

Direct measurement of inertial impact and propulsive force in a eukaryotic swimmer

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Cilia and flagella are highly conserved biological motors that drive the motility of diverse eukaryotic cells. In the microscopic world, an organism's swimming kinematics are generally assumed to be a direct, instantaneous proxy for its underlying motor force output. However, as organisms scale in size—such as during the evolutionary transition to multicellularity—this fundamental assumption may break down. Using the multicellular alga *Volvox* as a model biophysical system, we perform direct, time-resolved force measurements during free-swimming collisions to deconvolve a swimmer's inertial impact force from its motor's propulsive force. We discover a ~ 30 Hz propulsive pulse, which serves as the mechanical signature of collective ciliary action and hydrodynamic synchronization. Crucially, we demonstrate that while this high-frequency motor output drives a fluctuating velocity in the smaller *V. carteri*, it is mechanically filtered by the inertia of the larger *V. farrisii*, resulting in a smooth swimming trajectory. Our work reveals that as organisms grow larger, their own mass acts as a mechanical low-pass filter. This fundamentally decouples high-frequency motor dynamics from whole-organism swimming kinematics, highlighting how physical constraints shape the mechanobiology of motility as organisms evolve toward complex multicellularity.

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I. INTRODUCTION

Cilia and flagella are highly conserved, microtubule-based eukaryotic organelles driven by ATP hydrolysis via motor proteins like dynein [1]. When multiple flagella beat in close proximity, they can spontaneously synchronize their phases entirely due to hydrodynamic coupling mediated by the surrounding viscous fluid [2,3]. This hydrodynamic synchronization leads to the formation of metachronal waves, which have been directly quantified across the spherical surface of the colonial alga *Volvox carteri* [4,5].

A fundamental question in the mechanobiology of active matter is how the localized, high-frequency forces generated by these microscopic motors translate into the macroscopic swimming kinematics of the whole organism. In fluid dynamics, this relationship is governed by the Reynolds number

(Re), a dimensionless value that represents the ratio of inertial forces (momentum) to viscous forces (fluid friction). Microorganisms typically exist in a regime characterized by a very low Reynolds number ($Re \ll 1$) [6,7]. In this “Stokes regime,” viscous friction overwhelmingly dominates. If a bacterium stops its flagellar motor, it coasts less than the width of an atom before stopping; therefore, an organism's kinematics (its speed and trajectory) are considered a direct, instantaneous proxy for its motor's force output.

However, the volvocine lineage provides a premier model for studying the evolutionary transition from unicellular life to complex multicellularity [8,9]. As these organisms scale up in size and mass, they transition into an intermediate regime ($Re \sim 1$) where inertia—the tendency of an object to stay in motion—begins to compete with fluid friction. In this regime, the assumption that kinematics directly mirror motor dynamics may break down.

Understanding this decoupling requires direct, time-resolved measurements of the forces produced by the swimmer's motor. Early biophysical techniques focused on measuring quasistatic stall forces, for example, by holding an organism with a micropipette and measuring the deflection of a calibrated glass fiber [1]. While foundational, these methods could not resolve high-frequency motor dynamics.

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More recently, techniques have emerged to measure dynamic forces. For instance, Schulman *et al.* inferred dynamic force patterns indirectly from high-resolution flow field measurements around free-swimming *Caenorhabditis elegans* [10]. Conversely, Bøddeker *et al.* achieved direct, time-resolved force measurements on *Chlamydomonas* cells held by a flexible micropipette force sensor [11]. However, because the cells in this method are physically tethered to a tiny glass needle rather than freely swimming, the approach cannot capture the inertial impact forces associated with the deceleration of an unconstrained organism.

Consequently, the direct measurement of force across the low-to-intermediate Re transition, and, in particular, the capture of inertial forces, has remained a challenge. The transient impact force generated by the momentum of a swimming organism during deceleration has, to our knowledge, never been directly measured.

Here, we use two species of *Volvox* as a tunable biophysical system to probe this regime. By performing direct force measurements on *V. carteri* (Vc, $Re \approx 0.03$) and the larger *V. farrisii* (Vf, $Re \approx 0.8$), we directly test how organismal inertia filters the output of a complex biological motor. Our dynamic collision assay allows us to deconvolve the propulsive force generated by the ciliary array from the inertial impact force generated by the organism's momentum, revealing a fundamental decoupling between motor output and swimming behavior.

II. MATERIALS AND METHODS

A. Strain and culture

Volvox carteri f. *nagariensis* strain Eve 10 (= UTEX1885, ~ 2000 cells/colony) and *Volvox farrisii* strain MI01

(= NIES – 4029, ~ 5000 cells/colony) [photomicrographs of both species are shown in Fig. 1(a)] were cultured in Standard Volvox Medium under a 16 h light/8 h dark cycle at 26 °C [12]. Before experiments, mature colonies were collected by filtration through a 40- μm mesh.

B. Direct force measurement via optical lever

The propulsive force of swimming *Volvox* spheroids was measured using a scanning probe microscope (SPM-9700, Shimadzu). This technique relies on the “optical lever” principle [schematic shown in Fig. 1(b)], a highly sensitive method commonly used in Atomic Force Microscopy to measure sub-nanonewton forces [13].

In this setup, a microfabricated, flexible cantilever is submerged in the fluid chamber. A 670 nm laser is focused onto the back of this cantilever, and the reflected beam is captured by a quadrant photodiode. When a free-swimming *Volvox* collides with the cantilever, the organism's propulsive force bends the cantilever slightly. This minute bending deflects the laser beam across the photodiode, effectively magnifying the physical displacement.

A probe (CL-RT800PSA-1, Olympus) with two cantilevers of differing spring constants (0.15 and 0.57 N/m) was used. Measurements were performed in a custom glass dish containing 0.5 ml of *Volvox* suspension. The total laser intensity (I), cantilever deflection (D), and torsion (T) signals were recorded at 250 Hz with a digital oscilloscope (PicoScope 4424A, Pico Technology). Because the stiffness (spring constant, k) of the cantilever is known, the deflection signal (x) can be directly converted to force (F) using Hooke's law ($F = -kx$) and the manufacturer-provided inverse optical lever sensitivity (InvOLS). A robust protocol was used

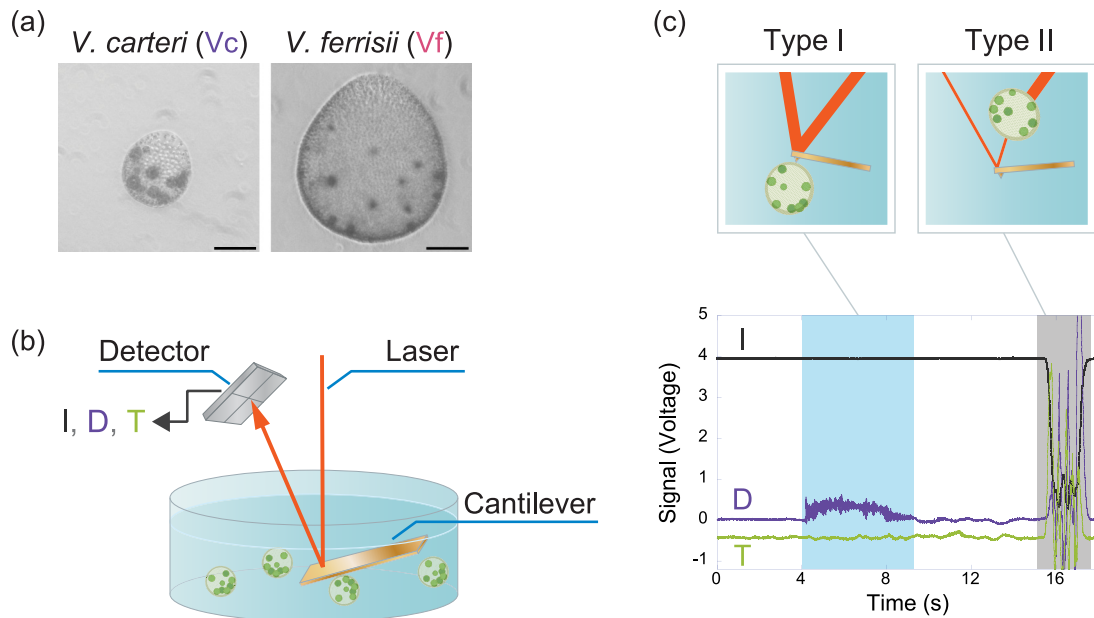


FIG. 1. Experimental setup and signal validation. (a) Photomicrographs of *V. carteri* and *V. farrisii*. Scale bars, 100 μm . (b) Schematic of the optical lever system. (c) Representative raw signals demonstrating the protocol for validating genuine collision events. A genuine collision (Type I, blue shaded region) is characterized by a clean deflection signal (D), with stable total laser intensity (I), and minimal torsion (T). Optical artifacts (Type II, gray shaded region) are caused by the organism swimming directly through the laser beam without contact, characterized by a sharp drop in total laser intensity (I) accompanied by spurious fluctuations in deflection (D) and torsion (T).

TABLE I. Physical and mechanical parameters. Spheroid mass and Reynolds number (Re) calculated from measurements and literature density values [3].

Parameter	<i>V. carteri</i>	<i>V. farrisii</i>
Spheroid diameter (μm)	200	800
Spheroid mass (ng)	4.2	269
Reynolds number (Re)	~ 0.03	~ 0.8
Peak force, F_{max} (nN)	3.3	9.8
Force consistency ($F_{\text{min}}/F_{\text{max}}$)	0.05	0.73
Oscillation rate (Hz)	~ 30	~ 30

to distinguish genuine collision events from optical artifacts [Fig. 1(c)]. Genuine events were identified by a clean deflection signal with a stable total laser intensity, ensuring that the organism was pushing the cantilever rather than simply swimming through the laser beam.

C. High-speed imaging

The swimming behavior of Vc and Vf was observed using a high-speed camera (FASTCAM SA5, Photron) mounted on an inverted microscope (IX70, Olympus). Because the ciliary beat frequency of *Volvox* is approximately 30 Hz, standard video frame rates (30–60 frames per second) would result in severe motion blur and aliasing, obscuring the fine motor dynamics. Therefore, images were acquired at 1000 Hz to capture millisecond-to-millisecond fluctuations in swimming speed.

Spheroids swam in a chamber formed by two coverslips separated by a 1-mm spacer, providing enough depth to minimize hydrodynamic wall effects. Illumination was provided by a 660-nm (red) LED. *Volvox* are highly phototactic, but their photoreceptors are relatively insensitive to deep red light. Using the 660-nm LED ensured that the organisms exhibited natural, undisturbed swimming behavior rather than a light-escape response. Velocity analysis and fast Fourier transform were performed using ImageJ (NIH) and KaleidaGraph (Synergy Software), respectively.

D. Estimation of Reynolds number

The Reynolds number quantifies the physical regime an organism experiences, defined as $\text{Re} = \rho v L / \mu$, where ρ and μ are the density and dynamic viscosity of the fluid, respectively, v is the characteristic swimming speed of the organism, and L is its characteristic length (diameter) (see Table I for parameters). We assume the properties of water at 25 °C ($\rho \approx 1000 \text{ kg/m}^3$ and $\mu \approx 0.001 \text{ Pa s}$). For *V. carteri*, with $L \approx 200 \mu\text{m}$ and $v \approx 150 \mu\text{m/s}$ [14], $\text{Re} \approx 0.03$. For *V. farrisii*, with $L \approx 800 \mu\text{m}$ and $v \approx 1 \text{ mm/s}$ [3], $\text{Re} \approx 0.8$.

E. Hydrodynamic simulation

To confirm that the differences in swimming behavior between the two species are purely a physical consequence of their mass and size, we developed a one-dimensional (1D) mathematical model. The model solves the equation of motion

for a sphere representing a *Volvox* colony:

$$m \frac{dv}{dt} = F_{\text{external}}(t) - 6\pi\eta r v. \quad (1)$$

This equation is a direct application of Newton's second law ($F = ma$). The left-hand side represents the organism's mass (m) multiplied by its acceleration (dv/dt). The right-hand side represents the sum of the forces acting on the organism: the pulsatile propulsive force generated by the cilia ($F_{\text{external}}(t)$), minus the Stokes drag ($6\pi\eta r v$), which is the fluid friction pulling backward against a sphere of radius r moving at velocity v in a fluid of viscosity η .

The mass m was calculated for each species using measured spheroid diameters (Table I) and a density of 1.003 g/ml, consistent with the 0.3% density excess previously reported for *Volvox* [3]. The equation was solved numerically using a standard explicit Euler integration scheme with a time step of $\Delta t = 0.1 \text{ ms}$. The propulsive force, $F_{\text{external}}(t)$, was modeled as a periodic function with a fundamental frequency of 30 Hz. To match the experimental force consistency data [Fig. 3(c)], the force profile for Vc was constructed as a highly pulsatile waveform ($F_{\text{min}}/F_{\text{max}} \approx 0.05$), while the profile for Vf was modeled with a larger continuous baseline component ($F_{\text{min}}/F_{\text{max}} \approx 0.7$).

III. RESULTS

A. Direct measurement of a $\sim 30 \text{ Hz}$ propulsive force

Analysis of collision events revealed that the propulsive force generated by the biological motors is not steady but oscillates periodically (Fig. 2). For Vc, the force trace shows a strong, regular oscillation, whose power spectrum has a dominant peak at $\sim 30 \text{ Hz}$. Remarkably, Vf exhibits a propulsive

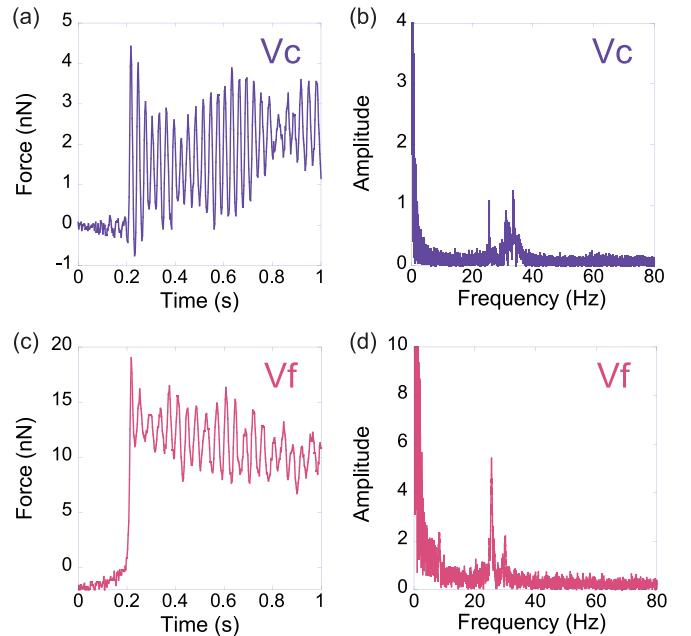


FIG. 2. The propulsive force of *Volvox* oscillates at $\sim 30 \text{ Hz}$. (a) Representative force trace for *V. carteri*. (b) Power spectrum of the trace in panel (a). (c) Representative force trace for *V. farrisii*. (d) Power spectrum of the trace in panel (c).

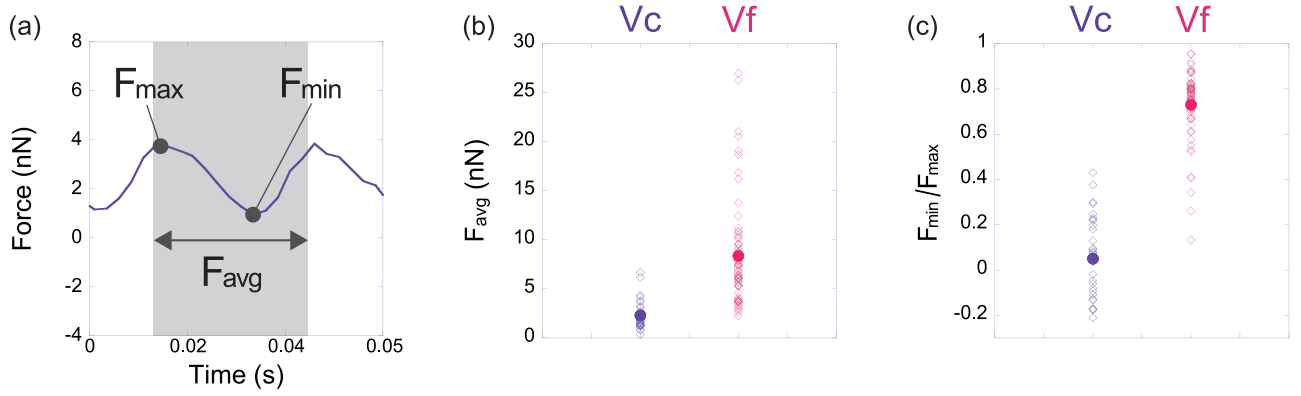


FIG. 3. Quantification of propulsive force properties. (a) Schematic defining metrics: peak force ($F_{\max}$), minimum force ($F_{\min}$), and time-averaged force (F_{avg}). (b) Distribution of time-averaged propulsive force (F_{avg}) for Vc (blue, $n = 35$) and Vf (orange, $n = 71$). (c) Distribution of the force consistency ratio ($F_{\min}/F_{\max}$).

force oscillating at a nearly identical frequency. This frequency is in excellent agreement with the ~ 32 Hz ciliary beat frequency that drives the metachronal waves in Vc [4,5].

The properties of this force pulse were further quantified by defining the peak force ($F_{\max}$), minimum force ($F_{\min}$), and time-averaged force (F_{avg}) [Fig. 3(a)]. As illustrated by the force trace in Fig. 2(a), these values were selected and calculated from a stable, periodic segment of the force trace during the steady oscillatory phase of the collision, well after the initial onset/impact. Specifically, $F_{\max}$ and $F_{\min}$ represent the average of the local peaks and troughs of the ciliary force oscillations over multiple consecutive cycles within this stable window, and F_{avg} is the time-integrated average over the same period. While the mean F_{avg} was larger for Vf than Vc, a more striking difference was observed in the force difference was observed in the force consistency ratio, $F_{\min}/F_{\max}$. The force from Vc is highly pulsatile (mean $F_{\min}/F_{\max} = 0.05$), whereas the force from Vf is nearly continuous (mean $F_{\min}/F_{\max} = 0.73$).

B. Inertial impact signature in *V. ferrisii*

Our dynamic collision assay allows for the first time the direct measurement of the inertial impact force (Fig. 4). The data reveal a stark difference in this parameter between the two species. Collisions with Vf are initiated by a large, transient impact force spike (F_{imp}), a direct consequence of its momentum ($p = mv$), which is absent in the low-Re Vc. The ratio $F_{\text{imp}}/F_{\text{prop}}$, where F_{prop} is the peak of the first propulsive cycle immediately following the impact, quantitatively captures this effect. For Vc, this ratio is ~ 1 , whereas for Vf, F_{imp} is significantly greater than F_{prop} .

C. Pulsatile force drives different kinematics

This same motor signature drives dramatically different free-swimming kinematics, measured via high-speed imaging (Fig. 5). The pulsatile force in Vc drives a fluctuating velocity with a corresponding ~ 30 Hz peak in its power spectrum. In stark contrast, the more massive Vf exhibits a nearly constant swimming velocity, with the 30 Hz peak strongly attenuated in its power spectrum.

D. Hydrodynamic simulation confirms inertial damping

The inertial filtering hypothesis is confirmed by our 1D hydrodynamic model (Fig. 6). Using the experimentally determined parameters, the model correctly captures the fluctuating velocity of Vc and the smooth trajectory of Vf when subjected to a 30 Hz force pulse.

IV. DISCUSSION

In this study, we presented the first direct, time-resolved measurements of the propulsive force generated by swimming *Volvox*, and the first measurement of inertial impact force in a microswimmer. Our central finding is a ~ 30 Hz oscillation

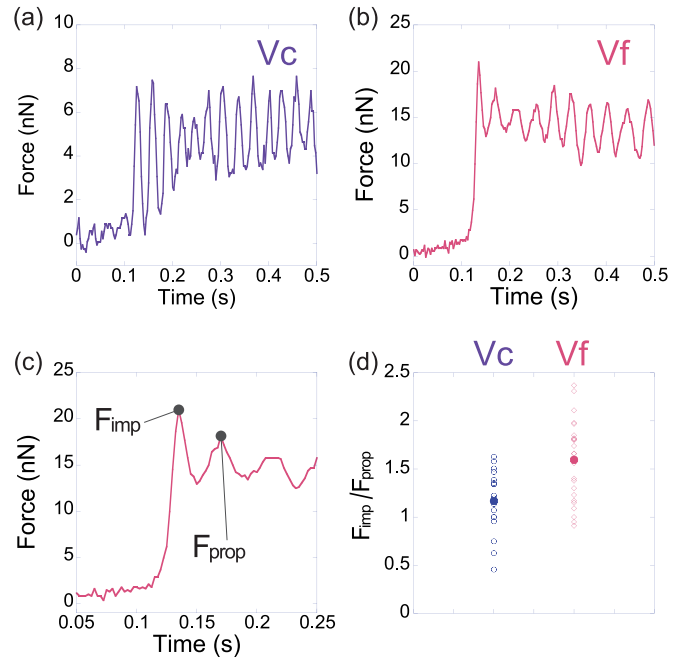


FIG. 4. Inertial impact force is a signature of Vf motility. Representative force traces showing the onset of collision for (a) Vc and (b) Vf. (c) Magnified view of the collision onset in panel (b). (d) The ratio $F_{\text{imp}}/F_{\text{prop}}$ is ~ 1 for Vc but significantly greater than unity for Vf.

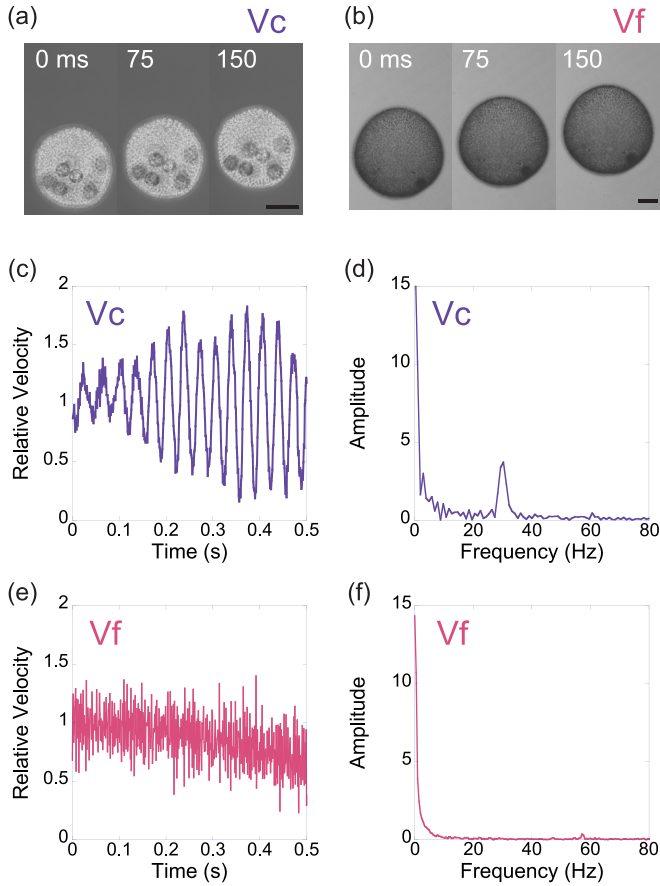


FIG. 5. Free-swimming kinematics show inertial damping of the force pulse. (a) Time lapse of V_c . (b) Time lapse of V_f . Scale bars, 100 μm . (c) The velocity of V_c oscillates strongly, corresponding to (d) a peak at ~ 30 Hz. (e) Velocity of V_f is nearly constant, and (f) the ~ 30 Hz peak is strongly attenuated.

in this force, which we identify as the mechanical signature of the collective ciliary action and hydrodynamic synchronization that generates metachronal waves [2]. The oscillation in the *total* organismal force is a direct consequence of these waves: Because a finite number of wavelengths fit on the spheroid's surface, the vector sum of the thousands of individual ciliary force vectors is not perfectly constant in time. The greater consistency in V_f is a result of biological scaling, averaging over a larger number of cilia (~ 5000 in V_f compared to ~ 2000 in V_c).

A key consequence of this pulsatile force is that an organism's swimming kinematics are not a reliable proxy for its underlying motor dynamics as it scales in size. Our results for V_c ($\text{Re} \approx 0.03$) are consistent with the low- Re principle where motion instantaneously follows force. However, our finding that V_f ($\text{Re} \approx 0.8$) possesses sufficient inertia to act as a mechanical low-pass filter represents a significant departure from this classic picture. This decoupling is explained by the competition between two timescales: the particle's viscous relaxation time, $\tau_r = m/(6\pi\eta r)$, and the period of the biological motor force, $\tau_f \approx 33$ ms. Here, m is the particle mass, η is the fluid viscosity, and r is the particle radius. Their ratio defines the Stokes number, $\text{St} = \tau_r/\tau_f$. For V_c , $\text{St} \approx 7 \times 10^{-5}$, while for V_f , $\text{St} \approx 1 \times 10^{-3}$. While both values are small, the Stokes

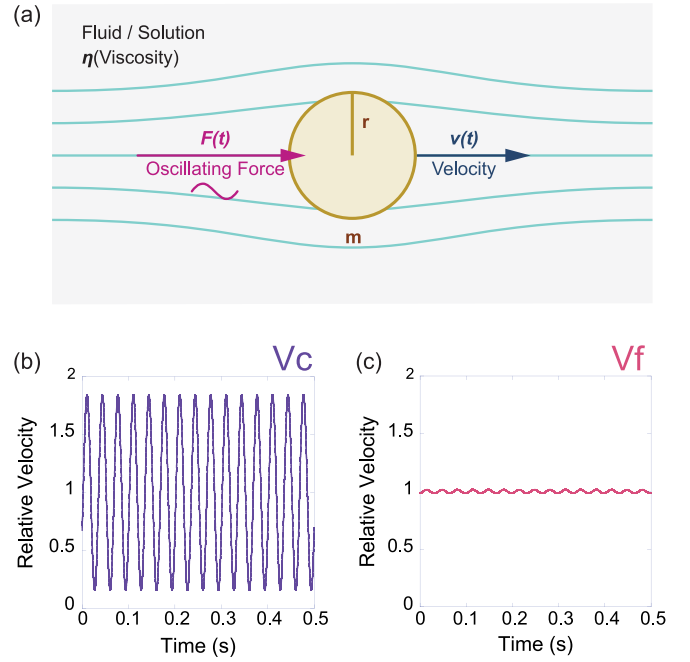


FIG. 6. Hydrodynamic simulation confirms inertial damping. (a) Schematic of the 1D model. Simulated velocity traces for (b) V_c and (c) V_f . The model correctly predicts significant velocity oscillations for V_c and a damped trajectory for V_f .

number for V_f is over an order of magnitude larger, sufficient to cause the phase lag and amplitude damping that constitutes the low-pass filtering effect. Future biophysical models of active matter must account for this decoupling when inferring motor activity from macroscopic behavior.

V. CONCLUSION

Our work establishes that beyond the strict Stokes regime, an organism's own mass can fundamentally decouple its internal motor dynamics from its outward swimming kinematics. Even though the fundamental biological motor (the 30 Hz ciliary beat) remains conserved across species, the physical manifestation of that motor output changes drastically due to organismal inertia. This provides a mechanical basis for understanding how physical constraints, such as inertia and fluid drag, act as selection pressures during the evolutionary transition to complex multicellularity in the volvocine lineage [8,9,15,16].

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DATA AVAILABILITY

The data that support the findings of this article are not publicly available upon publication because it is not techni-

cally feasible and/or the cost of preparing, depositing, and hosting the data would be prohibitive within the terms of this research project. The data are available from the authors upon reasonable request.

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- [1] J. Howard, *Mechanics of Motor Proteins and the Cytoskeleton* (Sinauer Associates, Sunderland, MA, 2001).
- [2] D. R. Brumley, K. Y. Wan, M. Polin, and R. E. Goldstein, Flagellar synchronization through direct hydrodynamic interactions, *eLife* **3**, e02750 (2014).
- [3] R. E. Goldstein, Green algae as model organisms for biological fluid dynamics, *Annu. Rev. Fluid Mech.* **47**, 343 (2015).
- [4] D. R. Brumley, M. Polin, T. J. Pedley, and R. E. Goldstein, Hydrodynamic synchronization and metachronal waves on the surface of the colonial alga *Volvox carteri*, *Phys. Rev. Lett.* **109**, 268102 (2012).
- [5] D. R. Brumley, M. Polin, T. J. Pedley, and R. E. Goldstein, Metachronal waves in the flagellar beating of *Volvox* and their hydrodynamic origin, *J. R. Soc. Interface* **12**, 20141358 (2015).
- [6] E. M. Purcell, Life at low Reynolds number, *Am. J. Phys.* **45**, 3 (1977).
- [7] E. Lauga and T. R. Powers, The hydrodynamics of swimming microorganisms, *Rep. Prog. Phys.* **72**, 096601 (2009).
- [8] J. G. Umen, *Volvox* and volvocine green algae, *EvoDevo* **11**, 13 (2020).
- [9] D. L. Kirk, A twelve-step program for evolving multicellularity and a division of labor, *BioEssays* **27**, 299 (2005).
- [10] R. D. Schulman, M. Backholm, W. S. Ryu, and K. Dalnoki-Veress, Dynamic force patterns of an undulatory microswimmer, *Phys. Rev. E* **89**, 050701(R) (2014).
- [11] T. J. Bøddeker, S. Karpitschka, C. T. Kreis, Q. Magdelaine, and O. Bäümchen, Dynamic force measurements on swimming *Chlamydomonas* cells using micropipette force sensors, *J. R. Soc. Interface* **17**, 20190580 (2020).
- [12] D. L. Kirk and M. M. Kirk, Protein synthetic patterns during the asexual life cycle of *Volvox carteri*, *Dev. Biol.* **96**, 493 (1983).
- [13] M. Krieg *et al.*, Atomic force microscopy-based mechanobiology, *Nat. Rev. Phys.* **1**, 41 (2019).
- [14] K. Drescher, K. C. Leptos, I. Tuval, T. Ishikawa, T. J. Pedley, and R. E. Goldstein, Dancing *Volvox*: Hydrodynamic bound states of swimming algae, *Phys. Rev. Lett.* **102**, 168101 (2009).
- [15] C. A. Solari, S. Ganguly, J. O. Kessler, R. E. Michod, and R. E. Goldstein, Multicellularity and the functional interdependence of motility and molecular transport, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 1353 (2006).
- [16] N. Ueki and K. Wakabayashi, Multicellularity and increasing Reynolds number impact on the evolutionary shift in flash-induced ciliary response in Volvocales, *BMC Ecol. Evol.* **24**, 119 (2024).
- Correction:* Errors in Refs. [10], [11], and [15] have been fixed.