

Mechanisms of temperature-dependent swimming: the importance of physics, physiology and body size in determining protist swimming speed

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SUMMARY

Body temperatures and thus physiological rates of poikilothermic organisms are determined by environmental temperature. The power an organism has available for swimming is largely dependent on physiological rates and thus body temperature. However, retarding forces such as drag are contingent on the temperature-dependent physical properties of water and on an organism's size. Consequently, the swimming ability of poikilotherms is highly temperature dependent. The importance of the temperature-dependent physical properties of water (e.g. viscosity) in determining swimming speed is poorly understood. Here we propose a semi-mechanistic model to describe how biological rates, size and the physics of the environment contribute to the temperature dependency of microbial swimming speed. Data on the swimming speed and size of a predatory protist and its protist prey were collected and used to test our model. Data were collected by manipulating both the temperature and the viscosity (independently of temperature) of the organism's environment. Protists were either cultured in their test environment (for several generations) or rapidly exposed to their test environment to assess their ability to adapt or acclimate to treatments. Both biological rates and the physics of the environment were predicted to and observed to contribute to the swimming speed of protists. Body size was not temperature dependent, and protists expressed some ability to acclimate to changes in either temperature or viscosity. Overall, using our parameter estimates and novel model, we are able to suggest that 30 to 40% (depending on species) of the response in swimming speed associated with a reduction in temperature from 20 to 5°C is due to viscosity. Because encounter rates between protist predators and their prey are determined by swimming speed, temperature- and viscosity-dependent swimming speeds are likely to result in temperature- and viscosity-dependent trophic interactions.

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Key words: *Colpidium striatum*, *Didinium nasutum*, temperature, viscosity, swimming speed.

INTRODUCTION

The ability to move is an essential component of an organism's fitness. An organism's speed (u) determines the rate at which it encounters objects within its environment, including food and predators. Encounter rates, when modeled as random walks, are proportional to u^2 (Visser and Thygesen, 2003; Visser and Kiørboe, 2006; Kiørboe, 2008). The swimming speed of an organism is the result of its power (P) available to generate thrust and retarding forces (F), resulting from drag. The resultant speed of an organism can be estimated *via* the classic equation $u=P/F$. For small, actively mobile aquatic organisms swimming can be costly. For example, cod (*Gadus morhua*) larvae can expend up to 80% of their standard metabolic rate on swimming (Ruzicka and Gallagher, 2006). Thus, any factor influencing an organism's ability to swim through its environment may have strong ecological implications.

The temperature of aquatic systems is highly variable on multiple scales, including temporal, e.g. changing climates (Schindler et al., 1996; Coats et al., 2006), seasonal changes (Balistreri et al., 2006) and diurnal changes (Morales-Baquero and Cruz-Pizarro, 1995), and spatial, e.g. thermal bars (Botte and Kay, 2000; Coats et al., 2006). Organisms may experience a change in water temperature over a long period, with the potential to adapt or acclimate (Wilson et al., 2000), or they may experience a rapid temperature change, with the risk for temperature-induced shock (Donaldson et al., 2008).

The magnitude of thrust an organism can generate is proportional to the amount of energy available for locomotion and the efficiency of the organism in converting this energy (*via* appendages) into mechanical propulsion. An organism's energy budget can be gauged by its metabolic rate (R), which is dependent upon both mass (Fenchel and Finlay, 1983; Gillooly et al., 2001; Brown et al., 2004; Savage et al., 2004; Makarieva et al., 2008) and temperature (Clarke, 1991; Savage et al., 2004; Apple et al., 2006). For poikilotherms, such as protists, internal body temperature closely tracks the temperature of their environment, thus metabolic rate is dependent on environmental temperature. The efficiency of ciliate protists in converting chemical energy into mechanical energy is low (Fenchel and Finlay, 1983). The proportion (ν) of protist energy budget used for propulsion is estimated to be less than 10%, but this is dependent upon taxa (Crawford, 1992). Thus here we consider the power available to a protist for swimming as proportional to its metabolic rate.

The magnitude of drag is governed by the physics of an organism's fluidic environment, density (ρ_f) and viscosity (μ), and the organism's morphology or length (l_c). The Reynolds number (Re) is used to determine the form of flow, and thus drag, acting upon an organism (Denny, 1993):

$$Re = \frac{\rho_f u l_c}{\mu} \quad (1)$$

Generally, Re values <1 describe viscous-dominated regimes, values between 1 and 450 describe intermediate regimes and values >450 describe inertia-dominated regimes (Fuiman and Batty, 1997; Borrell et al., 2005). For a small swimming organism, e.g. the ciliate protist *Didinium nasutum*, with $l_c \approx 1 \times 10^{-4}$ m, $u \approx 1.02 \times 10^{-3}$ m s $^{-1}$, $\mu \approx 1.01 \times 10^{-3}$ N s m $^{-2}$ (at 20°C) and $\rho_f \approx 1000$ kg m $^{-3}$ (at 20°C), $Re \approx 0.1$. Thus, protists operate in a viscous regime, where drag is linearly dependent on the viscosity of their environment (Denny, 1993; Vogel, 1994). The viscosity of water is temperature-dependent (Fig. 1); therefore, the drag on a protist is also predictably temperature-dependent.

For more complex organisms such as fish, adaption and/or acclimation effects of swimming speed to changing temperature can occur (Moran and Melani, 2001; Clarke, 1991). Fish propulsion is driven by muscular contractions controlled by a complex nervous system. Although fish muscle twitch rate has been shown to be temperature dependent (Batty and Blaxter, 1992; Wieser and Kaufmann, 1998), fish can adapt to changes in temperature by differential use of muscle types (Clarke, 1991). Nerve conduction is reduced by temperature, but this may be compensated *via* acclimation (Moran and Melani, 2001). Additionally, fish nervous systems can become 'shocked' *via* rapid changes in temperature (Clarke, 1991). Less studied is the impact of acclimation in determining the swimming speed of protists. We would not predict that protists acclimate to temperature in the same manner as fish. Protists are simpler unicellular organisms, with small cell volumes (thus internal temperature will closely track that of their environment), and they lack a complex nervous system. Protist propulsion occurs *via* cilia or flagella, the performance of which is directly related to cell temperature (Sleigh, 1956; Machmer, 1972; Riisgård and Larsen, 2007). However, protists inhabiting waters of differing temperature express a negative temperature-size trend (Atkinson et al., 2003). Temperature-dependent body size, $l(T)$, will affect the mass and therefore metabolic rate of protists. Additionally, $l(T)$ will also alter the length and thus drag (see below) of protists. Consequently, protists acclimated (cultured over multiple generations) to a particular temperature, e.g. 5°C, will have differing cell size, and thus predictably differing swimming speed, than protists that have been acclimated at 20°C and then rapidly exposed to 5°C. Thus acclimation effects may be present in protists *via* temperature-induced changes in body size.

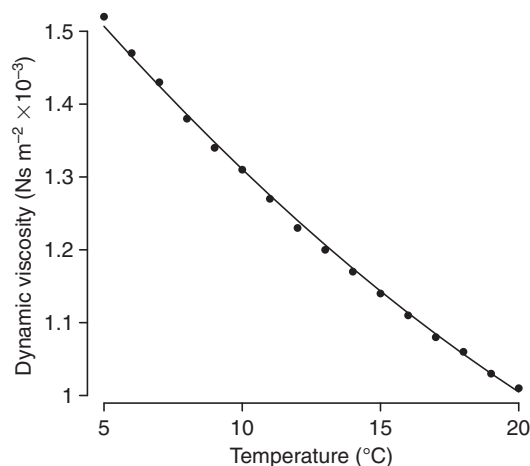


Fig. 1. The temperature dependency of viscosity. Individual data points are from an equation provided in Sengers and Watson (Sengers and Watson, 1986). Plotted line is the output of a statistically significant phenomenological model discussed in the text.

The combined impact of temperature on metabolic rates, physical properties of water and organism morphology can be predicted to result in measurable impacts of temperature on swimming speed. However, although the impact of temperature on swimming speed *via* physiological rates and processes is well documented, e.g. muscle twitch rate and nerve conduction as discussed above, the role of temperature dependent viscosity and morphology in determining temperature-dependent swimming speed is less well understood.

The temperature-dependency of swimming speed has been well recorded (e.g. Batty et al., 1991; Batty and Blaxter, 1992; Wilson, 2005; Riisgård and Larsen, 2009) and the relative importance of viscosity in determining fish swimming speed has been quantified (Von-Herbing and Keating, 2003). Fuiman and Batty observed an increase of 60% in fish (*Clupea harengus*) swimming speed when water temperature was increased from 6 to 13°C (Fuiman and Batty, 1997). These authors also manipulated the viscosity of water independently of temperature (by using a long-chain polymer, methylcellulose), showing that, for small fish, viscosity accounted for 54% of temperature-dependent swimming speed. Less well understood is the importance of viscosity in determining the swimming speed of microorganisms. Actively swimming protists, characterised by low Re , are likely to be highly susceptible to changes in viscosity. Many microorganisms use cilia or flagella to generate thrust, the beating (frequency and displacement) of which express a negative dependency on viscosity (Sleigh, 1956; Machmer, 1972; Riisgård and Larsen, 2007). Hagiwara et al. investigated the impact of changes in water viscosity on a rotifer (*Brachionus plicatilis*), and found that an increase in viscosity significantly reduced growth rates, population density, ingestion rate and swimming speeds (Hagiwara et al., 1998). Similarly, Winet observed a negative trend between water viscosity and the swimming speed of a ciliate protist (*Tetrahymena*) (Winet, 1976). Bacteria have been observed to have an optimum viscosity for swimming (Schneider and Doetsch, 1974).

Here we propose a model of the temperature dependency of swimming speed of a predatory protist, *Didinium nasutum* (Muller), and its protist prey, *Colpidium striatum* (Stokes). These particular species are the basis of ongoing investigations into the importance of temperature-dependent trophic interactions and environmental physics in determining predator-prey dynamics (Beveridge et al., 2010a; Beveridge et al., 2010b). The temperature-dependent mechanisms considered in the present study are metabolic rates, viscous drag and body size. From this model we generate several hypotheses. Empirical data are collected for each species from all possible combinations of three treatments: environmental temperature, environmental viscosity (manipulated independently of temperature) and whether protists are acclimated to the environment change. Experimental treatments are designed to segregate each of the above mechanisms that might lead to temperature-dependent swimming speed. Empirical data are used to fit and test the likelihood of model hypotheses in each treatment combination, thus highlighting which mechanisms significantly explain temperature-dependent protist swimming speed. Due to experimental constraints, we can only manipulate the impact of reducing temperature on protist swimming speed, as we can only experimentally increase the viscosity of a fluid independent of temperature, and not decrease it in an atoxic manner.

MATERIALS AND METHODS

Modelling the temperature dependency of protist swimming speed

To simulate the swimming speed of protists, we require a model for the drag and the power available for swimming protists. As protists operate in the viscous regime, drag (D) is modelled *via*

Stokes law (Denny, 1993), with protist morphology simplified to the nearest solid geometric shapes, *D. nasutum* to a sphere and *C. striatum* to a prolate ellipsoid:

$$D_{\text{sphere}} = 3\pi\mu l_s$$

$$D_{\text{ellipsoid}} = \mu \frac{2\pi l_1}{\ln\left(\frac{2l_1}{l_2}\right) - \frac{1}{2}} u, \quad (2)$$

where l_s is the diameter of a sphere, l_1 is the major axis of an ellipsoid and l_2 is the minor axis of an ellipsoid, where $l_1 > l_2$. We note that the equations we use for drag are for solid bodies. Although the drag on a ciliated body is likely to differ from that of a body with a solid surface (Vogel, 1994), this is still an unsolved problem, leaving us little choice but to simplify and note the fact.

Power is modelled as the fraction (y) of total organism metabolic rate (R), which is estimated and modelled as follows (Brown et al., 2004):

$$R = i_0 M^{3/4} e^{-E/kT}, \quad (3)$$

where i_0 is a normalisation constant, $M^{3/4}$ is the 'mass to the power 3/4' law, E is activation energy and k is the Boltzmann constant. This method allows us to model temperature-dependent metabolic rate via the Boltzmann factor ($-E/kT$), and mass dependency by the $M^{3/4}$ law. Although debate surrounds the $M^{3/4}$ law (e.g. Glazier, 2005), Fenchel and Finlay provide compelling evidence for its use with heterotrophic, free-living protozoa (Fenchel and Finlay, 1983).

To predict the impact of temperature on swimming speed, we considered three potential mechanisms for temperature dependency: (1) the temperature dependence of metabolic rate via the Boltzmann factor; (2) the temperature dependence of viscosity; and (3) the effect of temperature on protist size and thus length (impacting the flow of water around the organism) and mass (impacting metabolic rate). Thus, we obtain an expression for swimming speed as a function of temperature, in which temperature (T) appears four times. Substituting into $u=P/F$ Eqn 3 for P and Eqn 2 for F (rearranged so u is on the left), we arrive at our model:

$$u_{\text{Didinium}} = \frac{y(i_0 M(T)^{3/4} e^{-E/kT})}{\mu(T) 3\pi l_s(T)}$$

$$u_{\text{Colpidium}} = \frac{y(i_0 M(T)^{3/4} e^{-E/kT})}{\mu(T) \frac{2\pi l_1(T)}{\ln\left(\frac{2l_1(T)}{l_2(T)}\right) - \frac{1}{2}}}. \quad (4)$$

Pathways leading to temperature-dependent swimming speed in our model are depicted in Fig. 2.

Model simulations and hypotheses

Estimates for all parameters in Eqn 4 can be found in the literature – $y < 10\%$ (Crawford, 1992); $l(T)$ and $M(T)$ –2% body volume per 1°C increase (Atkinson et al., 2003); $E = 0.69$ eV and $i_0 = 0.001$ (Brown et al., 2004); and $\mu(T)$ (Sengers and Watson, 1986) – allowing us to generate *a priori* predictions with our model independent of our empirical data. Literature estimates of y , E and $l(T)/M(T)$ for our study organisms are varied, and so we estimated the temperature dependence of length (and thus mass) directly from observations of size at different temperatures. Additionally, as a simplification we modeled $\mu(T)$ via a quadratic model ($\mu = a + bT + cT^2$), fitted to and

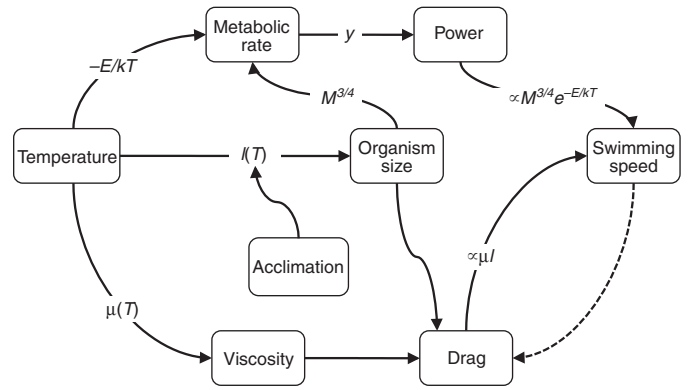


Fig. 2. Graphical depiction of the relationship between mechanisms predicted to result in temperature-dependent swimming speed as modelled in Eqn 4.

plotted against data in Fig. 1, which accurately describes $\mu(T)$ over the temperature range considered in this investigation (20 to 5°C; Table 1). We were unable to directly measure the temperature dependence of metabolic rate (E) and the proportion of metabolic rate utilised by swimming (y). We estimated E and y using a non-linear least squares method discussed in the following sections. Therefore, our model can be considered 'semi-mechanistic', as E and y are statistically derived via model fitting.

Our model (Eqn 4) was used to generate eight hypotheses from eight scenarios, based on all potential combinations of mechanisms that could result in temperature-dependent swimming speed (Table 2). Mechanisms in a scenario that were not modelled as a function of temperature were assigned their 20°C values (as our model simulates a reduction in temperature from 20 to 5°C). For example, scenario 4 includes the effect of temperature on viscosity but neglects the effects of temperature on metabolic rate and size. Fig. 3 illustrates the responses of *D. nasutum* and *C. striatum*, as predicted by all eight scenarios.

Table 1. Summary of the probability of significance of a linear model on the temperature dependency of viscosity

| Effect | d.f. | P | Coefficient |
|--------------------------|------|------------------------|-------------------------|
| Intercept | 1 | $< 2.0 \times 10^{-6}$ | 1.01×10^{-6} |
| Temperature | 1 | $< 2.0 \times 10^{-6}$ | -2.48×10^{-16} |
| Temperature ² | 1 | $< 2.0 \times 10^{-6}$ | 5.78×10^{-10} |
| Error | 23 | | |

Model structure and interpretation are detailed in the text.

Table 2. Summary of temperature-dependent functions included in different scenarios to generate model hypotheses

| Scenario | $\mu(T)$ | $R(T)$ | $l(T)$ |
|----------|----------|--------|--------|
| 1 | + | + | + |
| 2 | + | + | – |
| 3 | + | – | + |
| 4 | + | – | – |
| 5 | – | + | + |
| 6 | – | – | + |
| 7 | – | + | – |
| 8 (null) | – | – | – |

$\mu(T)$, temperature-dependent viscosity; $R(T)$, temperature-dependent metabolic rate; $l(T)$, temperature-dependent body size; +, included; –, not included.

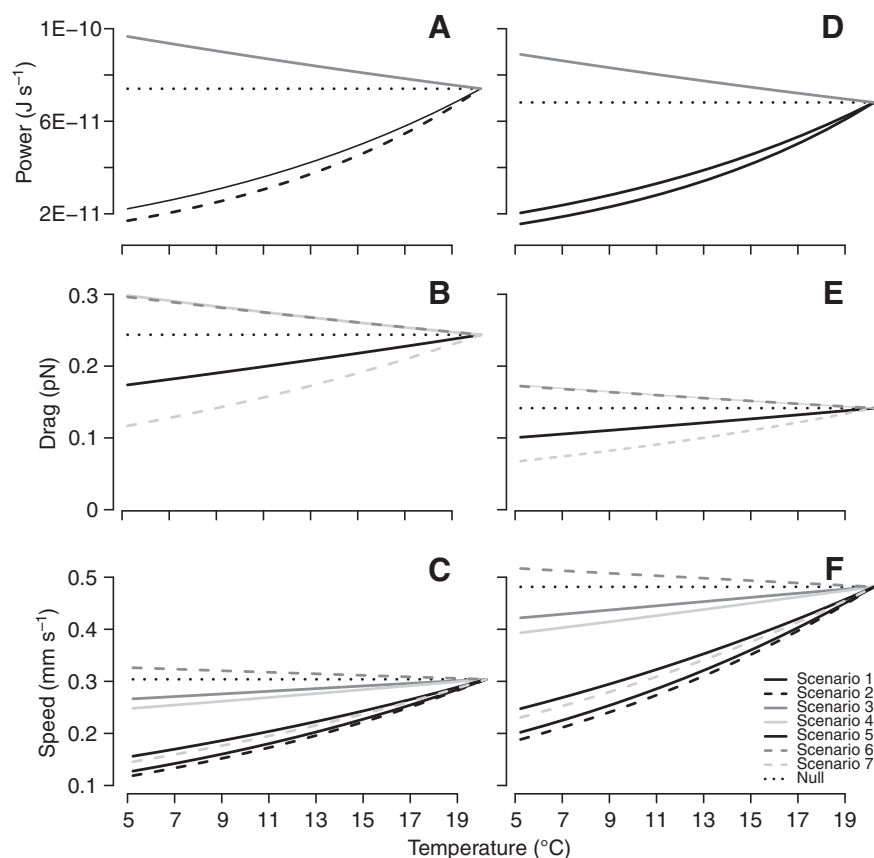


Fig. 3. Model (Eqn 4) predictions of protist temperature- and viscosity-dependent (A,D) power available for swimming, (B,E) drag and (C,F) swimming speed. Panels in the left-hand column are predictions for *Didinium nasutum* and panels in the right-hand column are predictions for *Colpidium striatum*. Predictions are based on seven scenarios plus a null hypothesis, summarised in Table 1 and discussed in the text.

Sampling and culturing

Protists were cultured in microcosms that consisted of a 200 ml glass jar, containing 100 ml of Chalkley's media (Tompkins et al., 1995), 0.55 g l⁻¹ dissolved protist pellet (Carolina Protozoa pelletsTM, Burlington, NC, USA), a foil lid, two wheat grains and a bacterial fauna (comprising species of *Bacillus* and *Serratia*).

We directly measured two response variables: cell size and swimming speed. Individual protists were pipetted onto standard microscope slides (equilibrated to treatment temperature) and filmed (Nikon Coolpix 4500, Tokyo, Japan) under a compound microscope (Nikon Optiphot-2, Tokyo, Japan, fitted with a cold light source) at 15 frames s⁻¹. Videos were analysed using open access software, ImageJ v1.4.3 (Rasband, 1997–2009) and a manual tracking plugin (MTtrack v1.2.0). To assay speed, 30 frames (2 s) of protist swimming were analysed per individual and total distance travelled was measured. It was not possible to track the trajectory of individual protists for more than 30 frames, as they would regularly swim out of the field of view. The size of protists was assayed in ImageJ by measuring the diameter of cells; for *C. striatum* (an approximate ellipsoid), the ratio between its major and minor axes was also determined.

Two environmental treatments were conducted: temperature of microcosms (four levels) and increased viscosity of the media independent of temperature (six levels). Temperature was manipulated in temperature-controlled rooms, maintained at 5, 10, 15 and 20°C (±1°C). In temperature treatments, environmental temperature and environmental viscosity (1×10⁻³ to 1.52×10⁻³ N s m⁻²) covaried naturally, allowing inference of E/kT and $\mu(T)$. In the viscosity treatment, environmental viscosity was manipulated while environmental temperature (and thus cell

temperature) was kept at 20°C, allowing inference of $\mu(T)$, whereas metabolic rate was assumed to remain constant. The most suitable compound for adjusting the viscosity of microcosm media independently of temperature is Ficoll[®] (Winet, 1976; Bolton and Havenhand, 1998; Abrusàn, 2004; Loiterton et al., 2004). Ficoll readily dissolves in water, exhibits Newtonian fluid properties in solution, requires small quantities to change viscosity (thus not impacting density) and is non-toxic. Microcosms at 20°C with Ficoll concentrations of 0, 0.5, 0.7, 1.5, 2 and 2.5% (by mass) produce a viscosity range of 1×10⁻³ to 1.52×10⁻³ N s m⁻², the same as for temperatures from 20 to 5°C. Viscosities were estimated from the mean of three measures using a Ubbelohde-type viscometer (calibrated Cannon C457, Cannon Instrument Company, State College, PA, USA). For ease of interpretation, treatments where viscosity was manipulated independently of temperature were converted into equivalent temperatures *via* parameter estimates in Table 1 (i.e. 2.5% Ficoll solution at 20°C, $\mu=1.52\times10^{-3}$ N s m⁻², equivalent to media at 5°C). Decreasing viscosity independently of temperature is not technically possible, thus E/kT cannot be manipulated independently of $\mu(T)$ and our data and model can only strictly predict swimming speeds responses for a reduction in temperature.

A third treatment, acclimation, had two levels: acclimated and non-acclimated. Acclimated protists were cultured in microcosms with environmental conditions as discussed above for 2 weeks (≈14 generations) and sampled in their cultured environment. Non-acclimated protists were cultured in standard media, with no Ficoll added, at 20°C for 2 weeks, then micropipetted into new media with a different environment (temperature or viscosity). Protists were allowed to equilibrate to their new

Table 3. Summary of treatment combinations used in the study to match, where possible, scenarios described in Table 2

| Species | Treatment combination | | | | <i>n</i> | <i>N</i> | Matched scenario |
|---------------------------|-----------------------|--------------|----------------------|--------------------|----------|----------|------------------|
| | Acclimated | Ficoll added | Temperature variable | Viscosity variable | | | |
| <i>Colpidium striatum</i> | + | – | + | + | 3 | 50 | 1 |
| | – | – | + | + | 3 | 50 | 2 |
| | + | + | – | + | 3 | 50 | 3 |
| | – | + | – | + | 3 | 50 | 4 |
| <i>Didinium nasutum</i> | + | – | + | + | 3 | 20 | 1 |
| | – | – | + | + | 3 | 20 | 2 |
| | + | + | – | + | 3 | 20 | 3 |
| | – | + | – | + | 3 | 20 | 4 |

Temperature could not be manipulated independent of viscosity, thus scenarios 5 to 8 could not be matched experimentally.

Ficoll added and temperature are the same (mirrored) variable, as the addition of Ficoll is the experimental manipulation to achieve a variable viscosity at a constant temperature.

n, number of unique source microcosms; *N*, number of individuals; +, included in treatment; –, not included in treatment.

environment for 10 min prior to sampling to ensure stabilisation of internal cell temperatures. The size of protists cultured in acclimated treatments will depend upon treatment temperature, whereas non-acclimated protists will have a size independent of treatment temperature, thus allowing quantification of $l(T)$ and an estimation of the importance of $l(T)$.

Replication occurred on two levels. First, three unique source microcosms were established for each treatment combination. This level of replication was required as protist population dynamics are variable and factors independent of temperature can affect swimming behaviour (Salt, 1979). The second level of replication was the number of unique protists filmed from each microcosm. A sample unit was created by taking a mean of the observations within each microcosm. The size of both protist species and the swimming speed of *D. nasutum* had an individual replication of 20. *Colpidium striatum* were more abundant on slides, with highly variable swimming speed, thus individual replication for *C. striatum* swimming speed was 50. Table 3 summarises all treatment combinations and their degree of replication.

Statistical analyses

Temperature- and viscosity-dependent protist size, $l(T)$, was estimated using a statistical linear model: $l = j + wT + qT^2$ [following protocol by Crawley (Crawley, 2007)]. Linear size dimensions were converted into mass by estimating cell volume from nearest geometric shapes (sphere, prolate ellipsoid) and assuming a protist cell density of 1000 kg m^{-3} , thus providing an estimate of $M(T)$ from $l(T)$.

In order to test among the different pathways by which temperature can affect swimming speed, we assessed which of the scenarios in Table 2 provided the best explanation of the empirical data. Each modelled scenario was fitted by the non-linear least squares (NLS) method (Crawley, 2007). The proportion of the metabolic rate required for swimming, y , and activation energy, E , were the fitted parameters for the NLS model. Each scenario of protist temperature-dependent swimming was fitted separately to each protist species, in each treatment combination. Akaike's information criterion (AIC) was used (following Bolker, 2008) to determine which scenario, and thus which hypothesis, best described the empirical data in each treatment for each species. (Note that information criteria provide an alternate approach to hypothesis testing.) All statistical analyses and model development were conducted using R (R Development Core Team, 2006).

RESULTS

Mechanistic model hypotheses

Fig. 3 illustrates all eight scenarios (Table 2) simulated by our model, using parameter estimates derived from the literature. Temperature effects on metabolic rate cause the most temperature dependency (78% of the response from 20 to 5°C) of protist swimming speed in our model (Fig. 3). When the temperature dependency of metabolic rate is removed (scenario 3) it causes the greatest deviation from scenario 1, which includes all possible temperature effects, compared to the removal of any other single mechanism.

Our model also suggests that the temperature dependency of viscosity is likely to explain a significant proportion, 16% of response from 20 to 5°C, of protist swimming speed [scenario 5, where $\mu(T)$ is absent, compared to scenario 1; Fig. 3]. Scenario 2, where size is not a function of temperature, differs little from scenario 1. This suggests that subtle changes (up to 10%) in cell dimensions contribute little (6%) to the temperature response of swimming speed. A reduction in size simultaneously decreases drag [via $l(T)$] but also reduces power [via $M(T)$]. This is confirmed by the shallow, negative slope between swimming speed and temperature modelled in scenario 6 where $l(T)$ is the only form of temperature dependency. Additionally, scenarios 5 and 7 and scenarios 3 and 4 differ little as differences are due to the inclusion or exclusion of $l(T)$.

Protist size

The cell length of both *C. striatum* and *D. nasutum* (Fig. 4) were independent of treatment, except for *C. striatum* when not acclimated to a change in viscosity. This relationship (temperature, $P_{1,117}=0.62$; temperature², $P_{1,117}=0.048$; Fig. 4H) was only marginally below the standard acceptance threshold of 0.05. Additionally, the gradient of the temperature response of *C. striatum* length was only $0.00054 \text{ mm } ^\circ\text{C}^{-1}$ (less than a 7% body length change from 20 to 5°C). Our model predictions (Fig. 3) clearly demonstrate that a change in body length of 10% would not be sufficient to cause a detectable change in swimming speed. Consequently both $M(T)$ and $l(T)$ were removed from Eqn 4 and replaced with mean estimates. The mean (\pm s.e.m.) diameter of *D. nasutum* was $0.11 \pm 0.01 \text{ mm}$, while the mean length of the major axis of *C. striatum* was $0.077 \pm 0.004 \text{ mm}$, with a major:minor axis ratio of 0.46 ± 0.016 . A full list of temperature dependent size models is provided in the supplementary material (supplementary material Table S1).

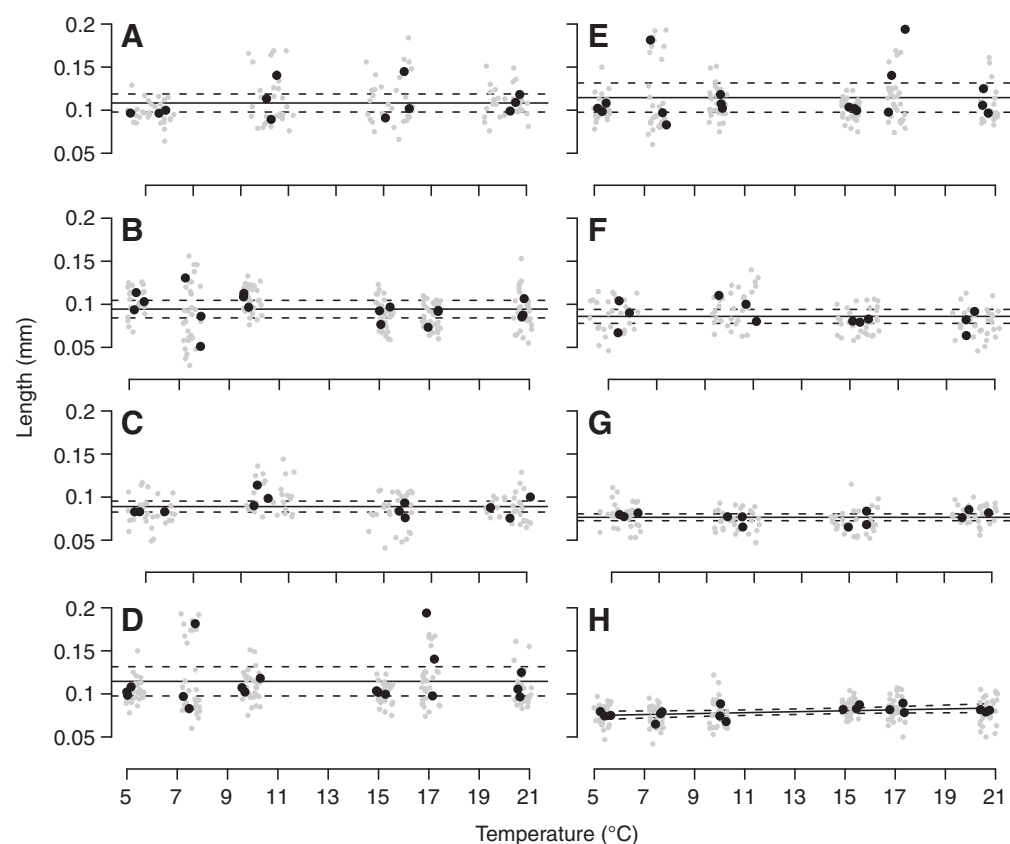


Fig. 4. Temperature (A,C,E,G) and viscosity, expressed as equivalent temperature, (B,D,F,H) dependency of *Didinium nasutum* (left-hand column) and *Colpidium striatum* (right-hand column) cell length. Panels A, B, E and F are from protists acclimated to their environment; panels C, D, G and H are from protists not acclimated to their environment. Grey circles represent individual observations; black circles represent mean microcosm estimates, upon which all statistics were conducted. Black lines represent mean across-treatment lengths ± 1 s.e.m. (dashed lines), except in panel H, where a significant temperature-length relationship was observed and fitted.

Measured swimming speeds

Swimming speed increased with temperature for both species of protists, in all treatments (Fig. 5). The overall mean swimming speed of *D. nasutum* ranged from 4.2×10^{-4} to $1.5 \times 10^{-3} \text{ mm s}^{-1}$. The overall mean swimming speed of *C. striatum* was slower and ranged from 1.6×10^{-4} to $5.7 \times 10^{-4} \text{ mm s}^{-1}$. Both species appeared to express a positive speed-temperature trend in all treatments, although within treatment variation was high for both species (grey circles, Fig. 5).

Model selection

Due to the lack of a significant $l(T)$ response, all model hypotheses where $l(T)$ was modelled were removed from the analysis (i.e. scenarios 1, 3, 5 and 6), reducing the total number of models tested from eight to four. Taking into account the lack of $l(T)$ response, the accepted minimum AIC model (MAICM) matched modelled scenario predictions with the exception of *D. nasutum* when acclimated to a change in temperature (Table 4). When *D. nasutum* was acclimated to a change in temperature, the MAICM did not contain a mechanism for $\mu(T)$, even though the viscosity of the environment in addition to temperature was varied. A full overview of each fitted model is provided in the supplementary material Table S2. Estimates of γ and E in MAICMs were relatively consistent within species and across treatments, again except for *D. nasutum* when comparing acclimated and not acclimated to a change in temperature (Table 4). Estimates of γ for *D. nasutum* ranged from 3.0×10^{-4} to 3.5×10^{-4} and E 0.19 to 0.69. Estimates of γ for *C. striatum* ranged from 8.2×10^{-5} to 3.4×10^{-4} and E 0.19 to 0.34.

DISCUSSION

Our experimentation and model show that both temperature-dependent viscous drag and temperature-dependent metabolic rates contribute to the temperature dependency of protist swimming speed.

We observed negligible temperature effects on protist size, and so acclimation effects *via* $l(T)$ were not observed. However, estimates of E for *D. nasutum* were affected by acclimation. Not accounting for the impact of metabolic and biophysical paths by which temperature can affect speed of movement will lead to inaccuracies in predicting the response of protist swimming speed to changing temperature.

We observed no significant trend (with the exception of *C. striatum* when not acclimated to a change in viscosity) between size and either temperature or viscosity over periods of up to 14 days. A non-significant trend for $l(T)$ for non-acclimated protists was predicted, as all protists were cultured in the same environment (20°C , $\mu \approx 1.01 \times 10^{-3} \text{ N s m}^{-2}$). However, a previous meta-analysis on the impact of temperature (over multiple generations) on protist size, including species of *Colpidium*, suggests a negative dependency of size on temperature (Atkinson et al., 2003). The conflicting findings presented here and in previous work (Atkinson et al., 2003) on temperature-dependent size in protists suggest the need for further research. Nevertheless, our model predictions (Fig. 2) suggest that moderate size changes will contribute little to temperature-dependent swimming speed when compared with the strong impact of $\mu(T)$ and $R(T)$. A decrease in protist size simultaneously reduces metabolic rate (Eqn 1) and viscous drag (Eqn 3), thus the resulting change in speed will depend on the magnitude of these two effects. A 10% reduction in *D. nasutum* diameter will reduce drag by 10%, mass by 27% and, consequently, metabolic rate by 21%, resulting in only a 6% change in swimming speed (Eqn 4).

When viscosity was manipulated (independently of temperature), so that $\mu(T)$ varied independently of $R(T)$, the MAICM for our data contained viscous drag as the only temperature-dependent mechanism (scenario 4). This was as predicted, as constant cell temperature will result in a metabolic rate independent of

Table 4. Summary of the minimum required temperature-dependent mechanisms to model (Eqn 4) protist temperature-dependent swimming speed

| Species | Treatment combination | | | Minimum required scenario | | | Model estimates | | |
|---------------------------|-----------------------|----------------------|--------------------|---------------------------|-------------------|----------------|-----------------|-------------------|------|
| | Acclimated | Temperature variable | Viscosity variable | Predicted scenario | Accepted scenario | Rejected | E | $y (\times 10^4)$ | AIC |
| <i>Colpidium striatum</i> | + | + | + | 1 | 7 | $l(T), \mu(T)$ | 0.68 | 3.54 | -170 |
| | + | - | + | 3 | 3 | None | n.a. | 3.0 | -260 |
| | - | + | + | 2 | 2 | None | 0.19 | 3.4 | -170 |
| | - | - | + | 4 | 4 | None | n.a. | 3 | -250 |
| <i>Didinium nasutum</i> | + | + | + | 1 | 2 | $l(T)$ | 0.38 | 0.98 | -190 |
| | + | - | + | 3 | 3 | None | n.a. | 0.92 | -310 |
| | - | + | + | 2 | 2 | None | 0.34 | 0.99 | -210 |
| | - | - | + | 4 | 4 | None | n.a. | 0.82 | -280 |

Scenarios from Table 2 were fitted to each treatment combination, with the most appropriate scenario accepted based on Akaike's information criterion (AIC). E , activation energy; $l(T)$, temperature-dependent body size; $\mu(T)$, temperature-dependent dynamic viscosity; n.a., not applicable; y , proportion of metabolic rate used for swimming; +, included in treatment; -, not included in treatment. A full table of all models fitted is available in the supplementary material (Table S2).

environmental conditions. Our finding of increased viscosity reducing swimming speeds is corroborated by work on other aquatic organisms: fish (Von-Herbing and Keating, 2003), rotifers, (Hagiwara et al., 1998; Larsen et al., 2008), sand dollar larvae (Podolsky and Emlet, 1993) and protists (Winet, 1976; Riisgård and Larsen, 2009). Our experimental design and model enabled us to go beyond testing for a phenomenological correlation between swimming speed and viscosity. Our data are in line with theory on the importance of viscous drag on small, slow-moving organisms (Eqn 3). When protists were exposed to a change in temperature, the MAICM contained both $\mu(T)$ and $R(T)$, except for non-acclimated *D. nasutum*. Δ AIC values between *D. nasutum* models including

and excluding $\mu(T)$ were low (Δ AIC=0.1; supplementary material TableS2). Bolker suggests that a Δ AIC value greater than four indicates significant differences between models (Bolker, 2008). Therefore, we provide strong evidence that Eqn 4, with the exclusion of $M(T)$ and $l(T)$ mechanisms can predict the swimming speed of *C. striatum* and *D. nasutum* at temperatures ranging from 20 to 5°C. The lack of a significant $l(T)$ response excluded our predicted mechanism for acclimation effects in protists. Consequently, observations and fitted models for *C. striatum* swimming speed were analogous between acclimated and non-acclimated treatments. However, for *D. nasutum* when acclimated to a change in temperature, the estimated value of E was higher (by a factor of 3.5) compared with *D. nasutum* when not acclimated to a change

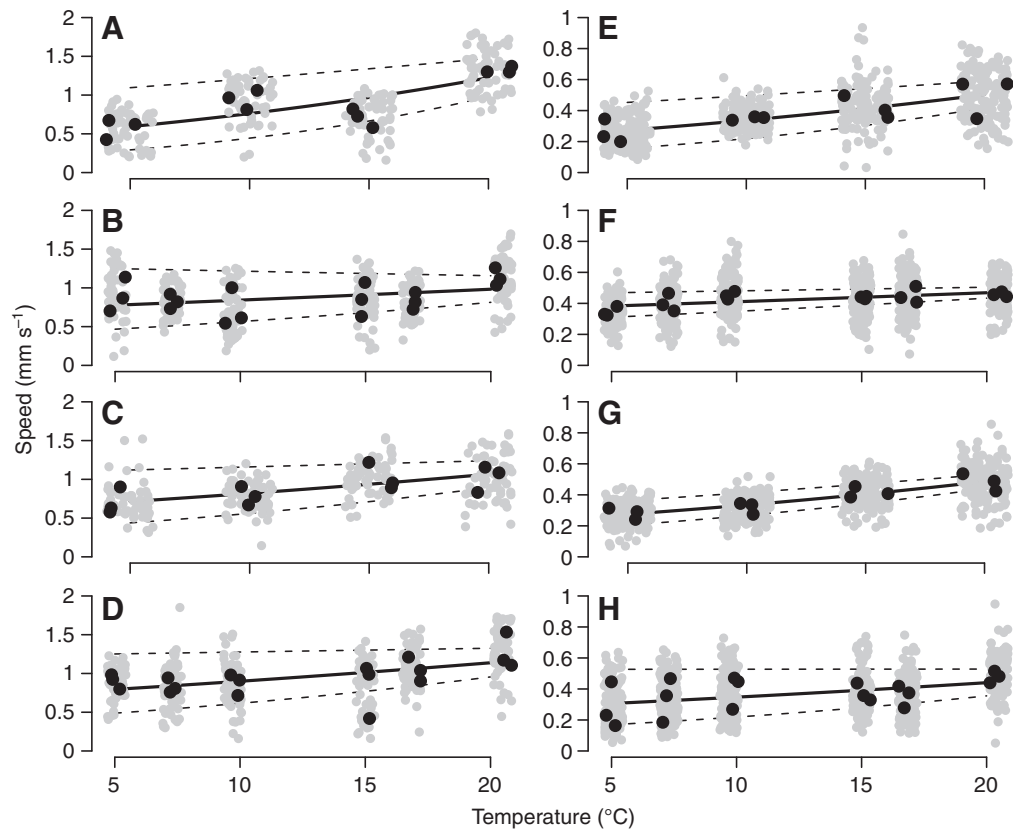


Fig. 5. Temperature (A,C,E,G) and viscosity, expressed as equivalent temperature, (B,D,F,H) dependency of *Didinium nasutum* (left-hand column) and *Colpidium striatum* (right-hand column) swimming speed. Panels A, B, E and F are from protists acclimated to their environment; panels C, D, G and H are from protists not acclimated to their environment. Grey circles represent individual observations; black circles represent mean microcosm estimates, upon which model fitting was conducted. Black lines are the most appropriate model predictions; dashed lines are the 95 and 5% confidence intervals of the y and E estimates. All models are discussed in the text. An artificial offset has been applied to the x-axis to aid visual interpretation of the model fit.

in temperature. Estimates of E for *D. nasutum* when acclimated and not acclimated to a change in viscosity were similar. This suggests that acclimation may have an effect on the metabolic rate/swimming speed power output of *D. nasutum*. A substantial effect of acclimation to temperature has been observed for other aquatic organisms [e.g. fish swimming (Clarke, 1991)]. The lack of viscosity acclimation is consistent with the feeding performance of polychaete larvae (which utilise cilia to generate a feeding current rather than thrust for swimming). A decrease in water temperature from 20 to 10°C resulted in a reduction in feeding rate, with up to 50% of this reduction attributed to changes in viscosity, irrespective of whether animals were acclimated to viscosity changes (Bolton and Havenhand, 2005).

The viscosity of water can naturally change independently of temperature *via* the release of mucous materials during phytoplankton blooms (e.g. Jenkinson, 1986; Seuront et al., 2006; Seuront and Vincent, 2008). For instance, during a spring bloom of *Phaeocystis globosa* in the English Channel, localised viscosity can be increased by up to 259% (Seuront et al., 2006). Although Eqn 4 was primarily developed to model the impact of temperature on swimming speed, at a constant temperature, Eqn 4 would predict: $u \propto \sqrt{1/\mu}$. Thus we can predict that a 259% increase in viscosity at a constant temperature will decrease swimming speed of small marine organisms by ~47%. Consequently, non-temperature-dependent viscosity as well as temperature-dependent viscosity is likely to be of high ecological importance. Additionally, our finding of $u \propto \sqrt{1/\mu}$ is comparable with the previously observed Arrhenius exponential relationship of swimming speed and viscosity (Larsen et al., 2008).

Our estimates of the proportion of metabolic energy required for protist swimming were consistent within species and across treatments ($\gamma = 3.0 \times 10^{-4}$ to 3.5×10^{-4} for *D. nasutum*; 8.2×10^{-5} to 3.4×10^{-4} for *C. striatum*). Although these estimates may appear low, they do not include the efficiency of cilia in converting biochemical energy into mechanical thrust. This efficiency has been estimated at ~1% (Fenchel and Finlay, 1983). Accounting for this efficiency, we can predict that 3 to 3.5% of *D. nasutum*'s and 0.82 to 3.4% of *C. striatum*'s total metabolic output is used to provide energy for swimming. These estimates are largely consistent with previous estimates of 0.1 to 0.7% (Fenchel and Finlay, 1983) and less than 10% (Crawford, 1992).

Using our model [Eqn 4, omitting $l(T)$] and parameter estimates (Table 4), we can deduce the relative importance of temperature-dependent metabolic rate and viscosity in determining the temperature response of *C. striatum* and *D. nasutum*. For a reduction in temperature from 20 to 5°C, viscosity accounted for ~40 and ~30% of the temperature response of *D. nasutum* and *C. striatum*, respectively. Our above estimates of the relative importance of viscosity in determining the temperature response of protist swimming speed are greater than our previous findings (of ~16%) based on parameter estimates from the literature. This is because, for both *C. striatum* and *D. nasutum*, our empirically derived estimates of E were lower than those in the literature, resulting in a smaller metabolic rate response and thus greater proportion of response attributable to viscosity-induced drag. Additionally, as viscosity is negatively exponentially temperature dependent and metabolic rate is positively exponentially temperature dependent, the relative importance of viscosity is greater at cooler temperatures. Our estimates of the relative importance of viscosity in determining the temperature dependency of swimming speed are similar to findings of the importance of viscosity for other taxa as highlighted in our introduction, e.g. 54% of fish larvae swimming response for a reduction in temperature from 13 to 6°C (Fuiman and Batty, 1997).

Here we have demonstrated that both the physics of the environment and an organism's temperature-dependent physiology can be used to predict the response of protist swimming speed to environmental changes. The temperature- and viscosity-induced swimming speed response reported here are likely to have ecological implications, predictably *via* predator-prey encounter rates. Future work on the impact of temperature changes on organisms, populations and communities should therefore consider the temperature dependency of both biological rates and the physical properties of the environment. Such implications need to be empirically substantiated if we are to fully understand the ecological consequences of environmentally induced behavioral responses.

LIST OF ABBREVIATIONS

| | |
|----------|--|
| AIC | Akaike's information criterion |
| E | activation energy |
| i_0 | metabolic rate at $T = -273^\circ\text{C}$ |
| k | Boltzmann constant |
| l | length |
| M | mass |
| MAICM | minimum Akaike's information criterion model |
| R | metabolic rate |
| Re | Reynolds number |
| T | temperature |
| u | speed |
| γ | proportion of metabolic rate used for swimming |
| μ | dynamic viscosity |
| ρ | density |

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