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Substantial energy expenditure for locomotion in ciliates verified by means of simultaneous measurement of oxygen consumption rate and swimming speed

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SUMMARY

In order to characterize the energy expenditure of *Paramecium*, we simultaneously measured the oxygen consumption rate, using an optic fluorescence oxygen sensor, and the swimming speed, which was evaluated by the optical slice method. The standard metabolic rate (SMR, the rate of energy consumption exclusively for physiological activities other than locomotion) was estimated to be 1.18×10^{-6} J h^{-1} cell^{-1} by extrapolating the oxygen consumption rate into one at zero swimming speed. It was about 30% of the total energy consumed by the cell swimming at a mean speed of 1 mm s^{-1}, indicating that a large amount of the metabolic energy (about 70% of the total) is consumed for propulsive activity only. The mechanical power liberated to the environment by swimming *Paramecium* was calculated on the basis of Stokes' law. This power, termed Stokes power, was 2.2×10^{-9} J h^{-1} cell^{-1}, indicating extremely low efficiency (0.078%) in the conversion of metabolic power to propulsion. Analysis of the cost of transport (COT, the energy expenditure for translocation per units of mass and distance) revealed that the efficiency of energy expenditure in swimming increases with speed rather than having an optimum value within a wide range of forced swimming, as is generally found in fish swimming. These characteristics of energy expenditure would be unique to microorganisms, including *Paramecium*, living in a viscous environment where large dissipation of the kinetic energy is inevitable due to the interaction with the surrounding water.

Key words: cost of transport, optic oxygen sensor, *Paramecium caudatum*.

INTRODUCTION

Paramecium inhabits an environment that is mechanically characterized by the dominant influence of viscous forces. In such a viscous environment, defined as low Reynolds number hydrodynamics, organisms move under conditions that greatly differ from those of our aerial environment, in which organisms move while being dominantly affected by the inertial force and the pressure of the surrounding fluid (Vogel, 1994). It is therefore not surprising that organisms perform a different mode of energy expenditure for locomotion in such a different mechanical environment.

As the viscous force dominates in the swimming of aquatic microorganisms, a large amount of energy should be dissipated through the interaction with the surrounding fluid. This means that the swimming of *Paramecium* is done with much lower efficiency than larger organisms, i.e. the ratio of efficient mechanical work to total energy expenditure for locomotion is low in *Paramecium*.

The total energy consumption of *Paramecium* was estimated on the basis of the oxygen consumption. Fenchel and Finlay summarized the data of the total oxygen consumption rate measured from the cells under various physiological conditions, such as growing or starved cells at different temperatures (Fenchel and Finlay, 1983). They obtained $0.19–4.4\times10^{-9}$ l O_2 h^{-1} for a single cell (*Paramecium caudatum*). These values can be converted to the power, $0.38–8.8\times10^{-5}$ J h^{-1} cell^{-1}, by the conventional transformation of liters of O_2 into 20.1 kJ (Schmidt-Nielsen, 1984).

Mechanical work done by swimming *Paramecium* can be estimated on the basis of Stokes' law. For a sphere with a diameter of 50 μm moving with a speed of 1 mm s^{-1}, which is one of the simplest models for *P. caudatum*, the power of swimming (the mechanical work done per unit time) has been calculated to be 3.4×10^{-9} J h^{-1}. This power calculated on the basis of Stokes' law, which is called Stokes power in this paper (*StP*), is only 0.004–0.09% of the total energy expenditure.

This very small percentage, however, does not correctly represent the efficiency of swimming of *Paramecium*. In order to estimate the efficiency, mechanical work should be compared with the energy used only for swimming. Little attention has been paid to the swimming behavior while measuring the oxygen consumption of microorganisms (Fenchel and Finlay, 1983; Scholander et al., 1952). We have therefore few data available in order to evaluate the amount of energy necessary for generating the locomotor activity of *Paramecium*. This is largely because it is difficult to simultaneously measure oxygen consumption and record the swimming behavior.

In this paper, we will present the energy expenditure of *Paramecium* in close relation to its swimming activity. For this purpose, paramecia were confined in a small volume of the chamber (<1 ml) and the oxygen consumption rate and the swimming speed were measured simultaneously from the same specimens. Oxygen consumption was measured by means of an optic fluorescence oxygen sensor (Okubo et al., 2008). Because this sensor has proved not to alter the amount of dissolved oxygen unlike oxygen electrodes, which consume a substantial amount of oxygen during the measurement procedure, it is ideal for measuring the oxygen concentration in a small volume of a sample. Swimming speed was

measured from the recording obtained by the optical slice method (Kato et al., 2003).

Our measurements revealed a linear relationship between the rate of the oxygen consumption and the speed of freely swimming *Paramecium*. By extrapolating from the regression line between oxygen consumption rate and swimming speed, we could estimate that the energy expenditure of the cell in the 'non-motile' state is about a quarter of the total energy consumed by the cell when swimming. This indicates that *Paramecium* uses a large amount (*ca*. 70%) of energy for swimming.

MATERIALS AND METHODS

Cells and culture

Paramecium caudatum Ehrenberg was cultivated at 23±1°C in hay infusion in Dryl's solution (2 mmol l^{-1} sodium citrate, 1.2 mmol l^{-1} Na_2HPO_4, 1.0 mmol l^{-1} NaH_2PO_4, 1.5 mmol l^{-1} $CaCl_2$, pH 7.2) (Mogami et al., 2001). We used cells at the early stationary phase of growth (18–22 days after incubation). Cells were collected by low-speed, hand-operated centrifugation (<170 ***g***) and were adapted to the experimental solution (KCM: 1.0 mmol l^{-1} KOH, 1.0 mmol l^{-1} $CaCl_2$, 0.25 or 1.0 mmol l^{-1} MOPS, pH 7.2 adjusted by HCl) for longer than 1 h. The K^+ concentration in the experimental solution was controlled by changing the amount of KOH added to the solution in order to achieve slower swimming by membrane depolarization (4 mmol l^{-1} K^+) (Machemer, 1989).

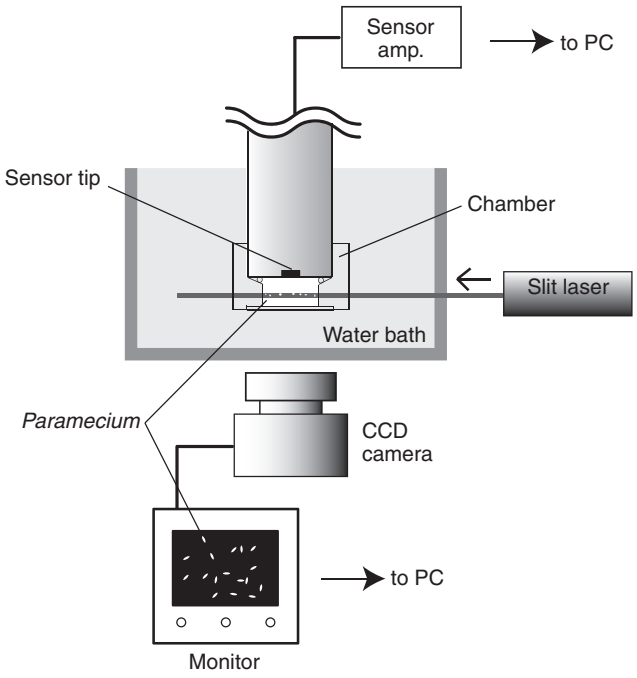

Fig. 1. Schematic drawing of a device for simultaneously measuring the oxygen consumption and the swimming performance of *Paramecium caudatum* in the same space. Oxygen concentration in the experimental chamber was recorded using an optic fluorescence oxygen sensor, and the swimming speed was measured by a computer-aided image analysis of the record obtained by the optical slice method, in which swimming cells were illuminated laterally by a slit laser, i.e. laser beam expanded as a slit of light perpendicular to the page, and the resultant dark-field images were fed to a computer for further analysis. In order to maintain the temperature during the measurement, the experimental chamber was placed in a water bath controlled at 23±1°C. Sensor amp., sensor amplifier with installed correction protocols for temperature.

Recording setup

We measured the oxygen consumption and the swimming speed of *Paramecium* simultaneously. As shown in Fig. 1, 0.74 ml of cell suspension with a density of 3.5×10^3–1.9×10^4 cells ml^{-1}, which is close to the density during the stationary phase, were transferred, without air bubbles, into the columnar space of the recording chamber. The chamber was made of Plexiglas with inner dimensions of 12 mm in diameter and 5 mm in depth. The bottom of the chamber was sealed with silicone grease by a coverslip, through which the swimming of the cells could be recorded. The oxygen concentration in the chamber was measured by an optic fluorescence oxygen sensor (FO-960, ASR, Tokyo, Japan). The principle of the sensor procedure is based on the quenching of fluorescence caused by collisions between molecular oxygen and fluorescent dye molecules in the excited state. This means that measurements can be done without any consumption of oxygen by the sensor itself. The sensor is therefore utilized especially for measuring oxygen consumption in a small volume. In our recording chamber, the sensor was placed at the top of the columnar space with its probe surface facing the specimen.

The swimming behavior of *Paramecium* in the columnar space was recorded using the optical slice method (Kato et al., 2003). The chamber was illuminated by a horizontal slit laser with a known beam thickness (half-maximum intensity width of 0.2 mm; SU-42C-635-10, Audio Technica, Tokyo, Japan), and dark-field images of cells that swam in the slit of light were recorded with a CCD camera (XC-77RR, SONY, Tokyo, Japan).

The recording chamber was placed in a water bath with circulating water of a constant temperature. Specimens in the chamber were illuminated by the slit laser placed outside the water bath (Fig. 1). All of the recording devices were further enclosed in a constant temperature box in order to avoid changes in the temperature of the small volume containing the specimen, which may result from contact with the larger body of the sensing device. Temperature throughout the experiment was 23±1°C.

Data processing

In each experiment, the partial pressure of oxygen (P_{O_2}, relative value to equilibrium with atmospheric air) was recorded every 30 s. Data were revised according to the temperature during measurements, following the built-in correction protocol. The rate of change in P_{O_2} ($\Delta P_{O_2}/\Delta t$) was calculated by numerical differentiation of the plot of P_{O_2} *vs* time (t) by the partial least-squares fitting. Thus, the oxygen consumption rate per cell ($\dot{V}_{O_2}$, in ml O_2 h^{-1}) was calculated as follows:

$$\dot{V}_{O_2} = SV_{ch}(\Delta P_{O_2} / \Delta t) / N, \quad (1)$$

where S is oxygen solubility (in ml O_2 ml^{-1}) at the temperature recorded, V_{ch} is the volume of the chamber (in ml) and N is the number of cells contained in the chamber.

To measure the swimming speed, dark-field images of swimming *Paramecium* were recorded by a video recorder (DV format) for about 40 s and fed into a computer. The positions of individual cells were determined by a laboratory-made, computer-assisted, tracking software (Bohboh, Bohboh Lab., Tokyo, Japan) (Shiba et al., 2002), and the mean speed was calculated from the changes in distance at specific time intervals. In each experiment, swimming trajectories lasting >0.67 s (or 20 frames) were picked randomly, and the mean speed was obtained from the measurement of 40 cells.

The probability of statistical significance (P) was determined using Student's t-test. The partial least-squares fitting was done using every seven data points obtained.

RESULTS

The $\dot{V}_{O_2}$ of the cells in the experimental chamber (Fig. 1) while being stirred continually, by a small magnetic bar put into the cell suspension, was not significantly different from that measured after stirring had stopped and *vice versa.* Stirring and post-stirring measurements were done 5–10 min after the stirring was 'on' and 'off', respectively. The ratio of the rate with stirring to that without stirring was 0.97±0.56 (±s.d.) and was not significantly different from 1.0 (N=8, P=0.87). This indicates that our procedure for measuring oxygen consumption using the sensor does not require any correction for heterogeneity of P_{O_2} in the chamber. Although the cells are focused on in order to record swimming some distance away from the sensor surface at the top of the columnar space, it seems safe to regard them as being representative in terms of the homogenous distribution of *Paramecium* cells usually found in a chamber of such small dimensions as used in Sawai et al. (Sawai et al., 2007).

Fig. 2A shows the time course of changes in P_{O_2} in the cell suspension. Time derivatives of the changes ($\Delta P_{O_2}/\Delta t$) were determined by the partial least-squares fitting, from which $\dot{V}_{O_2}$ was calculated using Eqn 1. Fig. 2B shows the time course of $\dot{V}_{O_2}$ thus obtained and the mean swimming speed (U) of cells simultaneously measured by the optical slice method. This gave a couple of pairs of $\dot{V}_{O_2}$ and U per each episode of replicated experiments.

The simultaneous measurement of $\dot{V}_{O_2}$ and U was also made at increased K^+ ion concentrations, aiming at reducing the swimming speed of cells. *Paramecium* has been known to reduce its swimming speed in response to the experimental depolarization of the membrane (Machemer, 1989). In the present study, we changed K^+ concentration from 1 mmol l^{-1} (standard solution) to 4 mmol l^{-1} for this purpose. In a depolarizing solution of 4 mmol l^{-1} K^+, cells swam slower as expected (0.76±0.16 mm s^{-1} N=14 in this solution compared with 0.99±0.26 mm s^{-1} N=54 in the standard solution, P<0.01). Fig. 3 shows plots of $\dot{V}_{O_2}$ vs U measured in solutions of different K^+ concentrations. These plots show a positive correlation (R=0.57, N=68) between increasing U and increasing $\dot{V}_{O_2}$. The linear regression of plots in the range of U=0.4–1.5 mm s^{-1} gave the following equation of $\dot{V}_{O_2}$ vs U (mm s^{-1}):

$$\dot{V}_{O_2} = 5.86 \times 10^{-8} + 1.42 \times 10^{-7}\, U. \quad (2)$$

Because the standard metabolic rate (SMR) is defined as the energy spent independent of locomotion (Hill et al., 2004), it can be estimated to be $\dot{V}_{O_2}$ at a zero swimming speed. The linear regression of $\dot{V}_{O_2}$ vs U gives us a primary approximation of SMR of a single *Paramecium* cell as 1.18×10^{-6} J h^{-1} by extrapolating $\dot{V}_{O_2}$ down to U=0 and equating 1 liter of oxygen with 20.1 kJ (Schmidt-Nielsen, 1984). The energy spent by *Paramecium* especially for swimming (swimming power, P_s) is obtained by subtracting SMR from the total energy expenditure converted from $\dot{V}_{O_2}$ using Eqn 2 at a given swimming speed; at its standard speed U_s=1 mm s^{-1} in our analysis, the calculated values of the total expenditure and P_s are 4.02×10^{-6} J h^{-1} and 2.84×10^{-6} J h^{-1}, respectively. When *Paramecium* swims at U_s and consumes oxygen at the rate expected from Eqn 2, SMR and P_s are therefore 29.3% and 70.7% of the total energy expenditure, respectively. This means that *Paramecium* uses a large part of its metabolic energy only for swimming.

DISCUSSION

We evaluated $\dot{V}_{O_2}$ only from measurements of cells up to 10^4 because of the limited resolution (signal-to-noise ratio) of the sensor. However, we did not find any significant correlation between the cell number and $\dot{V}_{O_2}$ in the range we tested (correlation coefficient=0.28, data not shown).

For statistical analysis of $\dot{V}_{O_2}$ vs U, there may be alternatives to Eqn 2, which we used for *Paramecium* and has the form:

$$\dot{V}_{O_2} = a + bU, \quad (3)$$

where a and b are least-squares parameters.

To assess the relationship with fish physiology, an empirical non-linear function of the following form was used with similar parameters of a and b:

$$\dot{V}_{O_2} = a + bU^c, \quad (4)$$

where the exponent c is generally about 2.5 for fish (Alexander, 2003). The non-linear least-squares fitting of Eqn 4 to the

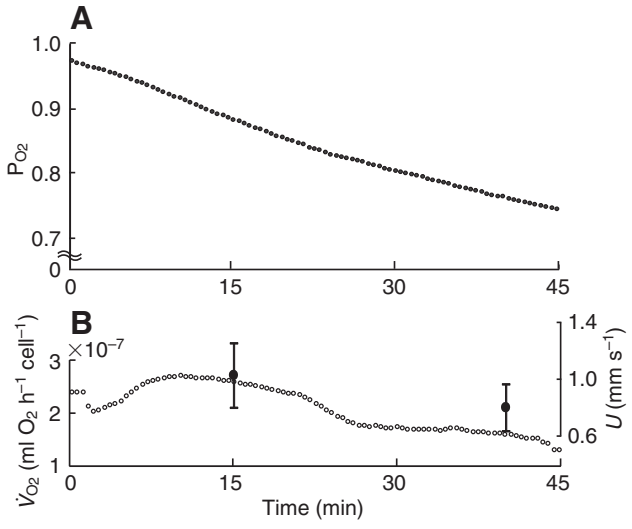

Fig. 2. Typical example of the oxygen consumption of swimming *Paramecium caudatum*. (A) Time course of the partial pressure of oxygen (P_{O_2}) in the experimental chamber containing 6.2×10^3 cells in the standard solution. (B) Time course of the oxygen consumption rate ($\dot{V}_{O_2}$) calculated by numerical differentiation of A (open circles) and swimming speed (U) (closed circles) measured by the optical slice method (mean±s.d.).

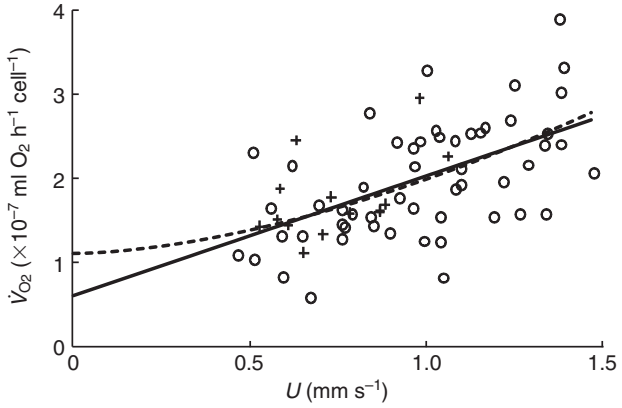

Fig. 3. Plots of the oxygen consumption rate ($\dot{V}_{O_2}$) against the swimming speed (U) of *Paramecium caudatum*. Open circles indicate data measured from cells in the standard solution (1 mmol l^{-1} K^+); crosses indicate data measured from cells in a higher K^+ solution (4 mmol l^{-1}). The correlation coefficient is 0.57 (N=68). The solid and broken lines show the regression lines obtained by the least-squares fitting of linear (Eqn 3) and non-linear (Eqn 4) functions, respectively.

Paramecium data presented in this study gave the following function:

$$\dot{V}_{O_2} = 1.09 \times 10^{-7} + 8.77 \times 10^{-8} U^{1.67}, \quad (5)$$

which is shown by a broken line in Fig. 3. Root mean squares of the fitting (a measure of goodness of fit) of the linear and non-linear functions were 5.45×10^{-8} and 5.48×10^{-8}, respectively, indicating that there is little difference in goodness of fit between the two functions. Indeed, both functions are indistinguishable for assessing the relationship between the rate of oxygen consumption and the swimming speed in the range of observations, although the intercept obtained on the basis of the non-linear function is about 54% larger than that obtained on the basis of the linear function (Fig. 3). However, it should be noted that the exponent c was calculated to be 1.67±2.66 (mean±95% confidence interval), which was not significantly different from the unity. For the simplification of the results, we took the resultant linear function (Eqn 2) as the primary approximation, in order to assess the relationship between the rate of oxygen consumption and the swimming speed of *P. caudatum*.

The power liberated by swimming *Paramecium* can be estimated to be the work per unit time, which is done against the viscous drag. As a ciliated microorganism swims through a fluid, energy is generally being dissipated by the viscosity in the fluid both inside and outside the cilia layer (Keller and Wu, 1977). While an estimate of the former is difficult as argued by these authors, the latter can be evaluated by hydrodynamics about a prolate spheroid moving and rotating in a viscous fluid. In low Reynolds number states, such as in the case of swimming *Paramecium*, energy dissipation due to translation (propulsion) can be calculated on the basis of Stokes' law. The power, termed 'Stokes power (*StP*)', at speed U is:

$$StP = D\eta U^2, \quad (6)$$

where η is the viscosity of the medium and D is the drag coefficient of the object. For a sphere of radius r, $D=6\pi r$. More realistically, *Paramecium* can be assumed to be a spheroid moving along its long axis. For a prolate spheroid with short rotating radius r_b and long axial radius r_a, D is calculated to be:

$$D = \frac{8\pi r_b}{\sqrt{\tau^2-1}\left(\frac{(\tau^2+1)}{2}\ln\left(\frac{\tau+1}{\tau-1}\right)-\tau\right)}, \quad (7)$$

where $\tau=r_a/(r_a^2-r_b^2)^{1/2}$ (Happel and Brenner, 1986). For *P. caudatum* with a mean size ($r_a=8.3\times10^{-5}$ m, $r_b=2.0\times10^{-5}$ m), swimming in water ($\eta=1.0\times10^{-3}$ Pa s) at U_s, *StP* is calculated to be 2.2×10^{-9} J h^{-1}. This value indicates that the efficiency of *Paramecium* swimming forward, i.e. the ratio of the power exerted to the environment to P_s, is only 0.078%. The rest of P_s is dissipated for rotation of the cell body (rolling and yawing, while yawing is significantly small compared with rolling in *Paramecium*) and inside the cilia layer as described above. Energy dissipation due to rolling (P_{roll}) of a prolate spheroid, i.e. rotation about its major axis, can be estimated by:

$$P_{roll} = 8\pi r_a r_b^2 C_M \eta \omega^2, \quad (8)$$

where C_M is the moment coefficient and ω is the rate of rolling (Chang and Wu, 1974). C_M is a function of the ratio of minor to major axis r_b/r_a and has been given by a numerical expression [equations 40b and 45 in Chang and Wu (Chang and Wu, 1974)]. For the rate of rolling $\omega=1$ rps$=2\pi$ s^{-1}, using the same values for r_a, r_b and η as described above, and $C_M=0.72$ at $r_b/r_a=0.24$, P_{roll} is calculated to be 8.5×10^{-11} J h^{-1}. This value is 3.9% of *StP*. Because P_{roll} thus evaluated is substantially small compared with *StP*, the rest of P_s is mostly dissipated inside the cilia layer. Studies investigating the swimming of larger-scale animals, however, have reported higher efficiency; about 10% and 5%, for a rainbow trout (*Salmo gairdneri*) swimming with undulating body and/or fins (Webb, 1971) and a squid (*Illes illecebrosus*) with jet propulsion (Webber and O'dor, 1986), respectively. These facts demonstrate that the energy cost of swimming in *Paramecium* is about two-orders of magnitude lower than in these larger animals.

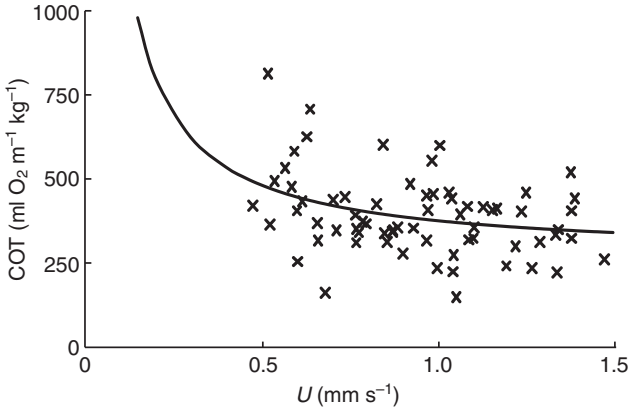

Fig. 4. Cost of transport (COT) of *Paramecium caudatum* as a function of the swimming speed (U). COT was calculated according to Eqn 9 using the mass of a single cell, $M=1.51\times10^{-10}$ kg [calculated by treating *P. caudatum* as a spheriodal body with a long rotating radius (r_a) and a short rotating radius (r_b) of 8.3×10^{-5} and 2.0×10^{-5} m, respectively]. The curve demonstrates a hyperbolic relationship of COT *vs* U derived from the linear relationship between $\dot{V}_{O_2}$ and U (Eqn 2).

Paramecium gains propulsive thrust from beating cilia, by which metabolic energy is converted to the mechanical work. Gueron and Levit-Gurevich computed the mechanical thrust of *Paramecium* cilia beating in metachronal coordination with neighboring cilia (Gueron and Levit-Gurevich, 1999). If we take 2×10^{-16} J to be the mechanical work per cilium per beat [cf. fig. 2 in Gueron and Levit-Gurevich (Gueron and Levit-Gurevich, 1999)], the total power of beating cilia on the entire surface of a single cell (10^4 cilia beating at 40 Hz) can be estimated to be 2.9×10^{-7} J h^{-1}. This means that the conversion of the mechanical power of ciliary beating to propulsive power (*StP*) may be far less efficient (0.77%) than that of the conversion of the P_s to the mechanical power of ciliary beating (10.1%). Remarkably low efficiency of energy expenditure is one of the characteristics of *Paramecium* swimming in the mechanical environment governed by a viscous drag, where large dissipation of the kinetic energy is inevitable in the interaction with surrounding water.

The efficiency of animal locomotion has also been discussed in terms of the cost of transport (COT, in ml O_2 m^{-1} kg^{-1}), which is defined as energy consumption for the translocation of the unit weight of an animal per unit of distance, and is formulated as follows:

$$\mathrm{COT} = \dot{V}_{O_2} / MU, \quad (9)$$

where M is the cellular mass for *Paramecium*. In this study, M was obtained based on the assumption that a spheroidal cell body has the mean density of 1.04 g cm^{-3} (Ooya et al., 1992). Fig. 4 shows COT as a function of U. As shown by the line in Fig. 4, a linear assessment of the relationship of $\dot{V}_{O_2}$ *vs* U (Eqn 2) gives a hyperbolic curve, indicating a monotonic decrease in COT with an increase in U. In the case of the swimming of fish, by contrast, COT curves

have a J-shape with a minimum COT within the range of the ordinary U (Claireaux et al., 2006). The J-shaped curve of COT in fish is possibly derived from the highly non-linear relationship of $\dot{V}_{O_2}$ *vs* U, which is represented by the exponent c in Eqn 4 significantly greater than the unity. The minimum COT corresponds to the optimum efficiency of locomotion (Videler, 1993). If we take non-unity c (1.67) into the calculation of COT in *Paramecium* swimming obtained from the least-squares fitting of Eqn 4, the minimum COT would be found at 1.45 mm s^{-1}, which is nearly the maximum speed. It is, therefore, argued that the efficiency of energy expenditure of swimming in *Paramecium* increases with speed. This is the second characteristic of *Paramecium* swimming, which we discovered for the first time from experiments carried out in this study, which have enabled us to measure oxygen consumption and swimming speed simultaneously.

The fact that *Paramecium* consumes a large amount of metabolic energy for swimming with extremely low efficiency suggests that it requires large changes in producing this metabolic energy when changing the propulsive thrust in response to external stimuli. In fact, Eqn 2 states that changes in U by 10% from U_s would change $\dot{V}_{O_2}$ by 7.1% of the total amount at U_s. It is, therefore, plausible that a subtle increase in the propulsive thrust might induce substantial effects on the other energy-requiring processes, such as cell proliferation, by reducing the energy supply to these processes in response to the increased demand for metabolic energy of locomotion.

Kato et al. hypothesized a close coupling of gravity-dependent changes in proliferation activity to gravikinesis (gravity-dependent modulation of the swimming speed) of *Paramecium* (Kato et al., 2003). *Paramecium* has been known to proliferate faster in microgravity (Planel et al., 1981; Richoilley et al., 1986) whereas it proliferates slower in hypergravity (Tixador et al., 1984; Planel et al., 1990; Richoilley et al., 1993; Kato et al., 2003). *Paramecium* also modulates its propulsive thrust depending on the swimming direction with respect to gravity; it increases the thrust when swimming upwards and decreases it when swimming downwards (Machemer et al., 1991; Ooya et al., 1992). Kato et al. considered how the energy supply to the proliferation activity would change in parallel with changes in the energy demand for modulating the thrust (Kato et al., 2003). They thought that a rapid increase in the demand for locomotion would complementally result in a decrease in the energy supply to proliferation and *vice versa*, as both proliferation and locomotion share a common metabolic resource within a cell. This hypothesis of what is essentially a counterbalance between proliferation and locomotion requires a substantial amount of energy change upon modulating the thrust either in microgravity or in hypergravity. The facts presented in the present study will make this requirement highly realistic.

SMR has not been, so far, distinguished from the total metabolic energy in unicellular organisms. It seems to be because of the far smaller amount of swimming power estimated by the theory of fluid dynamics than the total metabolic energy (Fenchel and Finlay, 1983). The estimation of mechanical work should have been done taking account of a very low efficiency of energy conversion due to a large dissipation of energy through the interaction with the surrounding fluid. In this study we empirically estimated the swimming power in *Paramecium*, and demonstrated that a large amount of energy is consumed for swimming and, as a result, only part of the total metabolic energy could be regarded as SMR. It is therefore suggested that the definition of SMR should be reconsidered in light of the energetics of microorganisms as found in the present study. This will cause us to re-examine energetic relations, such as allometric metabolism–mass relations, in unicellular organisms, which has long been discussed on a similar basis to that established in large animals.

LIST OF ABBREVIATIONS

a	least-squares parameter
b	least-squares parameter
c	exponent
C_M	moment coefficient
COT	cost of transport
D	drag coefficient
M	cellular mass for *Paramecium*
N	number of cells
P_{O_2}	partial pressure of oxygen
P_{roll}	energy dissipation due to rolling
P_s	swimming power
r	radius of sphere
r_a	long rotating radius
r_b	short rotating radius
S	oxygen solubility
SMR	standard metabolic rate
StP	Stokes' power
t	time
U	swimming speed
U_s	standard swimming speed
V_{ch}	volume in the chamber
$\dot{V}_{O_2}$	rate of oxygen uptake
η	viscosity of medium
ω	rate of rolling

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