Biophysical Journal, Volume 98

Supporting Material

Unsteady Motion, Finite Reynolds Numbers and Wall Effect on *Vorticella convallaria* Contribute Contraction Force Greater than the Stokes Drag

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Supplementary Material

The Stokes number and Reynolds number

We investigate characteristics of the contraction-induced flow by revisiting the Navier-Stokes equation that governs incompressible flow of a Newtonian fluid. The equation with the body force term omitted is

$$\rho\left(\frac{\partial \vec{u}}{\partial t}+\vec{u}\cdot\nabla\vec{u}\right)=-\nabla p+\mu\nabla^2\vec{u} \tag{S1}$$

where ρ is fluid density, $\vec{u}$ is flow velocity, t is time, p is pressure and μ is fluid viscosity. The equation is nondimensionalized with characteristic length scale L, velocity scale U and time scale τ. Then the variables and operator of the equation become the following: $\nabla^* = \nabla/L$, $\vec{u}^* = \vec{u}/U$, $t^* = t/\tau$ and $p^* = p/(\mu U/L)$, and the Navier-Stokes equation becomes

$$\frac{\rho L^2}{\mu\tau}\frac{\partial \vec{u}^*}{\partial t^*}+\frac{\rho LU}{\mu}\vec{u}^*\cdot\nabla^*\vec{u}^*=-\nabla^* p^*+\nabla^{*2}\vec{u}^*. \tag{S2}$$

Being the coefficient of the time derivative term of the nondimensional Navier-Stokes equation, the Stokes number (St) is defined as

$$\mathrm{St}=\frac{\rho L^2}{\mu\tau}=\frac{4R^2}{\nu\tau_{u,\max}} \tag{S3}$$

where ν is the kinematic viscosity of the fluid. The Reynolds number (Re) is defined as

$$\mathrm{Re}=\frac{\rho LU}{\mu}=\frac{2R|U_c|}{\nu}. \tag{S4}$$

In the case of contracting *Vorticella*, the diameter of the shrunken zooid is the characteristic length scale ($L = 2R$), the time to the peak contraction speed is the characteristic time scale ($\tau=\tau_{u,\max}$), and the moving speed of the zooid is the characteristic velocity scale ($U=|U_c|$).

Numerical integration of the history force

In contrast to the quasi-steady force and added mass force, the history force of the unsteady Stokes drag formula requires numerical integration because of its kernel. As the drag formula shows, the history force has a singularity as s approaches t, so it requires a special treatment in numerical integration. This singularity was evaded in numerical integration by following Kim et al.'s remedy, which is

$$\begin{aligned}
&\int_0^t \frac{\dot{U}_c}{\sqrt{t-s}}\,ds=\int_0^{N\Delta t}\frac{\dot{U}_c}{\sqrt{t-s}}\,ds\\
&=\frac{\Delta t}{6}\sum_{i=1}^{N-1}\left[\frac{\dot{U}_{c_{i-1}}}{\sqrt{N\Delta t-(i-1)\Delta t}}+\frac{2\left(\dot{U}_{c_{i-1}}+\dot{U}_{c_i}\right)}{\sqrt{N\Delta t-(i-0.5)\Delta t}}+\frac{\dot{U}_{c_i}}{\sqrt{N\Delta t-i\Delta t}}\right]\\
&\quad+\frac{0.9\Delta t}{6}\left[\frac{\dot{U}_{c_{N-1}}}{\sqrt{N\Delta t-(N-1)\Delta t}}+\frac{2\left(\dot{U}_{c_{N-1}}+\dot{U}_{c_{N-0.1}}\right)}{\sqrt{N\Delta t-(N-0.55)\Delta t}}+\frac{\dot{U}_{c_{N-0.1}}}{\sqrt{N\Delta t-(N-0.1)\Delta t}}\right]\\
&\quad+\frac{0.1\Delta t}{2}\left[\frac{8\sqrt{2}}{3}\frac{\dot{U}_{c_N}}{\sqrt{N\Delta t-(N-0.05)\Delta t}}-\frac{4}{3}\frac{\dot{U}_{c_N}}{\sqrt{N\Delta t-(N-0.1)\Delta t}}\right]
\end{aligned} \tag{S5}$$

where N is the number of time intervals and Δt is the size of the interval [1].

Discussion about flow visualization

Understanding the contraction-induced flow of *Vorticella* is indispensable to estimate its contraction force and to identify a biological reason of contraction. Vopel et al. measured the contraction-induced flow at several points around contracting *Vorticella* with a flow microsensor of which diameter was 50 μm and a response time was less than 1 sec [2]. However, the spatial and temporal resolution of their measurement does not seem high enough because the typical zooid size and contraction time of *Vorticella* are about 40 μm and a few msec, respectively.

The incredible speed of *Vorticella* contraction makes it difficult to study the contraction-induced flow experimentally. The micro-PIV/PTV technique is an ideal method to study the induced flow because it does not interfere with the flow and it has appropriate measurement resolution. The technique usually employs fluorescent tracers to remove unnecessary information from flow field, but the current study could not use such tracers. Exposure time less than 40 μsec is required to obtain clear images of contracting *Vorticella*, but a preliminary PIV experiment showed that this is not long enough to capture the motion of fluorescent beads. Another problem in using fluorescent beads is that *Vorticella* engulfs a significant number of beads. The zooid after consuming the beads appears too bright, like a huge agglomerate of fluorescent beads.

Recognizing the aforementioned problems, we tried bright field PTV experiments of which the results are shown in the result section. This is a compromise between a low signal-to-noise ratio and high temporal resolution. In our case, the depth of field (δ_z) is calculated with

$$\delta_z = \frac{3n\lambda_o}{\mathrm{NA}^2} + \frac{n}{\mathrm{M}\cdot\mathrm{NA}}e \tag{S6}$$

where n is the refractive index of the medium (1 for air), λ_o is the wavelength of light in a vacuum, e is the smallest resolvable distance of the camera sensor (0.5 μm), M is the magnification ratio (40×), and NA is the numerical aperture (0.6) [3]. Because the longest wavelength of intensity peaks of mercury arc lamps is 579 nm, the estimated depth of field is approximately 2 μm. Although this depth of field is comparable to the stalk diameter, obtained images are not as clean as ones that can be obtained with fluorescence because beads out of focus still exist in those images. Although the low quality of images resulted in error in tracking beads, pre-processing of raw images and post-processing of identified particle trajectories enabled qualitative analysis of the flow field induced by contracting *Vorticella*.

Calculation of total amount of energy available from calcium binding

Using *Zoothamnium*, Routledge et al. measured that the dry mass concentration of the spasmoneme (C_s) is 210 mg/mL and that the amount of calcium bound to 1 kg of dry mass of the spasmoneme (m_{Ca}) is 1.7 g [4]. The amount of calcium bound to 1 μm of the spasmoneme is given as

$$N_{Ca} = \frac{\pi}{4} d_s^2 \cdot C_s \cdot m_{Ca} \cdot \frac{1}{A_{Ca}} \tag{S7}$$

where d_s is the diameter of the spasmoneme (1.5 μm) and A_{Ca} is the atomic weight of calcium (40.1 g/mole). With given values, N_{Ca} is calculated to be 1.57×10^{-17} mole/μm. With Eq. 9, the total amount of energy available from the calcium binding of a 116 μm-long spasmoneme is 20.4 pJ.

Movie S1. *Vorticella convallaria* contracting in water. The zooid rotates after contraction is completed.

Movie S2. Water flow induced by contracting *Vorticella convallaria*. The movie compares experimentally visualized flow field and simulated flow field. The unit of velocity arrows is arbitrary, and the unit of the color bar is Pa.

Table S1. Comparison of total work and mechanical power output calculated by four methods: Stokes' law (Eq. 1), the Stokes drag formula with the wall effect corrected (Eq. 5), the unsteady Stokes drag formula (Eq. 4) and the computational fluid dynamic simulation.

PVP	W_{tot} (pJ)				P_{max} (nW) / time (msec)			
w/w %	Eq. 1	Eq. 6	Eq. 7	CFD	Eq. 1	Eq. 6	Eq. 7	CFD
0%	1.14	1.46	1.31	1.64	1.03/0.96	1.26/0.99	1.35/0.86	1.56/0.87
1%	1.74	2.17	1.85	2.25	1.05/0.90	1.26/0.92	1.23/0.80	1.36/0.83
2%	2.94	3.69	2.99	3.72	1.23/1.03	1.47/1.06	1.32/0.94	1.49/1.01
3%	3.02	3.77	3.02	3.79	1.00/1.04	1.19/1.08	1.05/0.95	1.19/1.03

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2. Vopel, K., et al., *Flow microenvironment of two marine peritrich ciliates with ectobiotic chemoautotrophic bacteria.* Aquat. Microb. Ecol., 2002. **29**: p. 19-28.
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