Flow and transport effect caused by the stalk contraction cycle of *Vorticella convallaria*

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Vorticella convallaria is a protozoan attached to a substrate by a stalk which can contract in less than 10 ms, translating the zooid toward the substrate with a maximum Reynolds number of ~ 1 . Following contraction, the stalk slowly relaxes, moving the zooid away from the substrate, which results in creeping flow. Although *Vorticella* has long been believed to contract to evade danger, it has been suggested that its stalk may contract to enhance food transport near the substrate. To elucidate how *Vorticella* utilizes its contraction-relaxation cycle, we investigated water flow caused by the cycle, using a computational fluid dynamics model validated with an experimental scale model and particle tracking velocimetry. The simulated flow was visualized and analyzed by tracing virtual particles around the *Vorticella*. It is observed that one cycle can displace particles up to $\sim 190\ \mu\text{m}$ with the maximum net vertical displacement of $3\text{--}4\ \mu\text{m}$ and that the net transport effect becomes more evident over repeated cycles. This transport effect appears to be due to asymmetry of the contraction and relaxation phases of the flow field, and it can be more effective on motile food particles than non-motile ones. Therefore, our *Vorticella* model enabled investigating the fluid dynamics principle and ecological role of the transport effects of *Vorticella*'s stalk contraction. Published by AIP Publishing. [<http://dx.doi.org/10.1063/1.4985654>]

I. INTRODUCTION

Due to its unique motility characteristics, the stalk contraction of the sessile ciliate *Vorticella* has drawn the attention of scientists since Antony van Leeuwenhoek first described it in 1676.^{1–4} As illustrated in Fig. 1, the bell-shaped cell body (or zooid) of *Vorticella* is anchored to a solid substrate by a $\sim 4\ \mu\text{m}$ -diameter stalk. This slender stalk coils in less than 10 ms, drawing the zooid toward the substrate, and then slowly relaxes over a few seconds, returning to its extended state. Because its stalk contracts to $\sim 20\%$ of its extended length, the zooid moves at a maximum speed of $60\text{--}90\ \text{mm/s}$.^{5–7} This maximum contraction speed corresponds to $\sim 1,200$ body lengths per second, making *Vorticella* one of the fastest animals relative to its body length.⁵

The sessile form of *Vorticella* has two main motile organelles: cilia around the mouth-like part of the zooid and the contractile stalk. The oral cilia generate a vortex flow of water to draw food particles toward the zooid.⁸ Sleight and Barlow observed that this feeding current could transport $\sim 3\ \mu\text{m}$ -diameter particles from distances of at least $450\ \mu\text{m}$ from the oral part.⁹ Using confocal micro-particle image velocimetry ($\mu\text{-PIV}$), Nagai *et al.* recently measured the maximum speed of the feeding current of a *V. picta* to be as high as $360\ \mu\text{m/s}$.¹⁰ Pepper *et al.*

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FIG. 1. Stalk contraction of a *Vorticella convallaria*. Time is shown in ms, and 0 ms indicates the initiation of the stalk contraction.

also measured the feeding current of a *V. convallaria* using particle tracking velocimetry (PTV) and investigated the effect of nearby boundaries on the size of the current.¹¹

The contracting stalk also causes water flow. Aside from its tethering role, the stalk moves the zooid toward and then away from the substrate. Stalk contraction has been thought to be for danger avoidance, and it is also presumed to complement the cilia in feeding.^{12–14} Although the cilia-driven feeding current transports food particles toward the zooid, stalk contraction may be necessary to introduce new particles into the feeding current. This is because the feeding current is inherently viscous dominant and mixing with fluids external to the current is very limited.^{11,15} However, this hypothesis has yet to be further investigated based on detailed analyses of water flow caused by the stalk contraction of *Vorticella*.

To the best of our knowledge, Vopel *et al.* first measured water flow induced during the stalk contraction-relaxation cycle of a solitary marine *Vorticella*.¹² They observed that the *Vorticella* induced only small fluxes of water toward the substrate during stalk contraction, whereas it transported large fluxes of water away from the substrate during stalk relaxation. They also suggested that the stalk contraction might be important for the feeding of *Vorticella*.

Recently, Ryu and Matsudaira investigated water flow caused by a contracting *V. convallaria* experimentally and computationally.⁷ By tracking microbeads around the organism using high-speed video microscopy, they visualized that the induced flow transported microbeads toward the substrate. They also created a computational fluid dynamics (CFD) model of the *V. convallaria* by modeling the zooid as a solid sphere moving toward the substrate with the stalk ignored. To rigorously mimic stalk contraction, they incorporated in their CFD model the measured stalk length profile of the *V. convallaria*. Their simulation and analysis showed that the convective inertia force of the contraction-induced flow should be considered, as the maximum Reynolds number was greater than unity. Here, the Reynolds number (Re) represents a ratio of the inertia force to the viscous force of the flow, as defined by $Re = 2RU/\nu$ where R and U are the respective radius and translation speed of the zooid, and ν is the kinematic viscosity of water.

Although water flow around *Vorticella* has been investigated as summarized, the role of stalk-contraction-related flow in the sustenance of *Vorticella* is still poorly understood. Because *Vorticella* lives on a no-slip solid surface where natural flow may be very limited within the boundary layer on the surface and because the ciliary feeding flow is limited in mixing, the protozoan may use its stalk contraction-relaxation cycle to refresh its food supply by stirring the surrounding water.^{16,17} Therefore, it is necessary to investigate rigorously the flow and resultant transport effect induced by the contraction-relaxation cycle.

In this paper, we simulated water flow induced by the stalk contraction-relaxation cycle of the solitary *V. convallaria* (the prototype *Vorticella*) that Ryu and Matsudaira studied,⁷ using an improved CFD model in order to better understand how *Vorticella* benefits from the contraction cycle. A new CFD model for the prototype *Vorticella* was created to include the zooid, stalk,

and substrate and then verified for grid independence using the grid convergence index. The model was validated with an experimental scale model of the *Vorticella* in which the water flow was measured with PTV. Simulated water flow caused by the stalk contraction-relaxation cycle was visualized and analyzed by tracking virtual massless particles in the CFD model. We observe that one stalk contraction-relaxation cycle causes net upward displacement of particles directly beneath the zooid and close to the stalk, whereas the remainder of the particles are displaced downward. Such particle motions are amplified as the cycle repeats.

This article is structured as follows. Section II introduces the CFD model of the prototype *Vorticella*. Section III explains the experimental validation of the CFD model using the scale model of the *Vorticella*. Section IV shows simulation and analysis of water flow caused by the stalk contraction-relaxation cycle, which is followed by discussion in Section V and conclusions in Section VI.

II. CFD MODEL

Previously, we developed a CFD model of a solitary *V. convallaria* to simulate water flow caused by its contraction and to evaluate its contractile force.⁷ To more rigorously investigate the flow induced by the stalk contraction cycle of *Vorticella*, we created a refined two-dimensional (2D) axisymmetric CFD model of the same *Vorticella* cell (i.e., the prototype *Vorticella*) using COMSOL Multiphysics®. The governing equations for the flow field are the continuity equation and the Navier-Stokes equations of incompressible Newtonian fluids for axisymmetric 2D domains

$$\frac{1}{r} \frac{\partial}{\partial r}(ru) + \frac{\partial w}{\partial z} = 0, \quad (1)$$

$$\begin{aligned} \rho \left(\frac{\partial u}{\partial t} + u \frac{\partial u}{\partial r} + w \frac{\partial u}{\partial z} \right) &= -\frac{\partial p}{\partial r} + \mu \left\{ \frac{\partial}{\partial r} \left[\frac{1}{r} \frac{\partial}{\partial r}(ru) \right] + \frac{\partial^2 u}{\partial z^2} \right\} \\ \rho \left(\frac{\partial w}{\partial t} + u \frac{\partial w}{\partial r} + w \frac{\partial w}{\partial z} \right) &= -\frac{\partial p}{\partial z} + \mu \left\{ \frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial w}{\partial r} \right) + \frac{\partial^2 w}{\partial z^2} \right\} - \rho g, \end{aligned} \quad (2)$$

where r and z are, respectively, the radial and vertical axes, u and w are the corresponding flow velocity components, p is the pressure, ρ and μ are the density and viscosity of water, respectively, g is the acceleration of gravity, and t is the time. In this study, Eqs. (1) and (2) were solved numerically using COMSOL Multiphysics®.

As shown by the mesh in Fig. 2, the zooid of the prototype *Vorticella* (radius $R_p = 16.4 \mu\text{m}$) and the substrate were, respectively, modeled as a rigid sphere and a solid plane forming the bottom of the mesh. The coiling stalk of the *Vorticella* was simplified to be a straight rod ($3.1 \mu\text{m}$ diameter) connecting the zooid and the substrate for the sake of easier modeling. The length of the stalk part was modulated according to the motion of the zooid part. The extent of the computational domain ($393.9 \mu\text{m} \times 800.6 \mu\text{m}$) was determined based on the scale ratio of $\lambda = 774$ between the dimensions of the prototype *Vorticella* cell and the dimensions of the experimental scale model used to validate the CFD model ($30.5 \text{ cm} \times 62 \text{ cm}$).

A structured quadrilateral grid was used to discretize the computational domain. For stable numerical convergence, the grid domain was split into three parts: top, middle, and bottom parts [Fig. 2(a)].⁷ The Arbitrary Lagrangian-Eulerian (ALE) moving mesh feature was implemented to move the middle part with the moving zooid, whilst the grids in the other parts were free to deform in the z -direction in accordance with the motion of the middle part. Therefore, we substantially eliminated any unwanted twisting of grid elements.

Boundary conditions of the CFD model (Fig. 2) were initially selected based on the experimental scale model (Fig. 3). The axisymmetric boundary condition was applied to Boundary ① which represents the axis of symmetry of the model. The no-slip moving wall boundary condition was applied to Boundaries ② and ③, which are the surfaces of the zooid and the stalk. Boundary ④ is the substrate of the model and required the no-slip wall boundary condition.

FIG. 2. 2D axisymmetric CFD model of the prototype *Vorticella* cell. (a) Whole computation mesh with the coordinate axes. (b) Magnified view of the vicinity of the zooid. Assigned boundary conditions are ①—axisymmetric boundary condition, ② and ③—no-slip moving wall boundary condition, ④—no-slip wall boundary condition, ⑤ and ⑥—no-slip boundary condition for experimental validation of the CFD model and open boundary condition for simulating water flow around the prototype cell, and ⑦—the pressure point constraint ($p = 0$).

Boundaries ⑤ and ⑥ are the edges of the CFD domain, which correspond to the tank wall of the experimental scale model. Thus, the no-slip boundary condition was set to these boundaries for experimental validation of the CFD model, but it was later changed to the open boundary condition for simulating flow around the prototype *Vorticella*. It was confirmed that the domain size of the CFD model was large enough so that this change in boundary conditions had a negligible effect on simulation results. Lastly, the pressure point constraint ($p = 0$) was applied to Point ⑦.

FIG. 3. Experimental scale model of the prototype *Vorticella*. (a) Experimental facility for the scale model and PTV measurement. Flow domain: 0.61 m long $\times$ 0.61 m wide $\times$ 0.62 m high. (b) The *Vorticella* model and tracer particles illuminated by the light sheet emanating from the right (cropped and brightened to enhance illustration).

Because the quality of CFD simulation depends on the grid independence of the mesh used, the grid convergence index (GCI) was utilized to verify the grid independence of our CFD model (see [supplementary material](#) for details).^{18,19} Briefly, the GCI of the CFD model was calculated as the grid of the model was refined. Then, the optimal mesh distribution (or grid size) was determined based on the balance between the GCI and computation time (Fig. S2 of the [supplementary material](#)).

III. EXPERIMENTAL VALIDATION OF THE CFD MODEL

A. Experimental model

In order to verify the CFD model, an experimental scale model of the prototype *Vorticella* was built in a 0.61 m-long by 0.61 m-wide water-filled glass tank. The ceiling of the 0.62 m-high flow domain was an acrylic plate [Fig. 3(a)]. The *Vorticella* model was a 25.4 mm-diameter ($=2R_m$) acrylic sphere mounted on a 2.38 mm-diameter stainless steel rod. The rod protruded through a hole of the same diameter in the center of the acrylic ceiling, allowing the *Vorticella* model to be translated perpendicular to the ceiling. The rod was attached to a linear translation stage located above the acrylic ceiling, and the motion of the stage was regulated by a Parallax Propeller microcontroller. Note that in Fig. 3, the model substrate is above the *Vorticella* model, whereas for the CFD model and in the remainder of the paper, the substrate is shown below the *Vorticella*.

Although the stalk contraction-relaxation cycle of an actual *Vorticella* is asymmetric, a symmetric displacement cycle was devised for CFD model validation for two reasons. First, the scaled duration of the relaxation period is very long, and it would require excessive experimental time and a much more accurate translation stage for the experiments. Second, implementing Re of the actual *Vorticella* at the experimental model scale would result in very low velocities, and natural convection in the experimental facility would interfere with accurate PTV measurements.

The devised oscillation cycle was a simple periodic function, patterned after the contraction period of the prototype *Vorticella*

$$\frac{L_m(t) - L_{m,\min}}{L_{m,\max} - L_{m,\min}} = \frac{|t - 2\pi|}{2\pi} - 0.1497 \sin |t - 2\pi| \quad \text{for } 0 \leq t \leq 4\pi, \quad (3)$$

where L_m is the length of the rod protruding from the ceiling, which corresponds to the stalk end-to-end length (L_p), $L_{m,\min}$ and $L_{m,\max}$ are, respectively, the minimum (17.5 mm) and maximum (90.4 mm) of L_m , and t is time in seconds [each cycle is 4π s, see Fig. 4(a)]. $L_{m,\max}$ and $L_{m,\min}$ were scaled from the corresponding stalk lengths of the prototype *Vorticella* ($2R_p = 32.8 \mu\text{m}$, $L_{p,\max} = 116.8 \mu\text{m}$, and $L_{p,\min} = 22.5 \mu\text{m}$, and scale ratio $\lambda = R_m/R_p = 774$).⁷

Equation (3) was used to drive the linear stage for multiple cycles, and it was verified that the position of the sphere closely followed the position given by Eq. (3). The maximum speed of the zooid model was 22.5 mm/s at $t = (\text{odd number}) \times \pi$ s. Since the water temperature in the tank was $\sim 23^\circ\text{C}$, the maximum Re of the experimental scale model was ~ 620 . This maximum Re was about two orders of magnitude greater than the maximum Re of a typical *Vorticella*,⁷ but the induced water flow remained laminar. Ideally, experimental validation of the CFD model would be at Re values similar in magnitude to those of the prototype simulations, but because of practical limitations discussed later in this paper, this was not possible. Nevertheless, the higher Re tests performed in the experimental scale model could be used to validate the CFD model because spatial and temporal requirements are more stringent for flow simulation at higher Re compared to lower Re flows.

B. Particle tracking velocimetry (PTV)

Correlation-based particle image velocimetry techniques were not adequate for the present experiments because spatial gradients of velocity were too high near boundaries. Instead, PTV was employed to measure the flow velocity field around the oscillating *Vorticella* model.

FIG. 4. Stalk end-to-end length profiles for *Vorticella* models. (a) Profile used for the experimental scale model and PTV measurement to validate the CFD model. Each cycle follows Eq. (3), and PTV imaging scheme is shown with time points 1, 2, and 3 for flow velocity vector comparison for Fig. 7. (b) Profile used for simulating water flow using the validated CFD model of the prototype *Vorticella*: Eq. (4) for the contraction phase and Eq. (5) for the relaxation phase.

A light sheet was generated with a 50 mJ 532 nm Nd:YAG dual-head laser with a pulse rate of 30 Hz. Because of low flow velocities, collecting evenly spaced images at 30 Hz was a practical alternative that enabled applying multi-image particle tracking techniques. Light sheet optics included a zoom beam expander to transfer the beam a relatively long distance to the experimental tank and an optical head to form a narrow waist of the light sheet in the interrogation region [Fig. 3(a)]. The thickness of the light sheet in the interrogation region was ~ 2 mm. The light sheet was carefully positioned so that it was parallel to the axis of motion of the model and reflection from the rod was minimized [Fig. 3(b)].

The flow was seeded with fluorescent tracer particles manufactured using a mixture of polyester resin and Rhodamine.²⁰ After sieving, particles with diameters of less than 43μ m were used. Because many of the particles either settled to the bottom or floated to the top before experiments began, the particles staying in the flow were considered to be neutrally buoyant [Fig. 3(b)]. PTV measurements in relatively quiescent locations in the tank showed that these particles neither settled nor rose at measureable velocities.

A 1280×960 pixel AVT Prosilica 1290 camera was used to collect images of the flow. The camera was fitted with a 550 nm long-pass filter. This filter removed direct reflections of 532 nm light but passed fluorescent light emitted by the tracer particles which were excited by the light sheet. Thus, it was possible to record flow within ~ 0.3 mm of most boundaries although tracers, which deposited on the top of the sphere before experiments began, fluoresced enough light to prevent measurements closer than ~ 0.8 mm in this region [Fig. 3(b)].

Before each realization, the *Vorticella* model was moved to its initial position [$L_m(t=0)$ of Eq. (3)]. LabView was used to drive the camera, to grab the PTV images, and to control the image collection process. The camera triggered the microcontroller to begin the cyclic motion of the model when the first image was taken. Because the maximum number of sequential images that our image system could collect at 30 Hz was 360 images, the first 1034 images (=2.75 cycles) were discarded and then 360 images (nearly one cycle) were saved [Fig. 4(a)].

A multi-frame PTV algorithm similar to those described by Cierpka *et al.*²¹ and Hassan and Canaan²² was employed. Searching was limited to four consecutive frames and conducted based on the tracking algorithm shown in Fig. S3 (see [supplementary material](#) for details). 500 realizations of velocity vector fields were measured for 24 phases (i.e., positions of the scale model). Approximately 1000 particle matches were found for each realization. The phases were spaced at 0.5 s intervals with the seventh phase being when $L_m(t=37.7\text{ s})=L_{m,\max}$ [Point 1 in Fig. 4(a)]. The vector field realizations associated with each phase were merged for phase-averaging, resulting in 24 sets (12 of which are presented in this paper) of 485 000 to 522 000 vectors per phase.

The combined average density of valid vectors was 20 vectors/mm² for all phases. To calculate average velocity vectors, an adaptive Gaussian window (AGW) was applied.^{23,24} The width of the averaging window was 1.24 times the average spacing of the particles which was based on the vector density. To reduce computational time, only vectors within a $1.4 \times 1.4\text{ mm}^2$ box surrounding each averaging point were included in the AGW average. It was determined that vectors outside of the boxes did not significantly affect the average and that the AGW average did not differ substantially from an unweighted mean of the vectors within the box. For comparisons with CFD results, averages were only calculated for boxes with at least 20 vectors.

C. Validation of the CFD model

To validate the CFD model, we compared the simulated flow field of the CFD model to PTV measurements from the experimental scale model (Fig. 5). For direct comparison, the GCI-verified CFD mesh was scaled up to the scale of the experimental model based on the scale ratio of 1:774, and the zooid part of the CFD model was set to move following Eq. (3), mimicking the experimental model. Since the scale and operating conditions of the scaled-up CFD model and the experimental model were equivalent, the CFD and PTV results could be directly compared for validation of the CFD model of the prototype *Vorticella*.

Figure 6 compares the axial flow velocity (w) from PTV and CFD results in the interrogation plane used for PTV at 12 phases with a 1 s interval from $t=34.7\text{ s}$. Red indicates positive (away from the substrate) velocity and blue indicates negative (toward the substrate) velocity. Black is approximately zero velocity and is used to distinguish between regions of positive and negative velocity. Overall, PTV measurements and CFD simulation of axial flow velocity are in very good agreement.

FIG. 5. Schematic diagram of the modelling approach. The CFD model was created based on the dimension of the prototype *Vorticella* and verified using the grid convergence index (GCI). Then, the CFD model was enlarged to the scale of the experimental *Vorticella* model for the validation purpose. The validated CFD model simulated water flow around the prototype *Vorticella*.

FIG. 6. Contour plots of axial flow velocity for 12 phases of the experimental cycle. The left and right plots in each panel are the phase-averaged velocity of PTV measurements and the equivalent CFD results, respectively. Negative velocities are toward the substrate boundary (the bottom of the figures).

Figure 6 shows that the measured and simulated flow field in the immediate vicinity of the sphere is symmetric. However, one should note that the contours directly to the right of the zooid model in the PTV measurements are unreliable because they are in the shadow of the sphere [the left of the zooid model in Fig. 3(b)]. This is only true directly to the right of the sphere since above and below the sphere, the light sheet passes by the sphere undistorted. In contrast to the flow distribution observed in the proximity of the sphere, the flow field distant from the sphere was not observed to be completely symmetric in the experimental model, such as $t = 38.7$ s. Although velocities in this region are quite small and approach the uncertainty limit of the PTV measurements, we suspect that slight asymmetries in the experimental tank or model sphere roughness might cause this asymmetry. The influence could be extremely small and might be an indicator that axial symmetry of the far-field is not very stable for this flow. Certainly, the far-field boundaries in this experiment are not axisymmetric since the tank is not cylindrical but a rectangular prism.

Figure 6 demonstrates the effects of streaming previously described and observed for the motion of an oscillating sphere in a fluid.^{25–28} In this study, the fluid is bounded, the translation distance of the sphere is significantly greater than the sphere diameter, and the asymmetry of the flow field causes net streaming away from the boundary along the axis of the sphere (note the region marked by positive velocities above the sphere). This streaming effect is caused by the generation of ring vortices on the backside of the sphere followed by shedding of the vortices when the sphere reverses directions at its position farthest from the boundary. A similar ring vortex detaches from the sphere when the sphere reverses direction near the boundary, but its ability to transport fluid away from the sphere is confined by the boundary. Although similar phenomena could cause significant secondary flow in the simulated *Vorticella* motion described later, the simulated motion has much lower Re and the translation profile is much less symmetric. Such differences will undoubtedly cause dissimilarities with the flow behavior later observed in the CFD simulations of the prototype *Vorticella*.

Figure 7 compares phase-averaged PTV (green) and CFD (red) flow velocity vectors at $t = 37.7$, 40.7 , and 43.7 s, which correspond to the sphere positions farthest from the substrate, half way, and closest to the substrate [Points 1, 2, and 3 in Fig. 4(a)]. Only data generated on the

FIG. 7. PTV (green) and CFD (red) velocities at 12π ($=37.7$ s) when $L_m=L_{m,\max}$, 13π ($=40.7$ s) when $L_m=(L_{m,\max}+L_{m,\min})/2$, and 14π ($=43.7$ s) when $L_m=L_{m,\min}$. Images were cropped with respect to the sphere center for comparison and correspond to Points 1, 2, and 3 in Fig. 4(a). Cartoon insets show the configuration of the model for each time point.

illuminated side of the experimental model are used for this comparison. The PTV and CFD results are very similar for $t=40.7$ s, when the sphere approaches the maximum speed and the velocity field is strongly influenced by motion of the model. At $t=40.7$ s, the only place showing a substantial deviation in velocity magnitude is along the rod. Because velocity measurements could not be made immediately adjacent to the rod and velocity gradients near the rod were high, PTV velocity measurements near the rod were biased low. For $t=37.7$ s and 43.7 s when the *Vorticella* model reverses its motion, PTV and CFD results are also in good agreement, but differences are greater compared to the 40.7 s result. There are two explanations for these greater differences observed during flow reversal phases. First, most of the flow field during flow reversal has very low velocities, which are difficult to accurately measure with PTV. Second, high velocities during flow reversal are mostly found in locations with high velocity gradients, which clearly increase scatter in the comparison between PTV and CFD velocities.

For more quantitative comparisons of the PTV and CFD results of Fig. 7, refer to Fig. S5 (supplementary material), which contains scatterplots of the velocity magnitude and direction for the region nearest to the substrate, the region farthest from the substrate, and the grid points adjacent to the rod. These scatter plots support the observations discussed above.

IV. CFD-BASED INVESTIGATION OF FLOW CAUSED BY VORTICELLA

A. CFD simulation

The results given in Sec. III C demonstrate that the enlarged CFD model of the prototype *Vorticella* is in agreement with the validation experiments (Fig. 5). For directly evaluating transport effects of the stalk contraction cycle of the prototype *Vorticella* at its true scale, the CFD model was set to follow the representative stalk length change profile of the prototype *Vorticella* cell [Fig. 4(b)]. For the contraction phase, we employed the contraction profile of the prototype *Vorticella*⁷

$$\frac{L_p(t) - L_{p,\min}}{L_{p,\max} - L_{p,\min}} = \left[\operatorname{sech}\left(\frac{t}{9.263 \times 10^{-4}}\right)^{1.243} \right]^{0.873} \quad (0 \leq t \leq 0.025 \text{ s}), \quad (4)$$

where L_p is the stalk end-to-end length of the *Vorticella*, and $L_{p,\max}$ ($=116.8 \mu\text{m}$) and $L_{p,\min}$ ($=22.5 \mu\text{m}$) are the L_p of the fully extended and coiled stalk, respectively. For the relaxation

phase, we used six L_p data sets of three relaxing *Vorticella* cells that Ryu measured previously²⁹ and fitted an equation similar to Eq. (4) against the data set to obtain the following profile:

$$\frac{L_p(t) - L_{p,\max}}{L_{p,\min} - L_{p,\max}} = \left[\operatorname{sech}\left(\frac{t - 0.025}{0.934}\right)^{1.016} \right]^{2.287} \quad (0.025 \text{ s} \leq t \leq 4 \text{ s}). \quad (5)$$

Based on Eqs. (4) and (5), the maximum speed of the zooid is found to be 57.7 mm/s for contraction and 84.8 $\mu\text{m/s}$ for relaxation, respectively. These maximum speed values correspond to Re of about 2 and 0.003, respectively.

It would be ideal to use the stalk length change profile of a specific *Vorticella* cell for the entire cycle. Because of the time scale difference between the contraction and relaxation phase, however, it is challenging to measure an entire cycle profile of a certain *Vorticella*. Instead, we used Eq. (5), which can be assumed to represent the relaxation phase, with the $L_{p,\max}$ and $L_{p,\min}$ values of the prototype *Vorticella* cell, to form one complete stalk contraction-relaxation cycle with a 4-s period [Fig. 4(b)]. Equations (4) and (5) were assigned to the no-slip moving wall boundary condition of the CFD model (i.e., Boundaries ② and ③ in Fig. 2).

B. CFD particle tracing

To visualize and analyze flow caused by the stalk contraction of the prototype *Vorticella*, neutrally buoyant particles were uniformly spread around the *Vorticella* in the CFD model in the area of about $100 \mu\text{m} \times 180 \mu\text{m}$ with particle spacings of $1.8 \mu\text{m}$ and $3.1 \mu\text{m}$ in the r and z directions, respectively. These flow tracers were grouped into four different colors, and their trajectories were numerically calculated based on the CFD simulation result using a time-dependent particle tracing algorithm (see [supplementary material](#) for details).

Figure 8 shows how these particles are translated by the prototype *Vorticella* during one contraction-relaxation cycle. During the contraction phase, the protozoan significantly displaces particles adjacent to the zooid and stalk (red and green particles) toward the substrate. Particles immediately beneath the zooid are displaced farthest. Particles distant from the substrate (red and cyan particles) move inward, while particles near the substrate (green and blue particles) move a

FIG. 8. Virtual, neutrally buoyant tracer particles (shown by colored dots) visualize water flow caused by one stalk contraction-relaxation cycle of the prototype *Vorticella* (top: contraction phase, bottom: relaxation phase). Contour lines in the 4.0 s frame show the local Péclet number of motile bacteria, which is estimated based on the net displacement of tracer particles at the end of the cycle.

little outward. Therefore, the stalk contraction generates a significant downward flux of tracer particles. However, these particle displacements are partially cancelled during the relaxation phase because the zooid drags the particles back toward their initial position. Consequently, most particles return close to their initial position at the completion of the entire cycle.

Pathlines of Fig. 9(a) show that particles show different trajectory patterns depending on their location with respect to the zooid. For instance, particles above the equator of the zooid show inward and then outward motion whereas those near the substrate show the opposite trend. Particles near the zooid are more affected by the zooid motion than distant particles are. Specifically, particles right below the zooid travel the longest distance following the zooid, as shown by contours of particle travel distance. These contours show that the closer the particles are to the zooid, the longer the distances they travel. Among particles around the zooid, lower particles are translated farther than upper particles because of the stalk. These particle trajectories during the contraction phase agree well with the experimentally obtained pathlines of polystyrene microspheres around a live *V. convallaria*.⁷ Although some particles near the zooid and the stalk can travel up to $188.5\ \mu\text{m}$, they return close to their initial position, marked by red dots, at the completion of the cycle.

Figure 9(b) compares the initial (red dots) and final (green dots) location of representative particle rows and thus shows their net displacement caused during one stalk contraction-relaxation cycle. A single cycle of the *Vorticella* has resulted in the upward net translation of particles around the stalk. However, flow tracers very close to the stalk have almost returned to their initial location because of the no-slip condition of the stalk, like those adjacent to the zooid. The rest of the particles show downward net displacement, and the net displacement decreases for particles located farther from the zooid, as illustrated by contour lines of the vertical net displacement. The maximum upward and downward displacements are $3.8\ \mu\text{m}$ and $-3.4\ \mu\text{m}$, respectively. Because these particles are massless with zero size, they trace fluid motion, and the evaluated net displacement of particles represents that of water around the *Vorticella* model.

The direction of the net displacement can be explained by the asymmetry in the flow field between the contraction and relaxation phase. During the contraction phase, the fast moving zooid drags the surrounding water toward the substrate, and the inertia of the water flow allows it to overcome the resistance of the no-slip substrate, as indicated by the maximum Re of ~ 2 . However, in the relaxation phase during which the slow moving zooid drags water away from

FIG. 9. Analysis of water flow caused by one stalk contraction-relaxation cycle of the prototype *Vorticella*. (a) Pathlines of representative tracer particles with contours showing the travel distance of the particles. (b) Net displacement vectors of the tracer particles with contours showing the vertical net displacement distance of the particles. (c) Averaged vertical displacement [Δz_{avg} of Eq. (6)]. For (a) and (b), red and green dots show the initial and final position of the particles, respectively. The unit of the color bars is μm .

the substrate, the inertia of the water flow is much less significant, as indicated by the much smaller Re , and thus the flow travels a shorter distance. As a result, water is displaced downward by the end of the cycle. In the case of water in the proximity of the stalk, the flow is governed more by the stalk. As the stalk moves, the boundary layer develops on the stalk, and the thickness of the boundary layer depends on the speed of the stalk surface. The boundary layer is thicker during the relaxation phase than during the contraction phase because of its lower speed. Therefore, it appears that the shortening stalk can only move water very close to its surface downward, but the relaxing stalk can displace water farther from its surface upward as well. As a result, nearby water returns close to its initial location whereas distant water is displaced upward.

To quantify the observed transport effect caused by the stalk contraction cycle, we averaged the net vertical displacement of particles in the radial direction at a certain z position

$$\Delta z_{avg}(z) = \frac{1}{\pi[R_o^2 - R_i^2(z)]} \int_{R_i(z)}^{R_o} [z(r, z, t = 4 \text{ s}) - z(r, z, t = 0 \text{ s})] \cdot 2\pi r dr, \quad (6)$$

where R_i is the radial position of the innermost particle and R_o is the maximum radial extent of particles ($= 98.4 \mu\text{m}$). For instance, $R_i = 3.4 \mu\text{m}$ for the particles above and below the zooid, and $R_i = 17.8 \mu\text{m}$ for the particles closest to the equator of the zooid. Figure 9(c) shows that the averaged net vertical displacement decreases from $0 \mu\text{m}$ at the substrate toward the zooid, reaches minimum (about $1 \mu\text{m}$ in magnitude) right below and around the zooid, and then increases above the zooid. This result illustrates that the stalk contraction cycle of the prototype *Vorticella* causes overall net downward transport of surrounding water and particles and that such downward transport is most significant near the location of the *Vorticella* zooid in the extended state. This downward net transport around *Vorticella* must be balanced by upward transport occurring farther away from the protozoan in order to satisfy the mass conservation of water flow.

To amplify the transport effect of *Vorticella*'s stalk contraction cycle, the motion of the particles was traced over a large number of repeated cycles. Because of indiscernible particle displacement at the end of one cycle, the CFD simulation result of one cycle was used for multi-cycle particle tracing. As Fig. 10 shows, the net displacement of particles caused by one cycle (as observed in Fig. 8) becomes exaggerated over repeated cycles. Particles above the zooid are moved downward, while particles near the stalk are dragged upward toward the zooid. Particles near the substrate are translated outward, while particles distant from the substrate and the zooid are translated inward. Therefore, repeated contraction-relaxation cycles result in upward flow near the stalk and rotating secondary flow around the *Vorticella*, as indicated by arrows in Fig. 10. In order to visualize the secondary flow, streamlines were found based on the net displacements of tracer particles and plotted (Fig. S6 of the [supplementary material](#)). The flow lines show that the resultant secondary flow has an elongated rotating pattern below the zooid and a counter-rotating pattern of which the spatial extent is much larger than the *Vorticella*. Also, note that for the asymmetric, low Re motion simulated for the prototype *Vorticella*, net streaming of the secondary flow above the zooid and along its axis of translation is towards the boundary. This is opposite to the direction of net streaming observed above the experimental scale model for the much higher Re , periodic motion. This suggests that at the low Re of the relaxation phase, ring vortices do not form and detach above the zooid, but the contraction phase does produce net rotation below the zooid. Contour lines in Fig. 10 show the net vertical displacement of the particles, illustrating the extent of the transport effect of *Vorticella*.

V. DISCUSSION

A. Biological significance

Microorganisms live in hydrodynamic environments where the Reynolds number (Re) is very low and thus the viscosity effect of surrounding liquids is dominant.^{30–34} Thus, they adopt

FIG. 10. Distribution of virtual, neutrally buoyant tracer particles (shown by colored dots) after multiple cycles. Contour lines show the net vertical displacement, and the unit of the color bar is μm . Arrows illustrate rotating secondary flow patterns inferred from net particle displacements.

rotating screw-like flagella, undulating whip-like flagella, or beating oar-like cilia for motility. Such motile organelles can overcome the reversibility of low Re flows and thus exert forces to the liquid based on asymmetric motion.

The coiling stalk of *Vorticella* is distinguished from the above low Re motile organelles because of its ultrafast contraction. During the contraction phase, the stalk coils quickly, pulling the zooid toward the substrate, and then it relaxes slowly, pushing the zooid away from the substrate. Although it has long been presumed that stalk contraction is for danger evasion, its ecological role is still uncertain. Because the moving zooid causes the surrounding water to flow, it has been suggested that stalk contraction is to induce transport near the habitat of *Vorticella*, where convective mixing within the boundary layer is limited.^{12–14,16,17}

In order to investigate the suggested hypothesis, we developed the CFD model of the prototype *Vorticella* and simulated water flow caused by its stalk contraction-relaxation cycles. Our results show that the asymmetry of the contraction-relaxation cycle resulted in net motion of the surrounding fluid. This observation suggests that the Re difference between the contraction and relaxation phases is responsible for any possible transport effects resulting from the stalk contraction-relaxation cycle: Re is as high as ~ 5 during contraction, whereas it is a few orders of magnitude less during relaxation.

Similar Re -difference-dependent transport has been observed in other biological systems. Koehl and her coworkers identified the fluid dynamics mechanism of hairy appendages of aquatic animals for capturing food particles or odorant molecules.^{35–40} A wide variety of organisms have appendages or legs bearing arrays of hairs to capture food or molecules from the surrounding fluid, and they investigated how water flows through these hairs using the concept of leakiness. Leakiness is defined as the ratio of the actual volume flow rate of water between a pair of hairs to the assumptive volume flow rate through a space of the same width with no hairs present.⁴¹ At $\text{Re} \sim 1$, little fluid is dragged along with the moving hairs, while at much lower Re , quite a bit of fluid is dragged along with the hairs and very little moves through the gap between adjacent hairs. Therefore, a change in the hair Re from 10^{-2} to 1 produces a substantial increase in the leakiness, which is related to boundary layer growth on and between the hairs. This leakiness is affected by the presence of a wall near the hairs, and this wall effect becomes negligible as the Re increases.^{36,42} This fluid dynamics mechanism is commonly found

in the feeding behaviors of certain copepods^{41–43} and in the olfactory antennae motions of crustaceans such as lobsters and mantis shrimps.^{37,39,40,44–46} We speculate that *Vorticella* takes advantage of the Re difference between its contraction and relaxation phases in a way similar to the hairy appendages discussed above.

It is worthwhile to interpret the ecological meaning of the simulated transport effect using the Péclet number, which is defined as $Pe = L_{p,max} U_c / D$, where U_c is a characteristic speed for particles transported by the contraction cycle of the prototype *Vorticella* [$\approx 1 \mu\text{m}/4 \text{ s}$ from Fig. 9(c)], and D is the diffusion coefficient of food particles of interest.¹⁵ Because the Pe number represents the relative importance of advective transport of food particles to their diffusive transport, large Pe indicates that advective transport of particles is more dominant. Because *Vorticella* preys on bacteria, two cases of non-motile and motile bacteria are considered here. If non-motile bacteria are assumed to be spherical particles of $0.1\text{--}10 \mu\text{m}$ diameter, their diffusion coefficient at room temperature is calculated to be $D = 0.02\text{--}2 \times 10^{-14} \text{ m}^2/\text{s}$ using the Stokes-Einstein relation: $D = k_B T / (3\pi\mu d)$, where k_B is the Boltzmann constant ($= 1.38 \times 10^{-23} \text{ J/K}$), T is the temperature ($= 298 \text{ K}$), and d is the diameter of bacteria.⁴⁷ Hence, the Pe of non-motile bacteria ranges from 10^3 to 10^5 . For motile bacteria, $D \approx 10^{-10} \text{ m}^2/\text{s}$,⁴⁷ and thus their Pe is approximately 0.2. This significant difference in the estimated Pe values suggests that the advection effect caused during the stalk contraction-relaxation cycle of *Vorticella* is more dominant for non-motile bacteria, whereas for motile bacteria, their diffusive motion is more important. In other words, non-motile bacteria would follow closely the particle trajectories shown in Fig. 9(a), whereas motile ones would diffusively deviate from the trajectories, probably during the slow relaxation phase (Fig. 11). This suggests that the stalk contraction-relaxation cycle of *Vorticella* could be more effective for motile bacteria because motile bacteria would freely swim away from the location to which they are transported at the end of the contraction phase, not returning to their initial positions, and because they would be introduced to the ciliary feeding current that *Vorticella* resumes at the end of the stalk contraction cycle. The local Pe contours of motile bacteria in Fig. 8, which are plotted based on the net displacement caused by one cycle, show that the suggested mechanism could be effective for most motile bacteria around *Vorticella*.

B. Model similitude

The goal of the CFD model was to simulate the motion of the prototype *Vorticella* cell, and it was ideal to experimentally validate the CFD model by applying the stalk length change profile of the entire cycle [Eqs. (4) and (5)] for both the CFD model and the experimental scale model. However, such experimental validation presented several major challenges, including maintaining fluid dynamic similitude, correctly representing the stalk contraction-relaxation cycle, and achieving accurate measurements with available equipment and facilities.

In the present case, Reynolds number similitude was required between the CFD model and the experimental scale model for validation. However, the prototype *Vorticella* is extremely small, and development of a working mechanical model of the prototype *Vorticella* at this scale was impractical, as was working with live *Vorticella*. An alternative was to develop an

FIG. 11. Schematic presentation of the transport effect of *Vorticella*'s stalk contraction on motile and non-motile bacteria. Both types of the bacteria are drawn toward the substrate during the fast stalk contraction, and motile ones freely swim away during the slow stalk relaxation while non-motile ones are returned to their initial location.

experimental model at a larger scale based on geometric similarity while maintaining dynamic similarity with the CFD model during validation. The smallest scale model that we could build, control, and measure flow velocities in with good accuracy had a zooid model that was 25.4 mm in diameter ($2R_m = 25.4$ mm). This zooid diameter was 774 ($=\lambda$) times larger than the prototype diameter ($2R_p = 32.8$ μ m), and Reynolds number similitude ($U_m R_m / \nu = U_p R_p / \nu$) required velocity to be 774 times smaller in the scale model than in the prototype ($U_m = U_p / \lambda$) with water as the working fluid. Here, U_m and U_p are the characteristic speed of the experimental scale model and the prototype *Vorticella*. Two issues would surface at such low velocities: natural convection and residual velocities would dominate flow behavior in the experimental tank, and the relative accuracy of mechanically controlling the motion of the model zooid would be poor. Reducing the model Re by using a working fluid with a much higher viscosity was not a viable option in the present study as explained below.

A second challenge for the experimental model was that the motion of the prototype *Vorticella* was complex, with an extremely short contraction phase [Eq. (4)] and a relaxation phase that was approximately 160 times longer [Eq. (5)]. Based on kinematic similarity, the characteristic time scale for the experimental model with water was found to be 774^2 times longer than that of the prototype *Vorticella* ($t_m = L_m / U_m = \lambda L_p / (U_p / \lambda) = \lambda^2 t_p$). Then, the 25 ms-long contraction phase of the prototype would require an approximately four hour long contraction phase in the experimental model. If glycerol was used as the working fluid for the experimental model, it would be possible to achieve $U_m \approx 1.5 U_p$ based on Reynolds number similitude and ~ 13 s-long contraction phase. However, the relaxation phase would be ~ 34 minute long. Ultimately, it was not possible for us to accurately control or measure such a wide range of zooid velocities and phase durations with available equipment.

Our solution to these problems was to scale up the GCI-verified CFD model to the dimension of the experimental scale model, maintaining the same grid topology, and then to validate the CFD model using the geometrically identical experimental model but at much higher Re than are characteristic of actual *Vorticella* scales (Fig. 5). In addition, the simulated periodic motion of the validation [Eq. (3)] was significantly simpler than the motion of the prototype *Vorticella* but represented a more demanding Re regime. The validated CFD grid was then used at much lower Re to simulate actual *Vorticella* behavior. While the experimental validation was not dynamically similar to the simulations of true *Vorticella* behavior, both had the same grid topology, both were laminar flow, and the experimental validation was performed at higher Re than the simulations that followed. The underlying assumption was that the physical requirements of the experimental validation were more demanding than the physical requirements of the subsequent simulations. Thus, a CFD configuration that accurately predicted the more demanding conditions of the scale model experiments could be expected to perform well when used to simulate the actual *Vorticella* behavior.

C. Strengths and weaknesses of the CFD model

A major benefit of using the CFD model is that it enables isolating any transport effect induced by the stalk contraction-relaxation cycle from that of the oral cilia. Although it was possible to visualize water flow caused by a contracting *Vorticella* using microspheres and high-speed video microscopy,⁷ it is challenging to measure detailed features of the water flow because of the cilia-caused feeding flow. The oral cilia always beat, cause circulating flow around the oral part of *Vorticella*, and stir particles around the protozoan. As a result, seeded microspheres cannot be uniformly spread around *Vorticella*, which is not ideal for flow measurement. If particles are fluorescent, furthermore, *Vorticella* engulfs some of the tracing particles, and the zooid becomes too bright for particles in water to be imaged properly. Therefore, using the CFD model can prevent these problems and enables investigating detailed features of the water flow caused by the stalk contraction cycle of the protozoan.

Our CFD-based approach can be improved by accounting for additional characteristics that may have an impact on the flow. First, it was only possible for us to consider one cell in the present *Vorticella* model. *Vorticellae* generally live in colonies, and it would be beneficial to

model a group of *Vorticellae* and then to apply the concept of leakiness for better understanding of hydrodynamic interactions of their stalk contractions. Second, the stalk length profile for the relaxation phase has been simplified as Eq. (5) for the sake of modeling. Actually, the zooid motion of relaxing *Vorticella* is more complicated than the rectilinear motion assumed for Eq. (5) because of unwinding of the coiled stalk. According to our observations, the re-extending stalk can display variations such as rotation of the zooid and pivoting of the whole organism, and the zooid does not always return to its initial position. Then, particles drawn toward the substrate during the contraction phase may not return close to their initial position, and thus the net transport effect of the stalk contraction of *Vorticella* could be more significant than the current estimation. Lastly, massless particles of zero size were used to trace simulated water flow. Although these virtual tracer particles visualize the water flow well, they have limitations in simulating motion of food particles caused by the water flow because food particles have finite size and mass.

VI. CONCLUSIONS

The ultrafast stalk contraction of the sessile ciliate *Vorticella* has been of scientific interest for many years, but the role of its stalk contraction-relaxation cycle still remains unknown. In this study, we hypothesize that *Vorticella* augments transport of substances critical for its sustenance, relying on water flow caused during the cycle. To investigate the hypothesis using simulation of water flow around a prototype *Vorticella convallaria*, a computational fluid dynamics (CFD) model of the *Vorticella* was developed, verified based on the grid convergence index, and validated with an experimental *Vorticella* model and particle tracking velocimetry (PTV) measurements. Then, the CFD model was set to mimic the stalk contraction-relaxation cycle of the prototype *Vorticella* with rectilinear motions of the zooid assumed. Tracing virtual massless particles near the *Vorticella* has revealed that one stalk contraction-relaxation cycle can translate particles over the maximum distance of $189\text{ }\mu\text{m}$, but most of the particles returned close to their initial position at the completion of the cycle. Also, particles show different net displacement depending on their location with respect to the protozoan: those near the stalk are moved upward whereas the rest downward, and the maximum net vertical displacement is $3\text{--}4\text{ }\mu\text{m}$. Repeated cycles are observed to amplify this transport effect. The observed net transport effect appears to be due to the asymmetry in the flow field between the contraction and relaxation phase, which is indicated by the difference in the Reynolds number, and it could be more effective on motile food particles, as indicated by their lower Péclet number. Our *Vorticella* model enabled quantitatively evaluating the transport effect of the stalk contraction-relaxation cycle of *Vorticella* by isolating it from that of oral cilia and suggesting a possible mechanism for *Vorticella* to utilize the transport effect for its sustenance.

SUPPLEMENTARY MATERIAL

See [supplementary material](#) for the GCI-based verification of the CFD model, the multi-frame PTV algorithm, the time-dependent particle tracing for the CFD model, and quantitative comparisons of the PTV and CFD results for validation of the CFD model.

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