparticles at the squid appendages. These mechanisms include direct interception, inertial impaction, diffusion, and gravitational deposition of particles, and their relative importance in a given system can be assessed by computing previously described nondimensional indexes (5). Using these indexes, we estimated the relative contribution of each mechanism to the capture of relevant particle types from the mantle-driven flow (26). Here, we assume the following conditions: Temperature $T = 20$ °C, density of seawater $\rho_s = 1.025$ g/cm$^3$, velocity of mantle-driven flow...
\[ V = 100 \times 10^{-6} \text{ m/s}, \text{ appendage diameter } D_c = 90 \times 10^{-6} \text{ m}, \]
and dynamic viscosity of seawater \( \mu = 1.08 \times 10^{-3} \text{ Pa·s}. \]
We determined a dominant role of direct interception by computing the ratio of the direct interception index to the other indices; specifically, we found the direct interception mechanism to be at least one order of magnitude more effective compared with any other mechanism. Table S1 lists the results, where \( N_I \) is the index of inertial impaction, \( N_G \) is the index of direct interception, \( N_D \) is the index of gravitational deposition, and \( N_M \) is the index of diffusional deposition.

Based on these results, we focused on estimating the effect of direct interception and ignored the other previously described capture mechanisms. The theoretical capture rate of particles by direct interception from the mantle-driven flow at the ciliated appendage was estimated by adapting a computational fluid dynamic (CFD) model for aquatic filter feeders (25). Briefly, the model predicts the capture rate of suspended particles by a cylindrical structure in a uniform flow. This model is valid for low to intermediate Reynolds numbers at the cylindrical structure, i.e., \( Re_c < 50 \), with \( Re = Ud_c/\nu \), where \( D_c \) is the diameter of the cylinder, \( U \) is the free-stream velocity, and \( \nu \) is the kinematic viscosity of the fluid. Here, \( Re_c \approx 0.01 \), given a kinematic viscosity of water at 20 °C of \( \nu = 1 \text{ mm}^2/\text{s} \) and the empirical measurement of the initiation flow \( U_{Ic} = 0.1 \text{ mm/s} \) past the light organ and appendage diameter \( D_c = 0.09 \text{ mm} \). For this Reynolds number regime, the model predicts that suspended particles with radius \( r_p \) will be intercepted at a per-second rate of \( I_c = 2Cu_cD_c \), with \( \lambda = 0.218r_c^{0.938} + 0.117r_c^{0.923} \), where \( C = 0.5 \times 10^{2}/\mu \text{L} \) denotes the number of particles/mm\(^3\) of fluid (corresponding to 500,000 particles/mL) and \( L = 0.6 \text{ mm} \) is the total length of the two appendages per ciliated field. Total number of captured particles \( I_T(t) \) over time \( t \) (in seconds) is estimated by \( I_T(t) = I_c t \).

**SEM.** For SEM, animals were first anesthetized in 2% (vol/vol) ethanol in ASW. Before fixation, the mantle was cut open near the light organ to expose the light organ to the fixative agent. Then, the animals were rapidly fixed in 1% (wt/vol) osmium tetroxide in marine PBS (mPBS) (50 mm sodium phosphate, 0.45 M sodium chloride, pH 7.4). After 30 min of incubation, this was followed by fixation in 4% paraformaldehyde in mPBS. Fixation was allowed to proceed for 12–14 h at room temperature. Animals were then washed twice for 10 min in mPBS and dehydrated through an ethanol series (53). The samples were dried and gold sputter coated (Tousimis Samdri 780 critical point drier; SeeVac Auto conductavac IV), mounted on stubs, and viewed with a Hitachi S-570 LaB6 scanning electron microscope. In the digitized images, light-organ dimensions were measured using ImageJ.

**High-Speed Video Recording.** For high-speed imaging of ciliary beat, the animals were first anesthetized in 2% (vol/vol) solution of ethanol in ASW. Then, the light organ was either imaged inside the animal through a window cut into the mantle (in vivo) or excised (ex vivo). Samples were placed in a plastic dish or depression slide with filtered ASW. For imaging of cilia–particle and cilia–bacteria interactions, microspheres or bacteria were added to a final concentration of \( 10^6–10^7 \text{ units/mL} \). The preparation was viewed using light microscopy (phase contrast, differential interference contrast, or fluorescent microscopy), paired with a high-definition camcorder (Sony HDR-CX560, image size 1,080 \times 1,920 pixels) to record at 120 frames per second, or with a high-speed camera (Phantom v710, 1,000 fps, image size 800 \times 600 pixels).

**Kinematic Analysis of Ciliary Beat.** Ciliary beat frequency (CBF) was determined from phase-contrast movie recordings of ciliary activity. An automated Matlab-based algorithm we have recently developed for in vitro analysis of ciliary beat (59) was used to detect regions of interest (ROI), i.e., regions where ciliary activity is present. In these ROI, the intensity value of each pixel over time was bandwidth filtered, windowed, and analyzed using fast Fourier transform (FFT). The dominant frequency of the average power spectrum over all pixels in a given ROI corresponds to the CBF.

Kymograph analysis was performed using ImageJ. Beat kinematics of single cilia were determined by manually tracing individual cilia in subsequent frames of high-speed movie recordings of the ciliated surface. The traces were centered and overlaid to visualize the motion of the entire beat cycle.

**Computational Model of the Vortical Flow Zone.** We used a 2D continuum model to numerically study the ciliated appendages. Two cylinders of diameter \( D \) are fixed at a distance \( \Delta \) apart, to mimic the frontal cross-section of the ciliated appendages. We prescribed tangential velocity boundary conditions on the surface of the cylinders (Fig. S6D). The velocity profile on the surface was chosen to match the distribution of cilia, their beat direction around the cylindrical appendage, and the empirical flow field, which consisted of two vortices and a sheltered zone. Since the long cilia beat in metachronal waves, we applied the boundary conditions at an effective diameter \( D_{att} = 0.95D \), which was roughly the midpoint of the highest and the lowest point on the wavy surface.

At zero Reynolds number, the fluid motion is governed by the Stokes equation \( \nabla \cdot \mathbf{u} = 0 \) and the incompressible condition \( \nabla \cdot \mathbf{u} = 0 \), where \( \mathbf{u} \) is the fluid velocity field, and \( \mu \) is the fluid viscosity. The boundary conditions are given by \( u_{\text{w}} = u_e \) and \( u_{\text{cylinder}} = u_c \), where \( u_e \) is the prescribed tangential velocity on the cylinder surface (Fig. S6E) and \( u_c \) is the unit tangent vector. In addition, we have a zero-force condition on the appendages. By symmetry, the net force in the \( x \) direction is automatically zero. The net force in the \( y \) direction is used to calculate the constant far-field velocity \( \mathbf{U} \). To obtain a nondimensional version of these equations, we used the length scale given by the cylinder diameter \( D \) and the timescale given by \( D/\max(u_c) \). We numerically solved these equations using the regularized Stokeslets method. To this end, regularized Stokeslets were uniformly distributed along the cylinder surfaces. The fluid velocity at an arbitrary point \( \mathbf{x} \) in the fluid domain generated by \( N \) Stokeslets is \( \mathbf{u}(\mathbf{x}) = \sum_{i=1}^{N} \mathbf{G}_i(x - x_i) \cdot \mathbf{f}_i \), where \( \mathbf{G}_i(x - x_i) \cdot \mathbf{f}_i \) is the velocity field induced by a regularized Stokeslet of strength \( f_i \) located at \( x_i \). We used the expression for \( \mathbf{G}_i(x - x_i) \) given by Cortez (60). The resulting flow field is depicted in Fig. S6F.

To investigate how particles with different sizes move in the flow field generated by the ciliated appendages, we considered the model proposed in ref. 39. In the model, a particle of diameter \( d \) follows the streamline crossing its center when away from the cylinders. The particle velocity gets perturbed by \( \Delta \mathbf{u} = \delta (D + d)/2 - |x_p - x_c|/(|x_p - x_c| - \delta) \) when in contact with a cylinder, that is to say, when \( (D + d)/2 - |x_p - x_c| > 0 \) is positive. Here, \( x_p \) and \( x_c \) are positions of the particle and cylinder, respectively, and \( \delta \) is the strength of the perturbation due to the steric interaction between the particle and the cylinder. For small \( \delta \), the particle could penetrate the cylinder surface. Here, we used \( \delta = 0 \) and tested that the results are numerically robust at this value. We seeded the particles upstream uniformly with a spacing \( d_s = 1/r_p \), where \( r_p \) is the density of the particles per unit length, along a seeding line of width \( 2\Delta \) located at a distance \( L_s = 5D \) from the center of the cylinders (to ensure that the particles were initially subject to a quasi-uniform flow). We released particles from their upstream positions and integrated their trajectories using an adaptive Runge–Kutta fourth- to fifth-order scheme with relative and absolute error tolerances of \( 10^{-10} \).

For a given separation distance \( \Delta \) and particle size \( d \), we calculated
the rate of particles captured between the cylinders. We consid-
ered the particles to be captured if the path lines of the parti-
cles cross the line connecting the centers of the cylinders (Fig.
S6G). We defined the capture rate \( r_c \), as the ratio between the
number \( c \) of captured particles and the total number of parti-
cles \( 2\Delta/d_s \); namely, \( r_c = c/(2\Delta/d_s) = c/(2\rho_p\Delta) \). The results
are depicted in Fig. 4H of the main text. Note that the actual
value of \( \rho_p \) does not affect the results because the number of
captured particles scales linearly with \( \rho_p \). Also, the results are
not sensitive to the initial position of particles as long as the par-
ticles are initially seeded sufficiently far upstream. We defined
the critical diameter \( d_s \), as the largest diameter a particle can
have and still reach the sheltered zone by following the flow
field. We found that the compression of streamlines near the
appendages results in particle streaming, where particles with
diameter greater than \( d_s \) get diverted by the streamlines into
the central outward jet (Fig. S6f). Particles of diameter equal
to, or smaller than, \( d_s \) travel with the near-surface streamlines
leading into the sheltered zone (Fig. S6h). The effect of \( D_{\text{out}} \)
on \( d_s \) is shown in Fig. S6f. Finally, we verified that the verti-
cal flows and size-dependent particle capture are not uni-
versal to all possible boundary conditions by providing a counter
example in Fig. S6 K–M. Clearly, for these made-up boundary-
ary conditions, no vortices were generated and no filtering by
size was observed. This counter example neither proves unique-
ness nor proves optimality of the boundary conditions (in other
words, spatial distribution of cilia) that produce the counterro-
ating vortices. However, it confirms that the size-selective parti-
cle capture observed here is sensitive to the spatial distribution
of cilia.

Computational Model of the Sheltered Zone. We considered a clus-
ter of cilia whose base points are rooted on the surface (\( x = 0 \)
plane) of a semiinfinite space \( x = (0, \infty) \), with \((x, y, z)\) being
Cartesian coordinates. The length of each cilium is \( l \) and the
spacing between the base points of neighboring cilia is \( D_y \) in the
\( y \) direction and \( D_z \) in the \( z \) direction. We assumed that each cil-
ium beats in the \( y \) direction and remains nondeformable as a
rigid bar during the beating cycle. The angle between the cil-
ium and the \( x \) axis was set to \( \theta = \alpha \cos(2\pi \phi/T) \), where \( \alpha \) is
the beating amplitude and \( \phi \in [0, T) \) is the phase of the cil-
ium during the beating cycle. We considered the cilium carpet
to be doubly periodic, and each periodic box had \( n_y \times n_z \) cilia
in the \( y \) direction and \( n_z \) cilia in the \( z \) direction. To compute
the flow field generated by the cilium carpet, we approximated
each cilium by a distribution of regularized Stokeslets along its
centerline together with an “image” distribution to satisfy the
no-slip boundary conditions at the base wall. The strength of
each Stokeslet was computed by imposing the no-slip boundary
conditions at the cilium, and the generated fluid velocity is
\( \mathbf{u}(x, t) = \sum_{j=1}^{\infty} \sum_{k=1}^{\infty} \int_0^T \mathbf{G}(x, x_{jk}(s, t)) \cdot \mathbf{f}(x_{jk}(s, t)) \) ds, where \( j \) and
\( k \) are indexes of the cilium in the \( y \) and the \( z \) direction, re-
spectively. We used the expression of \( \mathbf{G}(x, x_{jk}) \) given by Ainley et al.
(61). Due to the presence of the base wall, the infinite summa-
tion of the Stokeslets converges. Here, we approximated the in-
finite summations by using truncated sums \( \sum_{j=1}^{n_{\text{max}}} \) and \( \sum_{k=1}^{n_{\text{max}}} \). To
avoid the edge effects, we considered only the results obtained
for the middle periodic box.

To study the mixing effects of the short cilia with different
phase coordination, we uniformly seeded the fluid domain
near the cilia with passive tracers of two different colors in
a 1:1 ratio. We considered two cases of initial seeding: hori-
zontal strips and vertical strips. We computed the displace-
ment field \( \mathbf{d}(x) = \int_0^T \mathbf{u}(x(t), t) dt \) and used it as a discrete
map \( x(t + T) = x(t) + \mathbf{d}(x(t)) \) to study the long-term behav-
iors of the particle tracers. We adopted the mixing number
\( m = (\prod_{i=1}^{N_{\text{cl}}} \min((x_i - x_j)^2)^{1/N_{\text{cl}}}) \), following Stone and Stone
(36), where \( x_i \) and \( x_j \) are positions of tracer particles of dif-
cerent color and \( j = 1, 2, \ldots, N \), with \( N \) being the total number
of same-color particles (Fig. S8 D–F and Fig. 5 E–H).

Basically, we used the shortest distance between particles of dif-
cerent color as a measure for mixing, with a smaller mixing num-
brr indicating better mixing. We defined the mixing efficiency
as \( \eta_m = -\ln(m/m_0)/N_{\text{cl}} \), where \( m_0 \) is the initial mixing
number, and \( N_{\text{cl}} \) is the number of cycles.

We considered three different cases of this model: (i) cilia
beating in synchrony (SYNC); (ii) cilia beating in metachronal
waves, in which case the phase difference between the neighbor-
ing cilia in the \( y \) direction is a constant, with \( \Delta\phi \neq 0 \) (META);
and (iii) cilia beating at random phases (RAND), meaning that
the initial phase of each cilium is taken from a uniform distribu-
tion ranging from 0 to \( T \).

For these computations, each cilium was discretized into 20
regularized Stokeslets uniformly distributed along the centerline
and the regularization parameter was chosen to be 0.05 to match
a typical radius-to-length ratio. We prescribed the motion of each
cilium as a rigid bar beating in the \( y \) direction, as noted at the
beginning of this section. The beating amplitude was chosen to
be \( \alpha = 10^6 \). We normalized length by the cilium length \( l \) and
time by the beating cycle \( T \). The distances between neighboring
cilia were chosen to be \( D_y = D_z = 0.5 \). Each periodic box contained
\( n_y \times n_z = 8 \times 1 \) cilia, and cilia with the same \( y \) values were con-
sidered to beat in phase. For the metachronal wave case, we used
\( \Delta\phi = T/8 \) so that each periodic box contained one metachronal
wave. Each beating cycle was discretized into 50 time steps and
the particle tracer positions were integrated using a fourth-order
Runge–Kutta scheme. For the random-phase case, 10 Monte
Carlo simulations were performed to get a distribution of results.

Computational Model of Transport by Single Cilium. We compared
the fluid transport generated by a single cilium with different
beating patterns (Fig. S8 A–C). The beating pattern of a sin-
gle cilium was studied by Eloy and Lauga (62), who found that
for optimal transport the cilium exhibited a small curvature dur-
ing the effective stroke and large curvature during the recovery
stroke. Here, we used an asymmetric beating pattern adapted from
the rabbit tracheal cilium (9), which exhibits a nearly straight
effective stroke and large curvature during the recovery
stroke.

Using the regularized Stokeslet method with image distribu-
tions described in the previous section, we derived the flow veloc-
ity field for each of the two beating patterns. We further inte-
grated the flow generated by the single cilium over one cycle
to evaluate the transport efficacies of different beating patterns
quantitatively.
Fig. S1. Flow fields of the system associated with bacterial-cell capture. Mantle-driven, inhaled flow in *E. scolopes* at rest is laminar and passes by the ciliated epithelium. (A) Ventral view of juvenile *E. scolopes*. (B) Trajectories of microparticles reveal the flow regime of seawater that enters the mantle during normal inhalation and exits through the funnel during exhalation. Notably, the flow passes closely by the light organ where environmental, bacteria-sized particles are captured. (C) Velocity measurements of microparticles show that respiration-driven flow near the light organ reaches ca. 50–100 μm/s, which is six times slower than the ciliary flow velocities reached near the surface.

Fig. S2. Ciliary activity tightly controls adhesion of suspended materials to the surface. (A) In light organs with healthy ciliary beat, suspended microparticles (red; 10^6 particles/mL) are retained in low numbers and are found exclusively near the sheltered zone, which also accumulates mucus (arrow). (B) In light organs with impaired ciliary beat, the microparticles adhere indiscriminately in much greater numbers and across the entire surface of the organ.

Fig. S3. Dimensions and morphology of the ciliated surface. (A) Location of the ciliated light organ (white square) in the mantle cavity of a juvenile *E. scolopes*. (B) SEM of a light organ showing the position of the two pairs of ciliated appendages. The left pair is a diagram showing the types and distributions of cilia, and the right pair is shown in detail in C, where Δ is the center distance between the appendages, L is the total length spanned by the appendages, and D is the diameter of an individual appendage. (D) Scatterplot of D vs. Δ measured in the ciliated organs, showing consistent spacing of the two appendages by 1/2 to 1 times their diameter. These data are shown as a compact box plot in Fig. 4I of the main text.
Fig. S4. Flow visualizations in ex vivo ciliated surfaces confirm transport patterns of microparticles and bacteria. (A) Dye streaklines reveal the oscillatory flow generated by the long cilia. (B–E) The excised light organ is illuminated with a laser sheet. Fluorescent microparticles or bacteria suspended in the bath reveal the cilia-generated flow. (B) Pathlines and (C) velocities of particle tracers reveal the front-sectional flow pattern around appendages. (D) Path lines and (E) velocities of GFP-fluorescent V. fischeri suggest that these motile bacteria follow a similar flow pattern around the ciliated organ as passive microparticles.
Fig. S5. Time-lapse confocal recordings confirm similar trajectories of particles and bacteria in light organs not excised from the mantle cavity. (A) Trajectories of 4-µm (red) and 1-µm (green) particles near the ciliated appendages. The prolonged-exposure streaklines indicate relatively rapid flow on the right side where the long cilia are located and relatively slow flow on the left side, toward the sheltered zone. (B) Vortical transport of these particles and their accumulation in the sheltered zone (arrows) between appendages, showing accumulation of 1-µm (green) particles at higher rates than the 4-µm particles, despite their lower statistical chance of capture by direct interception. (C and D) Vortical transport and accumulation in the sheltered zone (arrows) of 1-µm (green) particles. (E and F) Vortical transport and accumulation in the sheltered zone (arrows) of V. fischeri cells (green). Blue, mucus coating on the appendages in all images.
Computational model of the vortical flow zone. (A) SEM imaging shows that the ciliated appendages form a torus-like ring. (B) Optical cross-section of the two appendages. D, appendage diameter; Δ, spacing between appendages. (C) Cross-sectional flow revealed by path lines of 4-µm (red) and 1-µm (green) particles, using fluorescent microscopy. Arrows indicate direction of ciliary beat derived from the flow field and video recordings. Note the beat reversal at the 7 o’clock and the 5 o’clock position around the left and the right appendage, respectively. (D) Model of a cross-section of appendages represented by two cylinders of diameter D and a spacing Δ. Dashed circles represent effective boundaries (Deff = effective diameter), upon which tangential velocity boundary conditions are applied. Magenta line denotes the location at which the beat of the ciliated field diverges into opposite directions. ut, tangential velocity; θ, angle defining location on perimeter of circular appendage. (E) The velocity profile is C1 continuous with a period of 2π and satisfies ut(θ) = 0 for θ = π/4 and π ≤ θ ≤ 3π/2, max(ut) = ut(0) = 1, and min(ut) = ut(π/2) = −1. The velocity profile is expanded by a second-order Fourier series for −π/2 ≤ θ ≤ π and a zero-value function for π ≤ θ ≤ 3π/2. The magenta line demarcates the effect of the beat reversal shown in D. (F) Field generated by tangential boundary conditions in E analogous to those observed empirically on the appendages of the light organ. (G) Example of particle trajectories in flow field for Δ/D = 0.95. Particles are seeded uniformly along a line of width 2Δ. Trajectories labeled in red demark particles diverted into the sheltered zone. (H and I) Fate of particles initially seeded uniformly along a horizontal line sufficiently far away from the cylinders. Particles approaching the appendages are directed to the outside and washed away; or, if their diameter is smaller than the critical diameter, captured in the sheltered zone (H, red trajectories); or, if their diameter is greater than the critical diameter, rejected (I). (J) Changing Deff will change the filtering effect quantitatively, but not qualitatively; specifically, if the effective diameter is increased (or decreased) by 0.05D, the critical diameter for a given separation distance Δ will increase (or decrease) by about 0.03D. (K) A tangential velocity profile not based on experimental observation. (L) Computed 2D flow field around appendages resulting from the made-up velocity profile in K. (M) Computed trajectories of particles in the flow field. Trajectories of particles eventually entering the sheltered zone.
are labeled red. Comparing the fate of small particles (Left) (particle diameter d = 1% of appendage diameter D) and of large particles (Right) (d = 15% D) shows that in this flow field, particle size has no effect on particle capture, demonstrating that applying a different tangential velocity profile from the one observed empirically (C and D) can lead to a nonvortical flow pattern with no particle sorting mechanism.

Fig. S7. Particle trajectories in sheltered zone reveal both diffusion-like and directional transport. (A) Particle trajectories in the fast-flowing regions covered by long cilia (Left) and the sheltered zone covered by short cilia (Right, white dashed rectangle). (B) Four particle trajectories recorded in the sheltered zone. Arrows indicate starting point and direction of the trajectories. (C) MSD analysis of the four trajectories shown in B. (D) The initial 100 ms of the four trajectories are used to analyze the contribution of diffusion. (E) The mean MSD (dashed line) of the first 100 ms is approximated by the linear trend 
$\text{MSD}(t) = 4Dt/t$, where $D \approx 7 \text{µm}^2\text{s}$ is the diffusion coefficient. This coefficient is of comparable magnitude to the theoretical value of $D = 2 \text{µm}^2\text{s}$ for a 1-µm tracer in water using the Stokes–Einstein equation (63). (F) Histogram of x and y velocity magnitudes during the first 100 ms. The diffusive behavior is further supported by a near-zero mean of these distributions. Mean values are $\langle u \rangle = -0.0033$ and $\langle v \rangle = -3.8742$ µm/s. (G) The first 400 ms of the two long trajectories are used to analyze the contribution of convection. (H) The mean of the MSDs (dashed line) of the first 400 ms follows a parabolic trend approximated by 
$\text{MSD}(t) = 4Dt + U^2t^2$, where $D \approx 4 \text{µm}^2\text{s}$, and $U \approx 20$ µm/s is the flow velocity, which is one order of magnitude smaller than the velocities reached in the neighboring, long-cilia–driven flow. (I) Mean autocorrelation of the first 400 ms shows similarity with a Dirac function, indicating that over time, directional transport cancels out.
Fig. S8. Computation of fluid transport by different cilia stroke kinematics. A–C, Left show asymmetric stroke kinematics of cilia in the rabbit small airway (9) while A–C, Right show symmetric stroke kinematics of the short cilia of the squid. (A) Stroke cycle. (B) Normalized flow velocities at end of the effective stroke (Top) and at end of the recovery stroke (Bottom). (C) Flow rate over one stroke cycle. Note that only the asymmetric stroke cycle (Left) generates a net directional flow. (D) Flow displacement field of an array of short squid cilia with a rigid stroke cycle and in RAND mode showing the divergence of particle trajectories. The colors represent the magnitudes of the displacement per stroke cycle while the arrows denote the directions. Two seeded particles that are initially in close proximity segregate over the course of 16 stroke cycles. This behavior results in overall mixing of the fluid. (E) The average flow field becomes more uniform and the mixing performance decays as we move farther from the cilia (x > 1). (F) the mixing pattern of an initially vertically stratified field after 16 cycles.
Mechanical selection: Long cilia-generated flow focuses mucus and bacteria near pores and rejects larger debris.

Flow-enhanced signalling: Short cilia near pores generate mixing flows that accelerate spread of biochemical signals, such as nitric oxide released by V. fischeri.

Behavioral response: Up-regulation of host chitotriosidase; V. fischeri bind to cilia and are primed to sense chitobiose.

Flow-enhanced signalling: Short cilia-generated mixing accelerates formation of steady-state chitobiose gradient originating from pores.

Behavioral response: V. fischeri form tight aggregates and migrate to pores.

**Fluid-mechanical mechanisms**
- Mechanical selection: Long cilia-generated flow focuses mucus and bacteria near pores and rejects larger debris.
- Biochemical/behavioral mechanisms:
  - Biochemical selection: “Winnowing away” of non-symbiont bacteria by mucosal antimicrobial effectors.
  - Behavioral response: Up-regulation of host chitotriosidase; V. fischeri bind to cilia and are primed to sense chitobiose.

**Biochemical selection**
- “Winnowing away” of non-symbiont bacteria by mucosal antimicrobial effectors.
- Expression of chitotriosidase around pore.
- Acidic mucus containing antimicrobial effectors.
- Chitobiose gradient.

**Behavioral response**
- Up-regulation of host chitotriosidase.
- V. fischeri bind to cilia and are primed to sense chitobiose.
- V. fischeri form tight aggregates and migrate to pores.

**Fig. S9.** Summary of findings in the context of symbiont-host association. The proposed fluid-mechanical mechanisms involved in symbiont-host association are proposed to occur in combination with known biochemical and behavioral mechanisms (refs. 18 and 19).

**Table S1.** Relative efficiency of possible mechanisms for capturing particles from mantle-driven flow (adapted from ref. 26)

<table>
<thead>
<tr>
<th>Particle type</th>
<th>Density, g/cm³</th>
<th>Diameter, µm</th>
<th>N_R/N_I</th>
<th>N_R/N_G</th>
<th>N_R/N_M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterium</td>
<td>1.075</td>
<td>1</td>
<td>3.9 × 10⁶</td>
<td>44.1</td>
<td>0.3 × 10³</td>
</tr>
<tr>
<td>Phytoplankton</td>
<td>1.095</td>
<td>5</td>
<td>0.6 × 10⁵</td>
<td>6.3</td>
<td>6.3 × 10³</td>
</tr>
<tr>
<td>Polystyrene bead</td>
<td>1.05</td>
<td>1</td>
<td>7.8 × 10⁵</td>
<td>88.1</td>
<td>0.3 × 10³</td>
</tr>
<tr>
<td>Polystyrene bead</td>
<td>1.05</td>
<td>4</td>
<td>1.9 × 10⁶</td>
<td>22.0</td>
<td>4.0 × 10³</td>
</tr>
</tbody>
</table>
**Movie S1.** The ciliated epithelium is exposed to inhaled flow and particles. In juvenile *E. scolopes*, inhaled flow and particles pass through the mantel cavity and past the ciliated light organ, before being exhaled through the water jet. In this recording of breathing at rest, suspended fluorescent microparticles (1–4 µm in diameter) are illuminated in a laser plane. In clips 1 and 2, particle tracking reveals path and velocity of inhaled matter. Clip 3 shows that 1-µm (green) and 4-µm (red) particles are in- and exhaled indiscriminately. In clip 4, DPIV is used to measure the velocity field at the nozzle of the water jet.

**Movie S2.** The ciliated epithelium features two distinct cilia populations and ciliary beat kinematics. High-speed ex vivo recordings of the ciliated organ reveal the uncoordinated beat of the short cilia and the metachronal beat coordination of the long cilia.
Movie S3. Cilia-generated flow field of the ciliated epithelium. Visualization of microparticle transport in both the ex vivo and the in vivo ciliated light organ shows that the majority of particles entrained into the long-ranging vortical flow zone are deflected from the surface whereas a small fraction of particles can enter and accumulate in the stagnation zone between the appendages.

Movie S4. The ciliated epithelium features two distinct microfluidic zones. Clip 1 is a close-up of the microparticle transport in the ex vivo light organ showing fast, directed transport in the vortical flow zone and, in contrast, slow, diffusion-like transport in the neighboring stagnation zone lined by short cilia. Clip 2 shows mucus and particles trapped in the stagnation zone being gently rocked by the motion of the short cilia.
**Movie S5.** In vivo recording of cilia-generated flow zones and size-biased particle capture. Confocal time-lapse recording of the in vivo ciliated surface (blue) shows distinct vortical flow and stagnation compartments, as well as preferential accumulation of 1-µm particles (green) compared with 4-µm particles (red) in the stagnation zone.

**Movie S6.** The ciliated surface appendage features circumferential ciliary beat. Cross-sectional view onto ciliated light-organ appendage shows the circumferential ciliary beat and the parting point at which beat direction reverses, creating a flow that passes the appendage left and right of the parting point.