The Relationship Between Kinematic Viscosity and the Swimming Speeds of *Paramecium aurelia*, *Tetrahymena* spp., and *Euglena* spp.

[redacted]

ABSTRACT

The effects of viscosity on the swimming speeds of three different protists, *Paramecium aurelia*, *Tetrahymena* spp., and *Euglena* spp., were observed and recorded. Swimming speed was modelled against viscosity using the power-law $s = k\nu^{-m}$, as suggested in literature. For *Tetrahymena* and *Euglena*, we found m to be 0.821 and 0.847 respectively. The data obtained from these two organisms fit the power-law models well, but with *Paramecium*, our proposed power-law model with m of 0.989 fits the data less closely. We suspect the presence of other factors unaccounted for in this study that significantly affected the swimming speed of *Paramecium*, although it is possible that the deviations from the model are simply due to statistical fluctuation.

INTRODUCTION

Viscosity is an intensive property that describes a fluid's resistance to flow when subjected to shear stress. In turn, shear is the component of net force parallel to the surface being analyzed (as opposed to the normal force, which is perpendicular to the surface).

Viscosity is affected by a number of properties that are susceptible to change in natural environments, such as temperature, concentration of solutes, and density of biological matter [1] [2], [3]. Thus, aquatic organisms are typically exposed to wide ranges of viscosities. Since viscosity is known to affect growth rates, swimming speeds, population density, and rates of food consumption [4], [5], [6], [7], an organism's ability to adequately navigate in and adapt to environments of varying viscosity is an important component of its fitness.

A fair amount of research has been conducted to determine the effect of viscosity on the swimming speeds of multicellular organisms such as fish [8], [9], [10], [11], copepods [12], and polychaete larvae [4]. However, there are relatively few studies detailing the effect of viscosity on microorganisms such as bacteria and protists [6]. At the scale of such small organisms, propulsion and motion are dissimilar. This is due to different forces dominating in each case. When an organism is large and able to move relatively quickly through water, the primary forces are those that the organism exerts on its environment – the inertial forces – and the organism can rely on its momentum to keep it moving. However, with microorganisms, inertial forces are negligible in comparison to viscous forces [13] – exerted on the organism by the environment – and different methods of propulsion must be used. The relationship between inertial and viscous forces for a moving body is quantified through the use of the Reynolds number (Re), given by

$$\mathrm{Re} = \frac{sl}{\nu}, \tag{1}$$

where s is the speed of the organism in the fluid in m/s*, l is its length in m, and ν is the kinematic viscosity of the fluid in m^2/s ($1\ m^2/s = 1{,}000{,}000$ cSt). Reynolds numbers are dimensionless, and indicate the ratio of inertial forces (given by sl in Eq. (1)), to viscous forces (given by ν).

The order of magnitude for Reynolds numbers for larger organisms such as fish typically ranges from 10^3 to 10^5 [14], whereas for microorganisms, Reynolds numbers are of order 10^{-2} [15]. Because dominant forces differ at high and low Reynolds numbers, results obtained from studies performed on fish cannot be easily applied to microorganisms. Previous studies show that while the speed of fish strictly decreases with increasing viscosity [8], [10], [11], the speed of bacteria does not respond in the same way; instead, it increases to a maximum before dropping off [16]. In this study, we add to the limited amount of research regarding the effects of viscosity on organisms at low Reynolds numbers by determining the relationship between viscosity and swimming speed of three freshwater protists: *Paramecium aurelia, Tetrahymena* spp., and *Euglena* spp.

Paramecium and *Tetrahymena* are ciliates that swim by means of hundreds of external cilia that beat synchronously, while *Euglena* are flagellates that propel themselves forward through the rotation of a single flagellum located at their anterior ends. Since many protists employ flagella or cilia for locomotion, selection of these model organisms provides us with some insight into the differential responses of protists to changes in

* In this case, s was chosen as the variable for speed instead of v to avoid confusion with the variable for kinematic viscosity, ν.

viscosity depending on their modes of locomotion. In addition to this, because *Paramecium* are approximately six times longer than *Tetrahymena* and *Euglena* (see Appendix B), this allowed us to test Larsen and Riisgård's [17] observation that viscosity has less of an impact on the swimming speeds of larger organisms. Based on results found in previous studies, we expected to find a power-law relationship between viscosity and swimming speed for all three organisms [5], [12], [18], [19], [20].

METHODS

A 0.900 wt% stock solution of methylcellulose (4000 cP at 2 wt%) was prepared, heat-treated at 55°C for 3 hours, then diluted with distilled water to produce solutions of different viscosities. Kinematic viscosities of resulting methylcellulose solutions were determined using a capillary tube viscometer (see Appendix A).

Paramecium aurelia, Tetrahymena spp., and *Euglena* spp. were cultured in nutrient broth (see Appendix D for broth composition) in a well-lit room at 25°C and maintained in these conditions for the duration of the experiment. *Paramecium* and *Tetrahymena* slides were created by pipetting 80μL of methylcellulose solution and 20 μL of organism in nutrient broth onto a microscope slide, then sealing it with a cover slip. Because organism density was lower for the *Euglena* culture, its volume needed to be increased in order to gather sufficient data. Thus, 60 μL of methylcellulose solution and 40 μL of *Euglena* in nutrient broth were placed onto a microscope slide, instead of 80 μL and 20 μL. *Euglena* were also treated at a smaller viscosity range (1 - 2.71 cSt) because preliminary observations showed irregular movement that was difficult to analyze when viscosity was increased past this point.

Prepared slides were placed on the compound microscope stage and left for 5 minutes to allow the organisms to acclimatize to the light from the microscope light source (see Error Analysis: Avoiding Reactions and Phototaxis). Videos of organism movement were then recorded at 40x magnification for *Paramecium* and 100x for *Tetrahymena* and *Euglena* with a Dino-Lite digital microscope at 15 frames per second. Logger Pro (v 3.8.6) was used to find the average speeds of organisms (see Appendix C). A total of 60 replicates over two trials were performed per dilution for each organism. Analysis was only completed on organisms that moved in relatively smooth, directional paths, or on organisms where intervals of smooth, directional movement could be analyzed (see Appendix C). Room temperature was monitored to ensure that it did not change significantly throughout the duration of each trial.

RESULTS AND OBSERVATIONS

Figure 1 shows the data obtained for each of the three organisms, averaged over two trials of 30 replicates each. Data were modeled using power-laws determined by linearizing each set of points with a log-log graph. While the data points for *Tetrahymena* and *Euglena* lie relatively close to the fitted model in the log-log graph, those of *Paramecium* appear to deviate more.

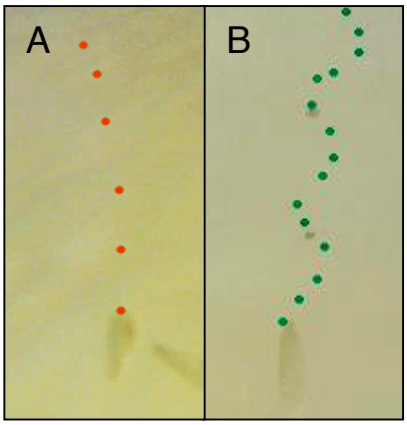

Figure 1. *Paramecium* demonstrating (A) smooth and directional (in 0% methylcellulose) and (B) sinusoidal (in 0.79% methylcellulose) path movement.

At low viscosities, organism movement was relatively linear (Figure 2a). However, at higher viscosities, non-linear path movement was much more pronounced, and all three types of organisms commonly travelled in sinusoidal paths (Figure 2b). This is consistent with descriptions of helical motion in ciliates and flagellates reported in previous studies [15], [21], [22], [23], as three-dimensional helical motion appears sinusoidal in two dimensions [21].

In a number of individuals in all three model organisms, precession (rotation of the axis of a spinning body) was observed. An example of this is shown in the time-lapse pictures in Figure 3. This is consistent with Jennings' descriptions of swimming patterns [23], [24] in microorganisms and is further explored below, in Error Analysis: Avoiding Reactions and Phototaxis.

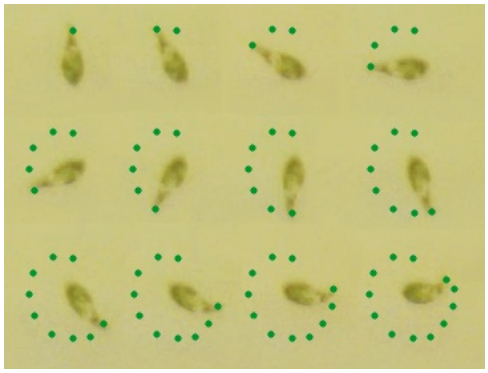

Figure 3. Time lapse of *Euglena* precession in 0% methylcellulose.

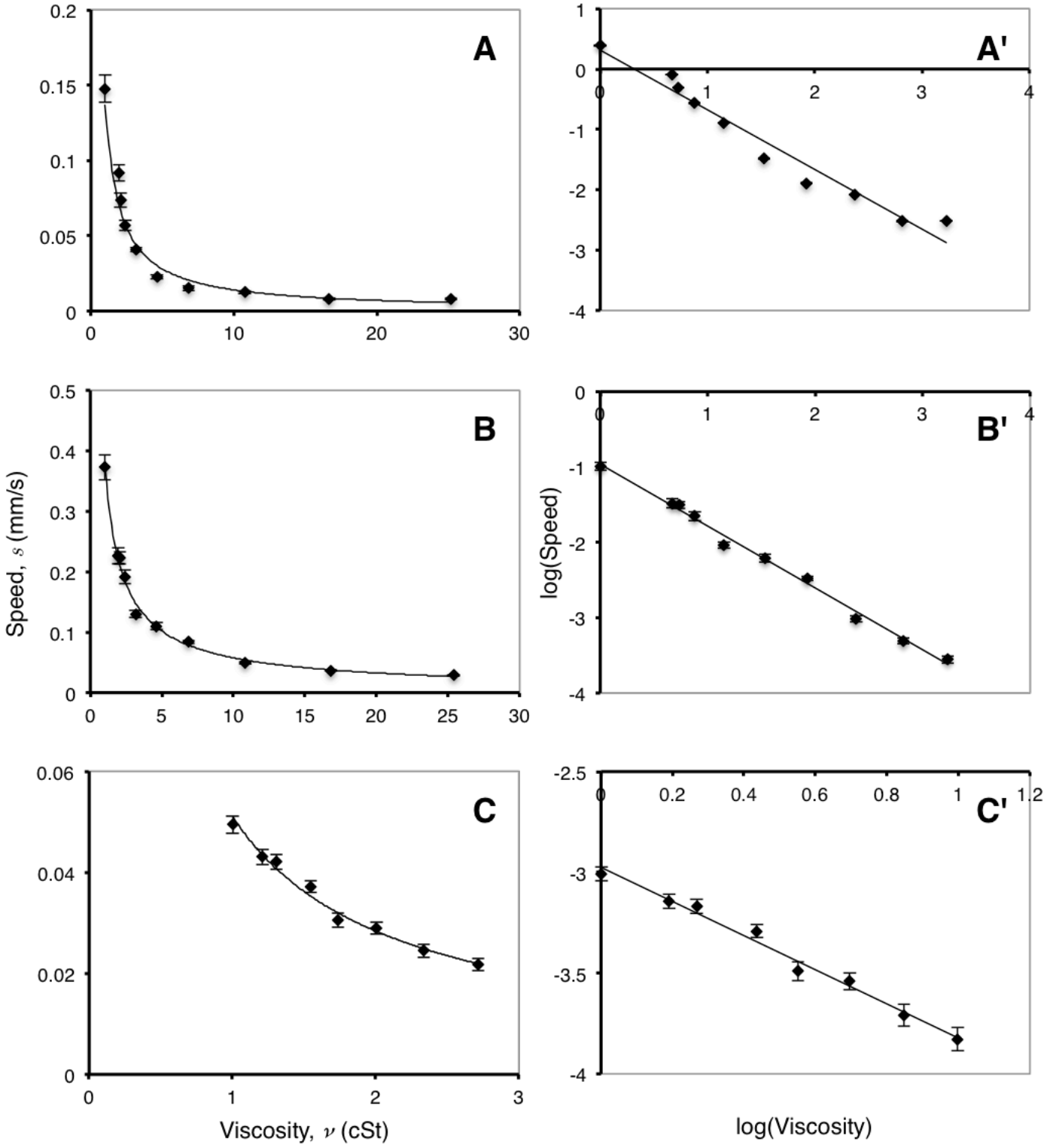

Figure 2. Power-law models for kinematic viscosity (ν) vs. swimming speed (s) in solutions of varying methylcellulose concentration for (A) *Paramecium aurelia*, $s = 1.370\nu^{-0.989}$, (R^2=0.960); (B) *Tetrahymena* spp., $s = 0.382\nu^{-0.821}$ (R^2=0.993); and (C) *Euglena* spp., $s = 0.0511\nu^{-0.847}$ (R^2=0.986). Error bars represent standard error of the mean (n = 60 for each data point).

DATA INTERPRETATION

We found that the data deviated much more from the model for *Paramecium* than for the other two test organisms. Qualitatively, we observe from Figure 1a that, excluding the first data point, the rest of the points appear to follow a curve other than the modelled power-law. Even when the first data point is treated as an outlier, no power-law seems to fit the data as well as expected. This suggests the presence of other significant factors that have an effect on *Paramecium* swimming speed.

It is worth noting that if the data for *Paramecium* are shifted downward slightly (by 0.00585 mm/s), the resulting power-law model fits the data much more closely (R^2 of 0.981 versus original R^2 of 0.959). Similar improvements, though to a much lesser extent, can be made with *Tetrahymena* and *Euglena*; however, shifted graphs are not included in this paper as they would seem to suggest an illogical non-zero swimming speed value for a horizontal asymptote. Nevertheless, this overall trend may suggest a key flaw in our experimental methods: when selecting organisms, we tended to choose only those individuals that exhibited movement. This practice may have introduced a positive bias to our data collection process, which is particularly noticeable in the *Paramecium*.

Although this is one hypothesis, there is also the possibility that it was solely by chance that the data for *Paramecium* resemble a different curve. In other words, the data should have followed the original model proposed, but it is simply by statistical fluctuation that is does not. More data on *Paramecium* and their swimming speeds in different viscosities should be recorded and analyzed in future studies, especially at lower viscosities. More information should be gathered to confirm or refute either hypothesis and to further study the possibility of some sort of curve besides a power-law governing the swimming speeds of the *Paramecium* with changing viscosity.

JUSTIFICATION OF A POWER-LAW

At low Reynolds numbers, drag is described by Stokes' law [12], [6],

$$F_D = C_D \varrho \nu s, \tag{2}$$

where C_D is a constant dependent on the shape of the object, ϱ is the solution density, ν is kinematic viscosity, and s is the speed of the object. For an object moving in fluid at constant speed, drag force (F_D) is equal and opposite to thrust force (F_T). We first assume that the organism's thrust force, F_T, is constant. In other words, we expect that the force exerted by the organism for propulsion is independent of its surroundings. Thus, at constant s and F_T, we expect $s = k\nu^{-1}$ through the simple manipulation shown below.

$$F_T = F_D = C_D \varrho \nu s \tag{3}$$

$$s = \frac{F_T}{C_D \varrho} \cdot \frac{1}{\nu} \tag{4}$$

$$s = k\nu^{-1} \tag{5}$$

However, experimental evidence shows that this is not the case: instead of exponent values of -1 for ν, we obtained values that were close, but not the same: -0.989, -0.821, and -0.847 for *Paramecium*, *Tetrahymena*,

and *Euglena* respectively. In addition, power-law models obtained by other similar studies report exponent values other than -1 [17]. This suggests that, contrary to our original assumption, F_T is not constant. In other words, the propulsive force of the organism is dependent on a property of its surroundings. In this study, we focused on the effects of viscosity. By considering that propulsive mechanisms at low Reynolds numbers should be more adapted to take advantage of viscous forces, it becomes easier to see how the effectiveness of cilia and flagella, and therefore the thrust force of the microorganism, might vary depending on the kinematic viscosity of the surrounding fluid. With this in mind, let us propose a model where drag is given by Eq. (2) but where an organism's thrust force is a power-law function of kinematic viscosity, given by

$$F_T = C_T \nu^n, \tag{6}$$

where C_T (which depends on size and shape) and n (which depends on ciliary beat frequency or flagellar rotation frequency) are organism-specific constants. The decision to model thrust force in this way is supported by suggestions in the literature that the frequencies of beating cilia and rotating flagella depend on a power of viscosity [17], [25]. At constant speeds when drag force is equal in magnitude to thrust force, we can equate Eq. (2) and Eq. (6), resulting in

$$C_D \varrho \nu s = C_T \nu^n, \tag{7}$$

$$s = \frac{C_T}{C_D \varrho} \times \frac{\nu^n}{\nu}, \tag{8}$$

$$s = k\nu^{n-1}, \tag{9}$$

where k is a constant that depends on fluid density and organism size and shape. If we let $m = 1 - n$, we obtain

$$s = k\nu^{-m}. \tag{10}$$

This relationship has been suggested through dimensional analysis [12] and has been discussed extensively in Larsen and Riisgård's [17] review of existing relationships of swimming speed to viscosity, where tabulated values of m range from 0.40 to 2.19.

COMPARISON OF VALUES OF m

Of our three model organisms, the largest Reynolds number applies to *Paramecium* and the smallest to *Euglena* (Appendix B, Table 3). If we assume that Larsen and Riisgård's observations hold true for *Paramecium*, then its higher Reynolds number should imply a lower effect of viscosity on swimming speed [17]. However, we find that the opposite is the case – *Paramecium* has the largest m (0.989, versus *Tetrahymena*, 0.821 and *Euglena,* 0.847; Figure 1) and has a speed that decreases the quickest with increasing viscosity, despite its size and Reynolds number. This suggests that the viscosity somehow affects the ability of the *Paramecium* to swim, counteracting the advantage given by its higher Reynolds number.

Thus, we find an exception to Larsen and Riisgård's suggestion that larger organisms are less affected by changes in viscosity. This exception may be due to the fact that some organisms are better adapted to increasing viscosities, as suggested in previous studies. For example, Gorski and Dodson [26] found that free-swimming *Daphnia pulex* sank much slower in response to a temperature-induced change in viscosity than predicted by Stokes' law, suggesting a behavioural adaptation that allowed the *Daphnia* to compensate for

changes in viscosity. In the same way, it is possible that *Tetrahymena* possess adaptations to changes in viscosity that *Paramecium* do not, even though they are both ciliary swimmers. More research is required to investigate what exactly is affected by changing viscosity that results in an unexpectedly higher value of *m* for *Paramecium*, although it is also possible that the range of Reynolds numbers dealt with in this experiment was simply too small to replicate Larsen and Riisgård's observations.

In comparing all three model organisms, the value of *m* for *Euglena* is greater than that of *Tetrahymena* but smaller than that of *Paramecium*. This does not provide us with any significant information about how ciliated and flagellated organisms might differ in their responses to changing viscosity with regard to swimming speed. A more comprehensive study is required, one which considers factors that arise from the inherent differences between cilia- and flagella-based motion such as fluid mechanics, frequency of ciliary beating and flagellar rotation, and interactions between individual cilia or flagella.

Error Analysis

Biological Variation

All of our test organisms were grown in nearly identical conditions, though some biological variation in size, shape, life stage, and mating type may have caused drag and thrust to vary between individuals. We minimized the influence of biological variation by averaging results obtained from 60 individuals per viscosity for each organism.

Avoiding Reactions and Phototaxis

As documented by Jennings, microorganisms can exhibit "avoiding reactions". Upon encountering negative stimuli [23], [24], the organism may back up, turn, and then proceed forward in a new direction. Through trial and error, the organism eventually distances itself from obtrusive environmental elements.[†]

Winet suggests that long-chain polymers such as methylcellulose align themselves in solution, appearing as long tubes to swimming microorganisms and mechanically inducing avoiding reactions [5]. This could explain the prevalence of stilted movement and precession (see Figure 2, Results) in many of our organisms at higher viscosities, instead of the smooth, directional movement that dominated at lower viscosities. Video recordings at higher concentrations included some organisms undergoing directionless rotation due to avoiding reactions instead of translational motion, and it became increasingly difficult to determine with any certainty whether the motion paths that we extracted and analyzed were accurate representations of the regular swimming patterns of these organisms at high viscosities, or of their avoiding reactions. Because of this, despite our attempts to remain consistent, it is possible that a number of the swimming speeds recorded at higher viscosities are less accurate.

Similarly, our results may have been impacted by the positive and negative phototaxic responses of *Euglena* and *Tetrahymena*, respectively [27]. In our study, we noticed that *Euglena* and *Tetrahymena* moved quite irregularly within a few minutes after putting the slide on the microscope. We reasoned that this was a phototaxic response exhibited due to the sudden increase in light intensity, but observed that the irregular movement ceased after several minutes of acclimatization. Because of this, we waited 5 minutes after each

[†] For a video of an example of this avoiding reaction, click here.

Tetrahymena and *Euglena* slide was placed on the scope before beginning data collection. However, it is still possible that phototaxic responses contributed to the error in our experiment. Current research indicates that with the exception of *Paramecium bursaria*, *Paramecium* spp. do not respond to light [28]; thus, it is unlikely that light had a significant impact on our results for this organism.

Errors in Video Analysis

Since we were unable to record movement in three dimensions, the speeds that we recorded are underestimates of the organisms' actual speeds. This unavoidable dimensionality caused some organisms to appear blurrier than others, which also affected the precision of our tracking method. Apparent movement caused by external vibrations may also have resulted in decreased accuracy in recorded organism swimming speeds.

Temperature

Although room temperature was monitored for the duration of the experiment, our experimental setup did not allow us to monitor the temperature of the liquid on the slides directly. Thus, it is possible that conditions on the slides deviated significantly from room temperature, undetected by us, throughout the video recording procedure (likely increasing due to the microscope light source). This may have in turn affected swimming speeds, either due to direct physiological effects of temperature or by the effects on viscosity by temperature. This provides us with a potential mechanistic explanation for the poor fit of *Paramecium* to the model in comparison to our other model organisms. Larsen, Madsen, and Riisgård [12] have suggested that larger organisms are more strongly affected by temperature-induced changes to viscosity than by changes due to the addition of solutes (such as methylcellulose). Thus, although temperature changes would have affected all three organisms, *Paramecium* – being the largest among them – may have been affected the most. This could account for its substantially greater deviation from the power-law model when compared to *Tetrahymena* and *Euglena*.

CONCLUSION

When we assume that organisms' thrust forces are not constant, but instead change with viscosity (as analyzed in the discussion), we can produce power-law models that effectively describe the relationship between swimming speed and kinematic viscosity of different microorganisms. Viscosity-speed data were obtained for *Paramecium aurelia*, *Tetrahymena* spp. and *Euglena* spp. and were fitted to power-law models with values of m of 0.989, 0.821, and 0.847, respectively. Further research may be required to investigate extraneous factors that may have caused data to deviate from the model, particularly with *Paramecium*.

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APPENDIX A: VISCOMETER CALIBRATION

There are two types of viscosity: absolute viscosity and kinematic viscosity. Absolute viscosity (μ) is the resistance of a liquid to shear stress, while kinematic viscosity (ν) is related to absolute viscosity by the equation

$$\nu = \frac{\mu}{\varrho}, \tag{11}$$

where ϱ is the density of the liquid [29].

In research, viscosity is commonly manipulated through the addition of solutes such as polyvinylpyrrolidone [16], [18], [12], Ficoll [4], and methylcellulose [16], [21]. Although methylcellulose solutions are non-Newtonian (meaning that viscosity is affected by the magnitude of shear stress), Winet [5] found in a similar study that this characteristic neither has a significant effect on the behaviour of the swimming speeds of microorganisms nor interferes with the fitting of a power-law to the data for the range of methylcellulose concentrations employed in this study. Consequently, despite its non-Newtonian nature, methylcellulose has been successfully used by many researchers in similar experiments [16], [21], [5], [19], [8] and was used in this study as well.

To determine the viscosity of our methylcellulose solutions, we constructed a capillary tube viscometer by marking off two horizontal lines near the top and bottom of a Pasteur pipet and fastening it to a metal stand. We then calibrated the viscometer by drawing various liquids with known viscosities up the pipet and allowing them to run through. Videos were recorded with a Canon Powershot SX150IS camera at 30 frames per second to determine the times taken for the meniscus of the liquid to fall from the top to the bottom mark.[‡] Results are shown in Table 1 below.

The kinematic viscosity of each liquid was plotted against the time it took to run through the viscometer. A liquid's viscosity and time taken are linearly related by the equation

$$\nu = kt, \tag{12}$$

where ν is the kinematic viscosity, k is the capillary viscometer constant (expressed in mm^2/s^2), and t is the time taken [33]. We used this model (Figure 5) to determine our viscometer's k-value to be 0.97 cSt/s. Using Eq. (12), we found the kinematic viscosities of our methylcellulose solutions, shown in Table 2.

Liquid (Temperature)	Kinematic viscosity (cSt)	Average time taken (s)
Acetone (25°C)	0.39	1.80
Water (20°C)	1.00	2.44
Seawater (25°C)	1.04	2.48
Skim Milk (21°C)	1.13	2.96
Isopropyl (25°C)	2.40	4.00
Canola oil (25°C)	57.00	64.91
85.5% glycerol (25°C)	74.85	80.55
91.7% glycerol (25°C)	185.55	170.30
95.2% glycerol (25°C)	314.52	271.10
98.0% glycerol (25°C)	502.88	465.63
100% glycerol (25°C)	718.42	706.51

Table 1. Liquids used for viscometer calibration, and average times taken to run through the viscometer. Viscosity values obtained from [30], [31], [32].

[‡] For videos of our viscometer in action, click here and here.

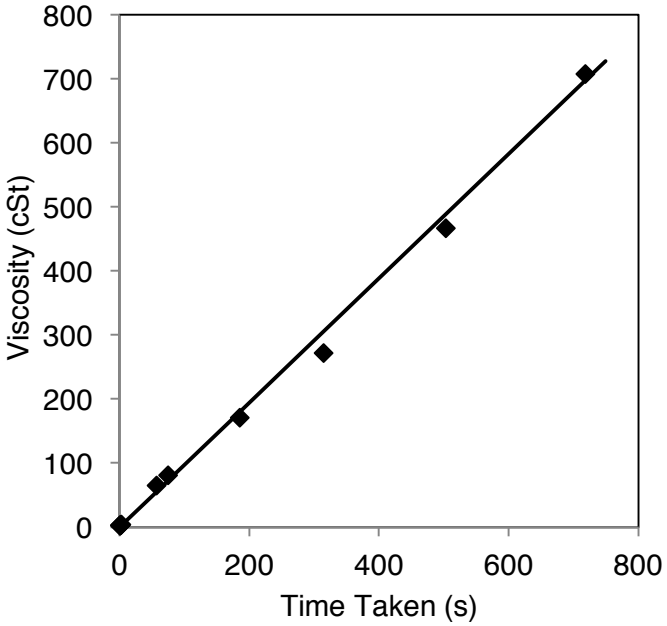

Figure 5. Kinematic viscosities and time taken for liquids to run through the viscometer, with $\nu = 0.97t$.

Methylcellulose (wt%)	Average time taken (s)	Kinematic viscosity (cSt)
0	1.03	1.00
0.0375	2.46	2.39
0.0750	2.67	2.59
0.113	3.28	3.18
0.169	3.84	3.73
0.225	4.82	4.67
0.281	6.29	6.10
0.338	8.46	8.21
0.450	15.6	15.1
0.594	33.2	32.2
0.747	71.4	69.3
0.900	151	147

Table 2. Methylcellulose solutions used, average times taken to run through the viscometer, and resulting calculated kinematic viscosities.

APPENDIX B: REYNOLDS NUMBERS

Reynolds numbers were calculated for each organism at 0% methylcellulose, using viscosities obtained from the viscometer (Appendix A) and organism lengths approximated by taking the average length of 10 individuals of each model organism in Logger Pro. Note the disparity between the Reynolds numbers for the larger *Paramecium* and the smaller *Tetrahymena* and *Euglena*, as well as between the ciliated and the flagellated organisms.

	Paramecium	*Tetrahymena*	*Euglena*
Kinematic viscosity (cSt)	1.00	1.01[§]	1.00
Speed (m/s)	1.48×10^{-3}	3.73×10^{-4}	4.94×10^{-5}
Length (m)	1.81×10^{-4}	2.94×10^{-5}	4.34×10^{-5}
Reynolds number	2.67×10^{-1}	1.10×10^{-2}	2.15×10^{-3}

Table 3. Calculated Reynolds numbers for *Paramecium*, *Tetrahymena*, and *Euglena* at 0% methylcellulose concentrations.

APPENDIX C: LOGGER PRO: DATA ANALYSIS PROCESS

Logger Pro (v. 3.8.6) was used to analyze videos of moving organisms. The field of view was 0.308 mm long for the *Paramecium* at 40x magnification and 0.0493 mm for *Tetrahymena* and *Euglena* at 100x magnification. In Logger Pro, videos were played back frame by frame and organisms were tracked throughout a time interval by marking the anterior end of the organism in each successive frame.[**] At high viscosities when movement was less pronounced, markers were placed more sparsely, with one or more frames in between. This was done to minimize error introduced by imprecise marker placement. Logger Pro then calculated the instantaneous *x*- and *y*-velocities of the organism by determining the distance and time differences between markers. It is important to note that some averaging of the data

[§] See Appendix D for the reasoning behind this slightly different kinematic viscosity.
[**]To view a video demonstration of this process, please click here.

was completed automatically by the program at this stage, since instantaneous velocities were calculated by taking the x- and y- positions of several markers into account at once. For this reason, organisms were tracked only for the periods in which they moved in relatively straight lines and where they were not observed to change orientation. This was to minimize underreporting of instantaneous velocities on curved paths where averaging would have significantly affected results. At higher viscosities, this was not as much of an issue in comparison to other confounding factors such as avoiding reactions. This was due to the fact that although organisms would occasionally move along curved paths, they also moved much slower, therefore allowing markers to be placed closer together and thus minimizing averaging effects.

Instantaneous *x*- and *y*-velocities were then copied to Microsoft Excel, where instantaneous speeds were found and averaged to find the organism's average speed across the time interval that it had been tracked. Each data point in Figure 1 represents the mean of the average speeds of sixty replicates for that viscosity.

APPENDIX D: NEW VISCOSITIES FROM NUTRIENT BROTH AND METHYLCELLULOSE MIXES

Shown below is the composition per liter of nutrient broth used to culture each organism.

Euglena

- 0.10 g KH_2PO_4
- 0.13 g K_2HPO_4
- 13 mg $FeCl_3$
- 0.30 g $MgSO_4 \cdot 7H_2O$
- 48 mg $CaCl_2$
- 0.10 g $Na_3C_6H_5O_7 \cdot 2H_2O$
- 0.30 g NH_4NO_3
- 4.0 mg H_3BO_3
- 4.0 mg $ZnSO_4 \cdot 7H_2O$
- 1.6 mg $MnSO_4 \cdot 4H_2O$
- 0.80 mg $COCl_2 \cdot 6H_2O$
- 0.16 mg $CuSO_4$
- 0.80 mg ammonium molybdate

Paramecium

- 25 g Cerophyl powder
- 7.6 g Na_2HPO_4
- 1.0 mL Stigmasterol

Klebsiella pneumoniae

Tetrahymena

- 2.5 g protease peptone
- 2.5 g yeast extract
- 5.5 g glucose
- 5.3 mg $FeCl_3$

Because a significant portion of nutrient broth was micropipetted onto each microscope slide along with the methylcellulose solution, the viscosities found for the methylcellulose dilutions in Appendix A are not representative of the actual viscosities experienced by the organisms. In order to determine the actual viscosities, we must use the Refutas equation, which allows us to factor in the viscosity of the nutrient broth. Use of this equation can be divided into three steps:

$$\mathrm{VBN} = 14.534 \times \ln[\ln(\nu + 0.8)] + 10.975\,, \tag{13}$$

$$\mathrm{VBN_{blend}} = [x_A \times \mathrm{VBN_A}] + [x_B \times \mathrm{VBN_B}] + \cdots + [x_N \times \mathrm{VBN_N}]\,, \tag{14}$$

$$\text{and } \nu_f = \exp\left(\exp\left(\frac{\mathrm{VBN_{blend}} - 10.975}{14.534}\right)\right) - 0.8\,, \tag{15}$$

where VBN is the Viscosity Blending Number of a liquid, calculated using ν, the liquid's kinematic viscosity. $\mathrm{VBN_{blend}}$ combines the VBNs of the liquids mixed by using the mass fraction of each component (x_N). The result is the final viscosity of the blended liquid (ν_f).

For the nutrient broth of *Euglena*, we observe that the majority of the additives are salts, so we approximate the broth as saltwater with a salinity of 0.99 ppt [34], and find its kinematic viscosity to be 1.00 cSt.

For *Paramecium*, nothing could be found regarding the viscosities of Cerophyl, Na_2HPO_4, or Stigmasterol. Therefore, we approximate the kinematic viscosity of the broth to be 1.00 cSt, equal to that of water.

Organism	Broth Component	Methylcellulose Component		Ratio of broth to methylcellulose components	Final kinematic viscosity of blended components (cSt)
	Kinematic viscosity (cSt)	Methylcellulose (wt%)	Kinematic viscosity (cSt)		
Euglena	1.00	0	1.00	4:6	1.00
		0.0375	1.38		1.21
		0.0750	1.59		1.31
		0.113	2.18		1.55
		0.169	2.73		1.74
		0.225	3.67		2.01
		0.281	5.10		2.33
		0.338	7.21		2.71
Paramecium	1.00	0.450	1.00	2:8	1.00
		0.0375	2.38		1.95
		0.0750	2.59		2.07
		0.113	3.18		2.40
		0.225	4.67		3.15
		0.338	8.21		4.61
		0.450	15.2		6.82
		0.594	32.2		10.8
		0.747	69.2		16.7
		0.900	147		25.2
Tetrahymena	1.01	0	1.00	2:8	1.00
		0.0375	2.38		1.96
		0.0750	2.59		2.08
		0.113	3.18		2.41
		0.225	4.67		3.17
		0.338	8.21		4.64
		0.450	15.2		6.86
		0.594	32.2		10.8
		0.747	69.2		16.8
		0.900	147		25.4

Table 4. Kinematic viscosities of blended methylcellulose and nutrient broth solutions as calculated using the Refutas equation.

Similarly, no viscosity information was available for the components of *Tetrahymena* nutrient broth with the exception of pure glucose, which has a kinematic viscosity of 880 cSt. If the remainder of the broth is approximated as water, the broth's kinematic viscosity can be calculated using the Refutas equation to be 1.01 cSt.

These values must then again be used in the Refutas equation to calculate the new viscosities of the solutions created on the microscope slides. Values are shown in Table 4.