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RESEARCH ARTICLE

# How do *Caenorhabditis elegans* worms survive in highly viscous habitats?

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## ABSTRACT

The nematode *Caenorhabditis elegans* is a filter feeder that lives in various viscous habitats such as soil, the intestines of slugs, and rotting materials such as fruits and stems. *Caenorhabditis elegans* draws in suspensions of bacteria and separates bacteria from water using the pharyngeal pump. Although these worms often live in highly viscous habitats, it is still unclear how they survive in these environments by eating bacteria. In this study, we investigated the effects of suspension viscosity on the survival rate of malnourished worms by combining live imaging and scaling analyses. We found that survival rate decreased with increases in viscosity because the high viscosity suppressed the amount of food ingested. The same tendency was found in two feeding-defective mutants, *eat-6*(*ad467*) and *eat-6*(*ad997*). We also found that the high viscosity weakened pump function, but the velocities in the pharynx were not zero, even in the most viscous suspensions. Finally, we estimated the amount of ingested food using scaling analyses, which provided a master curve of the experimental survival rates. These results illustrate that the survival rate of *C. elegans* worms is strongly dependent on the ingested bacteria per unit time associated with physical environments, such as the viscosity of food suspensions and the cell density of bacteria. The pump function of the *C. elegans* pharynx is not completely lost even in fluids that have $10^5$ times higher viscosity than water, which may contribute to their ability to survive around the world in highly viscous environments.

**KEY WORDS: Nematode, Pharynx, High viscosity, Survival rate, Malnutrition, Pump function**

## INTRODUCTION

Nematodes live on land and in aquatic environments from the poles to the tropics (Blumenthal and Davis, 2004; Parkinson et al., 2004; Borgonie et al., 2011; Erasmus and Onstott, 2011). Their habitat includes highly viscous environments, such as the intestines of other animals, rotting fruits and thick mud (Blaxter and Denver, 2012; Frezal and Félix, 2015; Peterson et al., 2015b; Samuel et al., 2016; Schulenburg and Félix, 2017; Woodruff and Phillips, 2018; Kanzaki et al., 2018). Thus, nematodes are able to adapt to highly viscous environments and continue to eat food in such environments. The nematode *Caenorhabditis elegans* lives in viscous habitats such as soil, the intestines of invertebrate animals and rotting plants (Blaxter and Denver, 2012; Frezal and Félix, 2015; Peterson et al., 2015b; Samuel et al., 2016; Schulenburg and Félix, 2017; Woodruff and Phillips, 2018; Kanzaki et al., 2018). However, it is still unclear how they survive in highly viscous environments by eating bacteria.

*Caenorhabditis elegans* has been intensively used for studies of calorie restriction associated with aging and longevity (Klass, 1977, 1983; Johnson and Wood, 1982; Kenyon et al., 1993; Lakowski and Hekimi, 1998; Lenaerts et al., 2008; Greer and Brunet, 2009; Ge et al., 2018) because it is easy to handle and observe as a result of its small size (about 1 mm in length), optical transparency, short life cycle and genetic tractability (Wood, 1988; The *C. elegans* Sequencing Consortium, 1998). Previous studies have reported that calorie restriction without starvation or malnutrition increases the life span of *C. elegans* worms (Klass, 1977, 1983; Johnson and Wood, 1982; Kenyon et al., 1993; Lakowski and Hekimi, 1998; Lenaerts et al., 2008; Greer and Brunet, 2009; Ge et al., 2018). However, the lifespan of malnourished or starved worms can be increased by increasing the concentration of food ($<10^8$–$10^9$ cells ml$^{-1}$) (Klass, 1977; Greer and Brunet, 2009; Ge et al., 2018). To investigate the lifespan of worms, survival assays have been performed with both solid and liquid culture media. Survival assays using liquid culture media have shown that wild-type *C. elegans* can survive for an average of 2–5 weeks, depending on the food concentration (Johnson and Wood, 1982; Klass, 1983; Kenyon et al., 1993; Lakowski and Hekimi, 1998). Survival assays using solid culture media have shown that wild-type *C. elegans* can survive for an average of 0–4 weeks, depending on the environmental temperature and food concentration (Klass, 1977; Lenaerts et al., 2008; Greer and Brunet, 2009; Ge et al., 2018). However, it is still unclear how the amount of food ingested by the worm affects its life span and how long worms can survive in highly viscous habitats.

*Caenorhabditis elegans* is a filter feeder, which draws in a suspension of bacteria and separates them using an organ called the pharynx, as shown in Fig. 1 (Avery and Shtonda, 2003; von Lieven, 2003). The pharynx consists of a three-part structure made up of muscle and marginal cells arranged symmetrically with respect to the central lumen and functions as a pump that generates pressure in the lumen (Albertson and Thomson, 1976; Avery and Shtonda, 2003; von Lieven, 2003; Fang-Yen et al., 2009). First, the muscles of the pharynx contract radially, opening the central lumen and creating room to suck in a food suspension from outside of the mouth. Next, the muscles relax, closing the central lumen and concentrating the bacteria in the tip of the pharynx, while the extra solvent is expelled to the outside of the mouth via smaller channels, which are extensions of the pharyngeal lumen (Avery and Shtonda, 2003; von Lieven, 2003; Fang-Yen et al., 2009; Kamal et al., 2019). This is the cycle of food condensation in the pharynx of *C. elegans*. Moreover, *C. elegans* can separate food depending on size (Kiyama et al., 2012). They actively ingest microparticles with diameters ranging from 0.5 to 2.0 µm, which are similar in size to bacteria.

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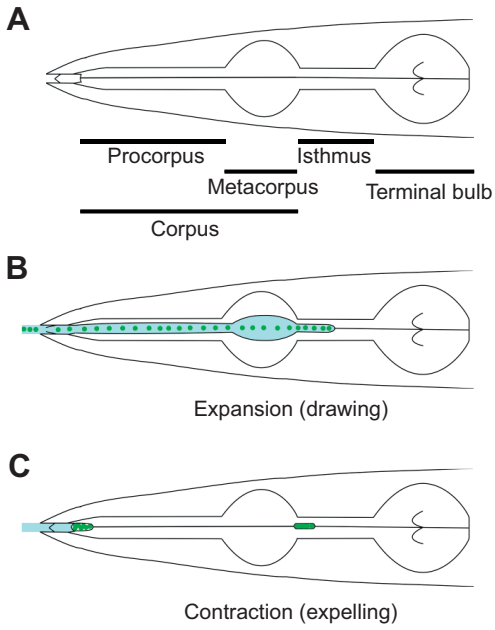


**Fig. 1. Pharyngeal structures and actions in the head of *Caenorhabditis elegans*.** (A) The pharynx is a tubular organ divided into three parts: the corpus, which is connected to the mouth and further divided into the procorpus and metacorpus, the isthmus, and the terminal bulb, which is connected to the intestine. (B,C) The pharynx generates pumping motions by expanding (B) and contracting (C) its muscles and lumen. As a result, the bacteria are trapped and transferred posteriorly to the intestine while the extra water is expelled to the outside.

Microparticles smaller than 0.5 µm or larger than 3 µm can be ingested, but this is rare.

Despite its biological and biomechanical importance, the effects of high viscosity on the survival rate and food condensation of *C. elegans* is still largely unclear. In this study, we investigated the effects of suspension viscosity on the survival rate of malnourished worms by combining live imaging and scaling analyses. We found that survival rate decreased with increasing viscosity. By investigating the pump function, we showed that the amount of ingested food is suppressed by high viscosity. Finally, we estimated the amount of food ingested by scaling the pump function, which provided a master curve of the experimental survival rates.

## MATERIALS AND METHODS

### Worm strain preparation

Wild-type strain N2, *eat-6*(*ad467*) and *eat-6*(*ad997*) worms were grown on the surface of nematode growth media (NGM) plates (0.3% sodium chloride, 1.7% agarose, 0.25% peptone, 1% cholesterol, 97.5% Milli-Q water) containing *Escherichia coli* strain OP50-1 in an incubator (CN-25C; Mitsubishi Electric Corporation) at 20°C (Wood, 1988). Approximately 5 mm square NGM plates containing dauer worms were cut with a knife, placed on new NGM plates in 60 mm plastic dishes (Corning Life Sciences, Tewksbury, MA, USA) seeded with OP50-1, and cultured for 1–2 days until they were gravid adults. Then, the eggs that the gravid worms had laid were hatched and cultured for 2–3 days until they were adults. In all experiments, we used adult hermaphrodite worms which hatched from the eggs that the post-dauer adults had laid and were within 2 days of becoming adults. For the experiments, the adult worms were extracted from 60 mm NGM plates by washing in 500 µl M9 buffer [0.3% potassium dihydrogen phosphate, 0.6% dipotassium hydrogen phosphate, 0.1% magnesium sulfide (1 mol $l^{-1}$), 0.5% sodium chloride] (Wood, 1988). The viscosity value of M9 buffer corresponds to the viscosity coefficient of water, 1.0 mPa s, because the physical property of M9 buffer is close to that of water. OP50-1 was used for all of the experiments in this study and was cultured in Luria–Bertani (LB) liquid media (1% bacto tryptone, 0.5% yeast extract, 1% NaCl) in a shaking incubator (BR-23FP, Taitec, Saitama, Japan) at 37°C and 200 rpm for 10–12 h (Wood, 1988). The $OD_{600}$ of 2% (v/v) OP50-1 (measured with a Biomate 3 spectrophotometer, Thermo Fisher Scientific, Waltham, MA, USA) was 0.87 ($6.7\times10^8$ cells $ml^{-1}$) (Chauhan et al., 2013).

### Methylcellulose solution preparation

Methylcellulose (MC) solution was used to increase the viscosity of the suspension surrounding the worms. MC is a harmless molecule with long, unbranched polymers, which creates a gel-like fluid when mixed with water (Tate et al., 2001). Two grades of MC were used: MC 400 (molecular weight 84,000: Fujifilm Wako Pure Chemical Corporation, Osaka, Japan) and MC 4000 (molecular weight 140,000: Fujifilm Wako Pure Chemical Corporation). Dispersions of MC were prepared in M9 buffer at concentrations of 0.5–5% [MC 400: 0.5%, 2.0%, 5.0% (w/w); MC 4000: 2.0%, 5.0% (w/w)]. MC was added to 10 ml M9 buffer and stirred vigorously in a shaking incubator (BR-23FP; Taitec) at 2–4°C and 300 rpm for 2–3 days to ensure the complete dissolution of MC (Herráez-Domínguez et al., 2005). We determined the viscosity values of MC solutions based on values in the literature (Herráez-Domínguez et al., 2005; Peyer et al., 2012; Kikuchi et al., 2017); these depend on the temperature and shear rate added by viscometers, and the temperature and the shear rate of these data were similar to those inside the pharynx of *C. elegans*. Therefore, the viscosity values obtained from the literature were $1.0\times10^1$, $3.8\times10^2$, $3.1\times10^3$, $2.4\times10^4$ and $1.0\times10^5$ mPa s at MC concentrations of 0.5%, 2.0% and 5.0% (w/w) for MC 400, and 2.0% and 5.0% (w/w) for MC 4000, respectively. These values cover viscosity environments where *C. elegans* worms would live (Picioreanu et al., 2000; Borrell, 2006; Zhang et al., 2008; Gómez-Díaz et al., 2009; Blaxter and Denver, 2012; Peterson et al., 2015a; Frezal and Félix, 2015; Samuel et al., 2016; Schulenburg and Félix, 2017; Kanzaki et al., 2018; Woodruff and Phillips, 2018).

### Survival rate assay

To measure the survival rate in suspensions with various viscosities, 10 worms were transferred to 25 µl M9 buffer or MC solution containing 0.2%, 0.5%, 1% or 2% (v/v) OP50-1 in a high oxygen permeable PTFE chamber (9×9 $mm^2$; Bio-Rad Laboratories, Hercules, CA, USA) on a microscope slide (76×26 $mm^2$, thickness 0.8–1.0 mm; Matsunami, Osaka, Japan). The chamber was sealed by a coverslip (18×18 $mm^2$, thickness 0.17 mm; Matsunami). We counted the number of living worms using a stereoscopic microscope (SXZ10, Olympus Corporation, Tokyo, Japan) at 24, 48 and 72 h. Worms were defined as living when movement of the body and the pharyngeal pump was observed. To distinguish between quiescence and death, we gently tapped the samples on the table before measuring the survival rate to assess whether worms in the samples could respond to the mechanical stimulus. As a precaution, we observed each worm twice every 10 min to prevent us from mistaking quiescence for death.

### Quantification of amount of food ingested

To measure the amount of particles accumulated in the intestine, 10–15 worms were fed 0.13% (v/v) fluorescent particles of 0.5 µm diameter (excitation wavelength of 505 nm, fluorescence wavelength of 514 nm; Thermo Fisher Scientific) mixed with 2% (v/v) OP50-1 in 100 µl M9 buffer or the MC solutions in a 14 mm glass-bottom dish

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(Matsunami) for 40 min at 20°C. To stop the worms feeding and to fix them, they were then soaked in 200 µl 99% ethanol (Fujifilm Wako Pure Chemical Corporation) for 1 min. Next, they were washed three times in 1 ml M9 buffer followed by 10 min centrifugation at 1413 ***g*** (3220; Kubota Corporation, Tokyo, Japan) to remove extra particles and defecated particles. The washed worms were transferred to a small volume (60–100 µl) of M9 buffer in a 14 mm glass-bottom dish (Matsunami). The fluorescence of the particles accumulated in the worms was observed with a fluorescence microscope (IX71, Olympus Corporation) equipped with a 4× dry objective lens (UPLFLN 4X, NA=0.13, WD=17 mm; Olympus Corporation). The fluorescence intensity was measured using ImageJ software (Schindelin et al., 2012).

### Measurement of pumping motions

To measure the pumping motions in the pharynx, 5–10 worms were transferred to 20–40 µl M9 buffer or MC solution containing 2% (v/v) OP50-1 in a chamber (9×9 $mm^2$, Bio-Rad Laboratories) on a microscope slide (24×60 $mm^2$, thickness 0.12–0.17 mm; Matsunami). The suspension was covered by a coverslip (18×18 $mm^2$, thickness 0.17 mm; Matsunami) to minimize dehydration. We observed pumping motions with a DIC microscope (BX51WI, Olympus Corporation) equipped with 60× (PLAPON 60XOPH, NA=1.42, WD=0.15 mm, Olympus Corporation) or a 100× oil immersion objective lens (UPLSAPO 100XOPH, NA=1.4, WD=0.13 mm, Olympus Corporation, Japan) and recorded high-speed image sequences of 2–3 s duration at 500 or 1000 frames $s^{-1}$ using a high-speed video camera (SA3, Photoron, Japan). We manually measured the frame numbers corresponding to the start and the end of the procorpus contraction, the metacorpus contraction, the isthmus contraction, the procorpus relaxation, the metacorpus relaxation and the isthmus relaxation, and the inner diameter at these moments of the central parts of the procorpus, metacorpus and isthmus.

### Measurement of flow in the pharynx

To visualize flow in the pharynx, 5–10 worms were transferred to 20–40 µl M9 buffer containing 0.03% (v/v) fluorescent particles (excitation wavelength 505 nm, fluorescence wavelength 514 nm; Thermo Fisher Scientific) mixed with 0.2% (v/v) OP50-1 in a chamber (9×9 $mm^2$, Bio-Rad Laboratories) on a microscope slide (24×60 $mm^2$, thickness: 0.12–0.17 mm; Matsunami). The suspension was covered by a coverslip (18×18 $mm^2$, thickness 0.17 mm; Matsunami) to minimize dehydration. We observed the flow of the particles with a confocal microscope (BX51WI, Olympus Corporation) assembled a Nipkow lens-type confocal unit (CSU-X1, Yokogawa Electric, Tokyo, Japan) with 20× dry objectives (LUCPLFLM 20XPH, NA=0.45, WD=6.60–7.80 mm; Olympus Corporation) and recorded high-speed image sequences of 2–3 s duration at 250 frames $s^{-1}$ using a high-speed video camera (SA3, Photron, Tokyo, Japan) (Kikuchi et al., 2019). We manually traced the positions of the particles during pumping motions in the procorpus and metacorpus. From the frame number and pixel number, we calculated the velocity of the particles flowing in the corpus and the mean velocity in the posterior part of the procorpus by dividing the displacement of the particles by the number of frames.

### Statistics

Statistical analyses were performed by using Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA) on macOS Sierra (Apple Inc., Cupertino, CA, USA). Statistical methods and sample numbers are described in each paragraph or figure legend.

## RESULTS

### Survival rate of worms at different viscosities

First, we investigated the effects of high viscosity on the survival of malnourished worms at two different food concentrations: 0.5% and 2% (v/v) *E. coli*. We measured the survival rate of worms after 24, 48 and 72 h in suspensions with viscosities ranging from $10^0$ mPa s to ∼$10^5$ mPa s. Survival rate at 24 h was almost identical regardless of viscosity with 0.5% and 2% (v/v) *E. coli* ($P$=0.33 and 0.99, respectively, Kruskal–Wallis $H$-test) (Fig. 2A,D); at 72 h with a food concentration of 0.5% (v/v), survival rate decreased with increases in viscosity ($P$=5.6×$10^{-3}$, Kruskal–Wallis $H$-test) (Fig. 2C). At a higher food concentration of 2.0% (v/v) at 48 and 72 h, the rate was not

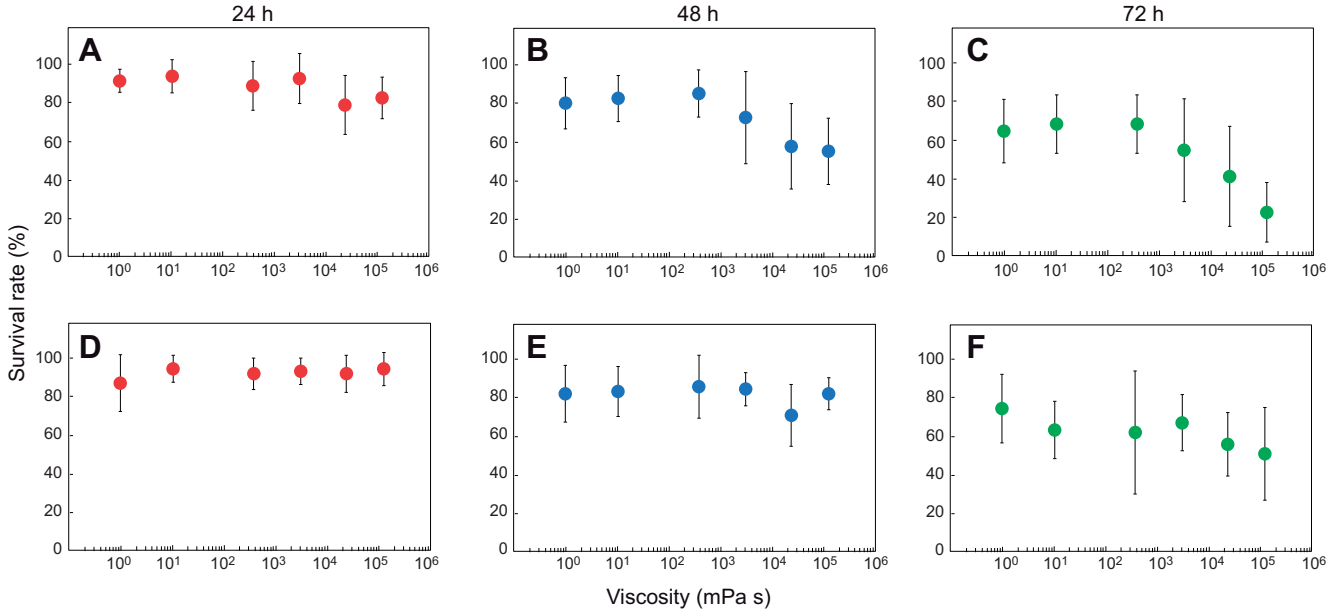


**Fig. 2. Effects of viscosity on the survival rate of the wild-type strain.** (A–C) 0.5% (v/v) *E. coli* at 24 h (A), 48 h (B) and 72 h (C). (D–F) 2% (v/v) *E. coli* at 24 h (D), 48 h (E) and 72 h (F). Data are means±s.d., $N$=8 assays in each condition.

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strongly affected by viscosity ($P$=0.54 and 0.41, respectively, Kruskal–Wallis $H$-test) (Fig. 2E,F). These results illustrate that the survival rate of worms decreases with a lack of ingested food, and this effect is enhanced by the surrounding viscosity.

### Accumulation of particles in the intestine at different viscosities

We measured the fluorescence intensity of 0.5 µm diameter fluorescent particles that accumulated in the intestine after 40 min feeding time in suspensions of various viscosities, from $10^0$ mPa s to ∼$10^5$ mPa s, to quantify the effects of viscosity on the amount of food ingested (Fig. 3A–F). A limitation of our experiments is that we could not directly measure the intake of particles by the pharyngeal pump and were unable to account for the effect of defecation. We found a negative correlation between viscosity and fluorescence intensity ($P$=2.5×$10^{-4}$, Kruskal–Wallis $H$-test) (Fig. 3G). These results indicate that the accumulation of the particles was significantly suppressed by increases in viscosity.

### Survival rate of two feeding-defective mutants

To clarify the relationship between survival rate and the amount of food ingested regardless of the health status of worms, we used two feeding-defective mutant strains, *eat-6*(*ad467*) and *eat-6*(*ad997*). These mutants have damaged sodium pumps, which are fundamental to the electric function of most animal cells, and defective pharyngeal muscles, and thus take in less food with their pharyngeal pumps (Avery, 1993; Davis et al., 1995; Doi and Iwasaki, 2008). We measured survival rates and the amount of food ingested by the two mutants in suspensions of various viscosities in the same way as for the wild-type strain. Their survival rates were lower than those of the wild-type strain in suspensions with high viscosity (Figs S2–S4). The mutants are likely to have more difficulty under viscosity stress than the healthy wild-type strain (Davis et al., 1995; Doi and Iwasaki, 2008), so this result is not surprising. Based on these data, next, we correlated the survival rate and the amount of food ingested, estimated from the fluorescent intensity of the particles accumulated in the body, and found that the survival rate decreased with decreases in the amount of food ingested in all worms (Fig. 4). These results indicate that the survival of worms depends on food intake regardless of health status.

### Pump function for food consumption at different viscosities

We measured the pumping motions in the pharynx in a viscosity range of $10^0$ mPa s to ∼$10^5$ mPa s. Fig. 5A–F shows the time course of pumping motions in high viscosity (3.1×$10^3$ mPa s) conditions observed using DIC microscopy and high-speed imaging. The pumping motions were similar among suspensions, as previously

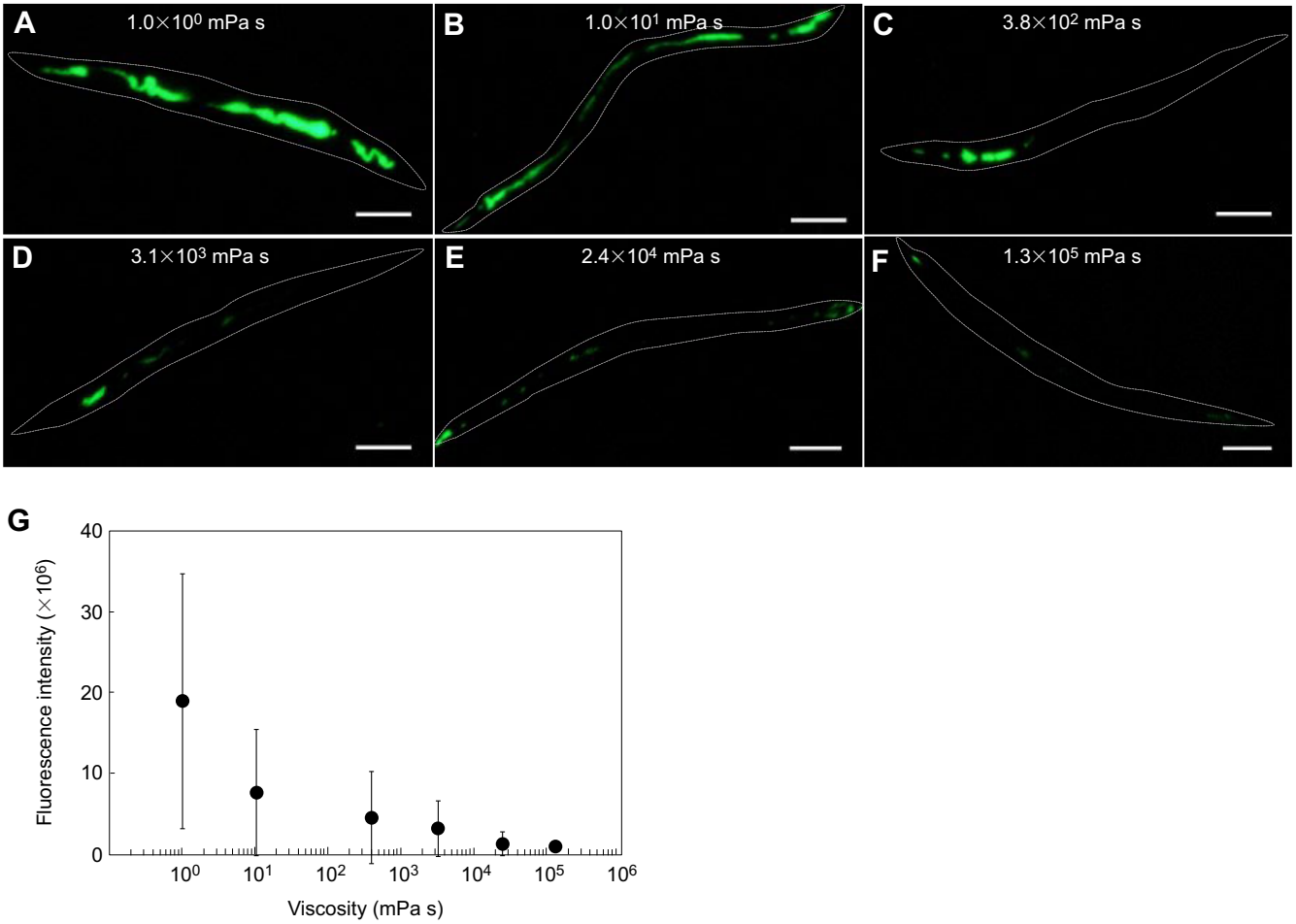


**Fig. 3. Accumulation of fluorescent particles in the wild-type strain.** (A–F) Fluorescence images of *C. elegans* worms which accumulated yellow–green particles of 0.5 µm diameter under various viscosities: (A) 1.0×$10^0$ mPa s, (B) 1.0×$10^1$ mPa s, (C) 3.8×$10^2$ mPa s, (D) 3.1×$10^3$ mPa s, (E) 2.4×$10^4$ mPa s and (F) 1.3×$10^5$ mPa s. The gray dotted lines in each image show the outline of the worms. Scale bars: 100 µm. (G) Effects of viscosity on the uptake of fluorescent particles by the wild-type strain. Data are means±s.d., $n$≥7 worms in each viscosity.

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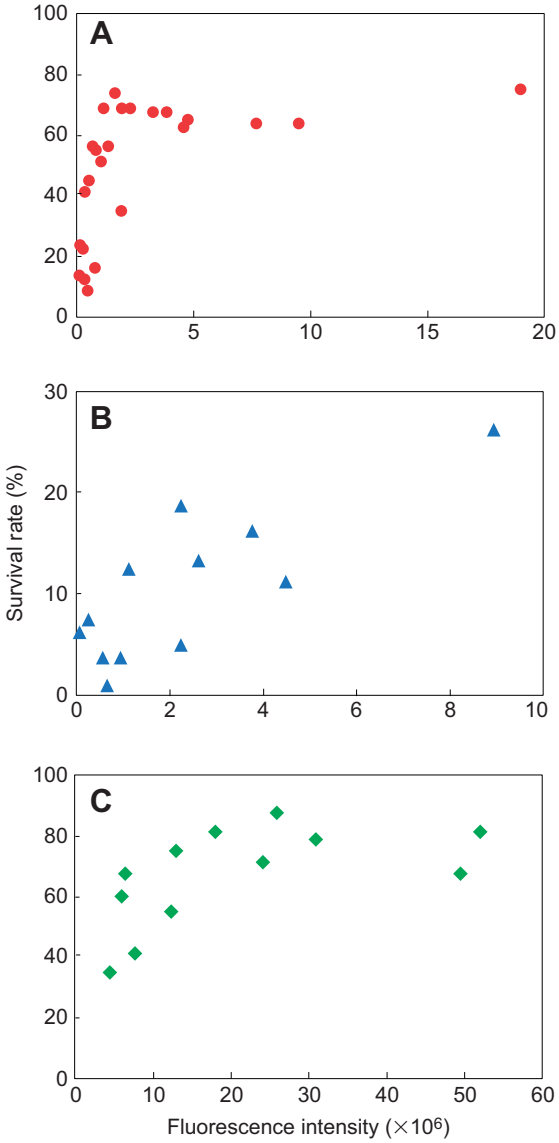


**Fig. 4. Relationship between survival rate at 72 h and amount of food ingested.** The amount of food ingested over 40 min is represented by the fluorescence intensity. (A) Wild-type strain (N2). (B) *eat-6*(*ad467*). (C) *eat-6*(*ad997*).

reported (Avery and Shtonda, 2003). We measured the diameter of the central parts of the procorpus, metacorpus and isthmus, and the frequency of pumping from image sequences. There was no significant correlation between viscosity and maximum diameter of the isthmus ($P$=0.054, Kruskal–Wallis $H$-test), although we obtained a significant correlation between those of the procorpus and metacorpus ($P$=0.015 and 0.031, respectively, Kruskal–Wallis $H$-test) (Fig. 5G). These results indicate that the maximum diameter of the isthmus was almost constant regardless of viscosity. Fig. 5H shows the relationship between viscosity and the minimum diameter of the procorpus, metacorpus and isthmus ($P$=1.8×10$^{-8}$, 6.8×10$^{-6}$ and 1.6×10$^{-4}$, respectively, Kruskal–Wallis $H$-test); all diameters significantly increased with increases in viscosity. The frequency of pumping was significantly decreased with increases in viscosity ($P$=6.7×10$^{-11}$, Kruskal–Wallis $H$-test) (Fig. 5I).

Next, we performed scaling analyses to estimate the volume of food suspension ingested into the pharynx. To construct the model, we assumed that the pharynx was a straight tube with a circular cross-section. Although this assumption may oversimplify the detailed cross-sectional configuration, it still provides reasonable scaling of the channel volume of the pharynx (Avery and Shtonda, 2003). By considering that the volume of food suspension ingested into the pharynx is proportional to the volume change in the pharynx and the frequency of pumping motions, we can derive the total ingested volume $V_{in}$ during the feeding time $T$ as:

$$V_{in} = (V_{max} - V_{min})N = \frac{\pi}{4}(D_{max}^2 - D_{min}^2)LfT, \quad (1)$$

where $V_{max}$ and $V_{min}$ are the maximum and minimum volume of the pharynx, respectively; $N$ is the number of pumping motions during the feeding time $T$; $D_{max}$ and $D_{min}$ are the maximum and minimum inner diameter of the pharynx, respectively; $L$ is the length of the pharynx; and $f$ is the frequency of pumping motions. The values of $D_{max}$, $D_{min}$, $L$ and $f$ were obtained from the experiments, and $T$ is an experimental condition. $V_{in}$ showed a tendency to decrease with increases in viscosity (Fig. 5J). Although $V_{in}$ may not be exactly proportional to the uptake of the fluorescent particles, its tendency to decrease with viscosity is in qualitative agreement with the data shown in Fig. 3G (the correlation coefficient of two plots was 0.96). These results clearly indicate that the performance of the pump is weakened by increases in viscosity, which decreases the amount of food ingested. Although we made some bold hypotheses, we also estimated the pump characteristics and the optimal food concentration for the pharyngeal pump. For details, please refer to the Appendix.

### Correlation between amount of food ingested and survival rate

To estimate the total number of *E. coli*, $N_{in}$, taken in by the pharyngeal pump, we assumed that the cell density of *E. coli* cells, $\rho_{E.coli}$, in the ambient suspension is constant and uniform. Because the total volume of the suspension ingested in the pharynx is $V_{in}$, $N_{in}$ is defined as $N_{in}=\rho_{E.coli}V_{in}$. The number of *E. coli* taken in by the pharyngeal pump per second is defined as $N_{in}/T$. Fig. 6 shows the relationship between $N_{in}/T$ and the survival rate at 24, 48 and 72 h (Fig. 2 and Fig. S1). The survival rate was almost unaffected by $N_{in}/T$ (Fig. 6A), but decreased in the case of $N_{in}/T<3$, and the results of the various conditions collapsed into a single master curve (Fig. 6B,C).

## DISCUSSION

In this study, we found that the survival rates of *C. elegans* at 24 h were almost identical regardless of viscosity (Fig. 2A,D) and the survival rates at 72 h decreased with increases in viscosity (Fig. 2C,F). The amount of food ingested was suppressed at higher viscosity (Fig. 5J). These results indicate that one factor in the death of worms at high viscosity is starvation. This could be shown by comparison of the master curves of the relationship between the survival rate and the amount of food ingested at 24 and 72 h (Fig. 6A,C). Of course, higher viscosity may influence other processes such as defecation and swimming. However, the same tendency was also found for the feeding-defective mutants *eat-6*(*ad467*) and *eat-6*(*ad997*), whose deficiencies are not only expressed in the pharynx but also in most cells, including in the body wall and vulval muscles (Doi and Iwasaki, 2008) (Fig. 4B,C). This indicates that the survival rate of *C. elegans* worms depends on the amount of ingested food regardless of their health status.

It has been reported that the pumping rate and the timing of muscle contraction and relaxation depend on the nervous system (Avery and Horvitz, 1989). We found that the change in viscosity of food suspensions affected the frequency of pharyngeal pumping

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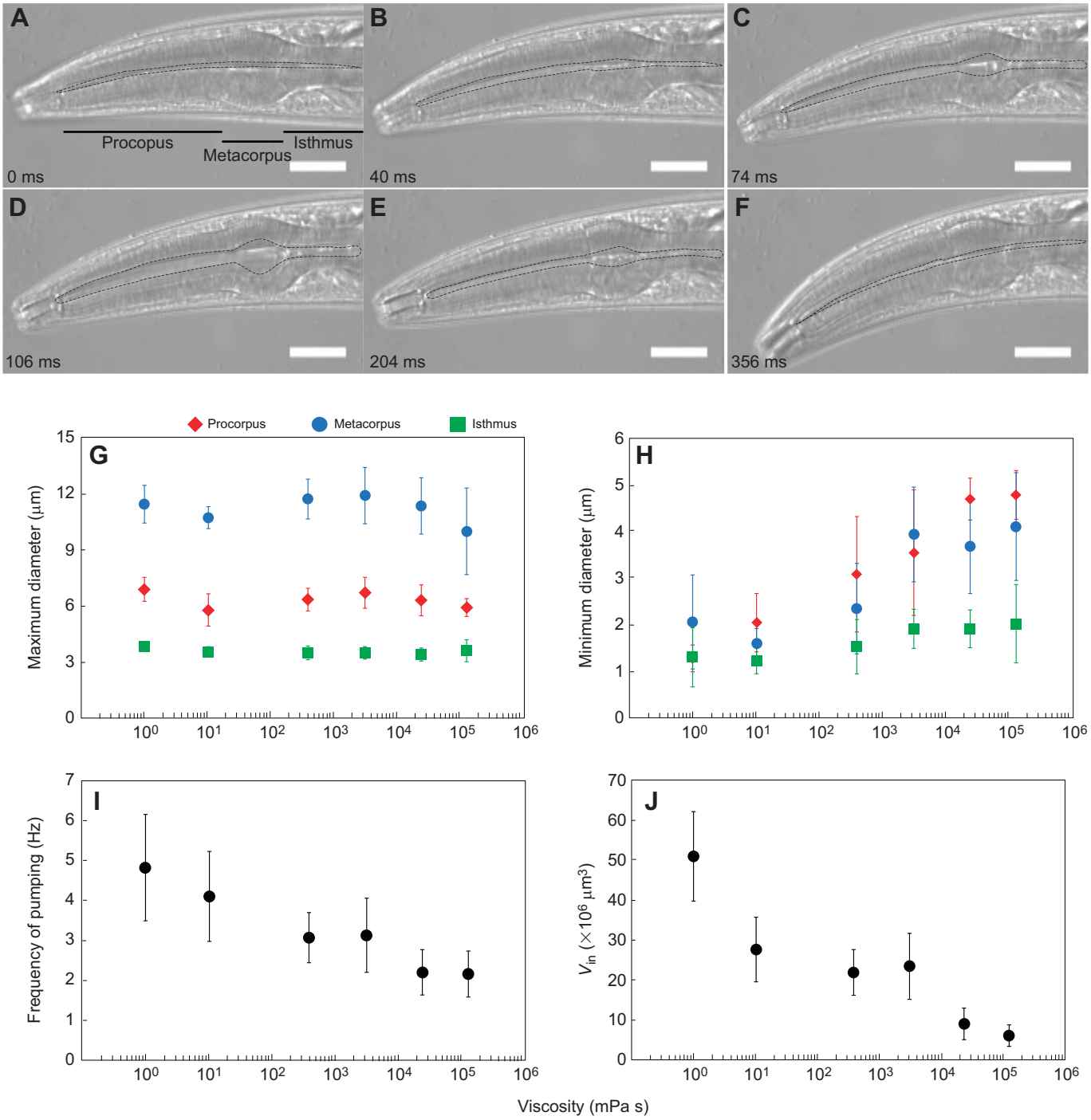


**Fig. 5. Pumping motion of the wild-type strain.** (A–F) DIC images of the pharyngeal pump of *C. elegans* at a viscosity of 3.1×10$^3$ mPa s. Time from the start of expansion of the pharynx is shown in the lower left corner of each image. The black dotted lines in each image show the outline of the lumen of the procorpus, metacorpus and isthmus. (A) The pharynx is fully closed at 0 ms. (B) A slight expansion of the procorpus and metacorpus is visible, and the isthmus is still closed at 40 ms. (C) Expansion of the procorpus and metacorpus continues, and the expansion of the isthmus is clearly visible at 74 ms. (D) Expansion of the procorpus and metacorpus is complete, and expansion of the isthmus continues at 106 ms. (E) Contraction of the procorpus, metacorpus and isthmus continues at 204 ms. (F) Finally, the pharynx is fully closed at 356 ms. Scale bars: 20 µm. (G–J) The effects of viscosity on the maximum (G) and minimum (H) diameter of the procorpus, metacorpus and isthmus, the frequency of pumping (I) and the total volume of food suspension ingested into the pharynx ($Q_{in}$; J), estimated by Eqn 1. Data are means±s.d., $n$≥5 for G, $n$≥5 for H, $n$≥11 for I, $n$≥5 for J.

(Fig. 5I). This was mainly caused by a significant increase in the minimum diameter of the procorpus (see Fig. 5H). We found that high viscosity had little effect on the maximum diameter (Fig. 5G). When muscles in the procorpus contract, the diameter of the procorpus increases to a maximum; because this remained unchanged, the high viscosity did not affect the contraction of procorpus muscles. In contrast, when muscles in the procorpus are relaxed, the diameter of the procorpus is decreased to its minimum diameter. As the minimum diameter was significantly changed, high viscosity did affect the relaxation of procorpus muscles. These

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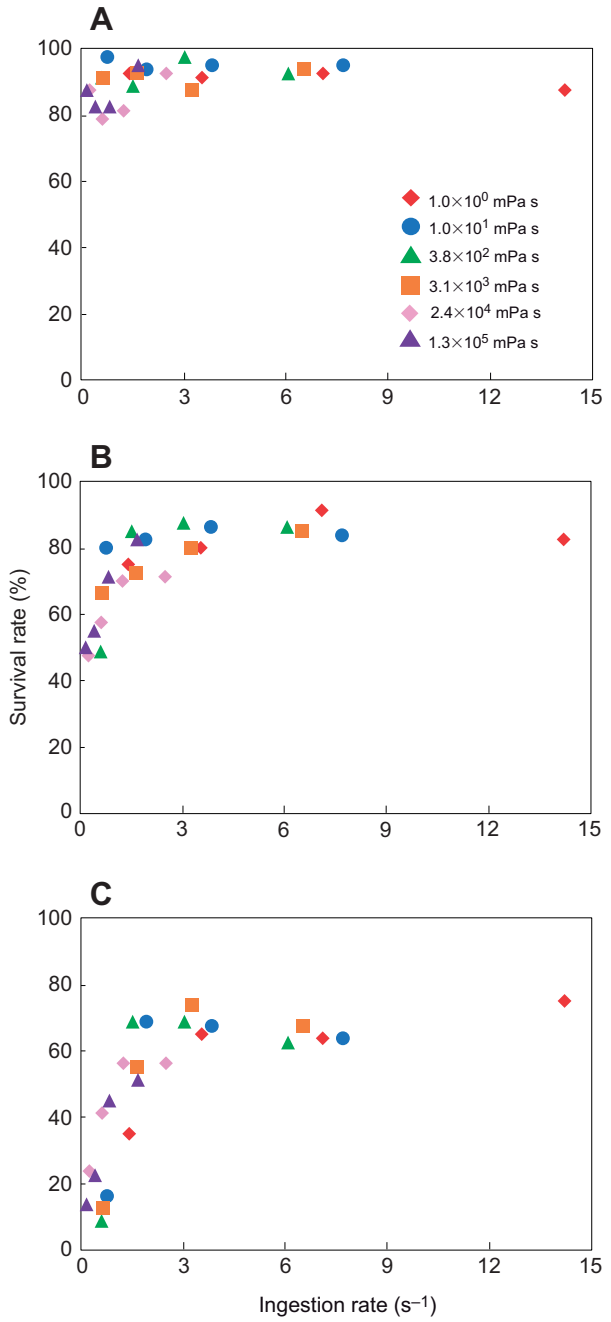


**Fig. 6. Relationship between survival rate and rate of ingestion of *E. coli*.** Ingestion into the pharynx was quantified as the number of bacteria taken in per second at (A) 24 h, (B) 48 h and (C) 72 h.

results indicate that the procorpus muscles generate sufficiently strong forces to overcome the viscous drag induced by high viscosity. The relaxation of muscles is a passive phenomenon and was significantly affected by the viscous environment. This result indicates that the pump volume changes slowly with the strong viscous drag, which leads to a decrease in the pumping frequency with greater viscosity.

Our results indicate that the pump function of the *C. elegans* pharynx is not lost even in conditions with $10^5$ times higher viscosity than water (see Appendix). In nature, *C. elegans* worms live in rotting plants, such as fruits and stems, and the intestines of other animals (Blaxter and Denver, 2012; Frezal and Félix, 2015; Peterson et al., 2015b; Samuel et al., 2016; Schulenburg and Félix, 2017; Woodruff and Phillips, 2018; Kanzaki et al., 2018). Because the viscosity in these places can be much higher than that of water, it seems that it would be difficult for the worms to suck in the surrounding fluid (Picioreanu et al., 2000; Zhang et al., 2008; Peterson et al., 2015a; Borrell, 2006; Gómez-Díaz et al., 2009). It has not been clear how *C. elegans* worms can survive in highly viscous habitats. To answer this question, we investigated the correlation between the survival rate and the amount of ingested food. We estimated the number of *E. coli* sucked in using scaling analyses, which provided the master curve of survival rate (Fig. 6C). Based on these results, we propose that the survival rate of worms is strongly dependent on the amount of ingested food associated with physical environments, such as the viscosity of food suspensions and the density of bacteria. Based on the master curve, we estimated the volume and caloric intake the worms need per day to survive. As shown in Fig. 6C, worms need ≥3 bacteria cells per second to maintain >70% survival rate after 3 days. It has been reported that the dry mass of a cell of OP50-1 is about 220 ng and the cell contains about 100 ng protein, 7.5 ng fatty acid and 15 ng carbohydrate (Brooks et al., 2009). The physical combustion heat of protein, fatty acid and carbohydrate is 5.7, 9.4 and 4.1 kcal $g^{-1}$ (where 1 kcal=4.184 kJ), respectively (Atwater and Bryant, 1900). By using these values, we estimated the calorie content of a cell of OP50-1 is $7.0\times10^{-7}$ kcal. The number of bacterial cells that worms need to take in per day so as to maintain >70% survival rate after 3 days is $2.6\times10^5$ cells $day^{-1}$, or 182 cal $day^{-1}$.

The survival rate after 72 h in suspensions with high viscosity and high food concentration was >50% (Fig. 6C). These results suggest that worms survive even in highly viscous environments, provided that they have access to a sufficient number of bacteria. Although some of the natural environments of worms, such as rotting plants and intestines of other animals, have a higher viscosity than water, these environments probably provide abundant food for worms to survive because they contain many bacteria and biofilms (Picioreanu et al., 2000; Zhang et al., 2008; Peterson et al., 2015a; Borrell, 2006; Gómez-Díaz et al., 2009). Therefore, worms are able to eat sufficient bacteria, even in highly viscous environments.

In conclusion, the survival rates of worms decreased with increases in viscosity because the amount of ingested food was suppressed by the high viscosity. This was true in normal worms as well as in feeding-defective mutants. We further noted that pump function was weakened by viscosity, particularly the relaxation of the procorpus muscles. Finally, we estimated the amount of food ingested and found that the survival rate of *C. elegans* worms is strongly dependent on the bacteria ingested per unit time associated with their physical environments, such as the viscosity of food suspensions and the cell density of bacteria. The pump function of *C. elegans* pharynx was not completely lost, even in suspensions with $10^5$ times higher viscosity than water, which may be the reason why they have thrived around the world.

## APPENDIX

### Estimation of pump characteristics

To quantify the effects of the viscosity of food suspensions on the status of the pharyngeal pump, we measured the flow velocity of 0.5 µm diameter particles, flowing in the posterior procorpus. Fig. A1 shows the flow of the particles during expansion and contraction of the corpus. They mainly flowed around the center of the pharynx during one cycle of pumping, as previously reported (Avery and Shtonda, 2003; Fang-Yen et al., 2009). Fig. A2A shows the relationship between the viscosity of food suspensions and the

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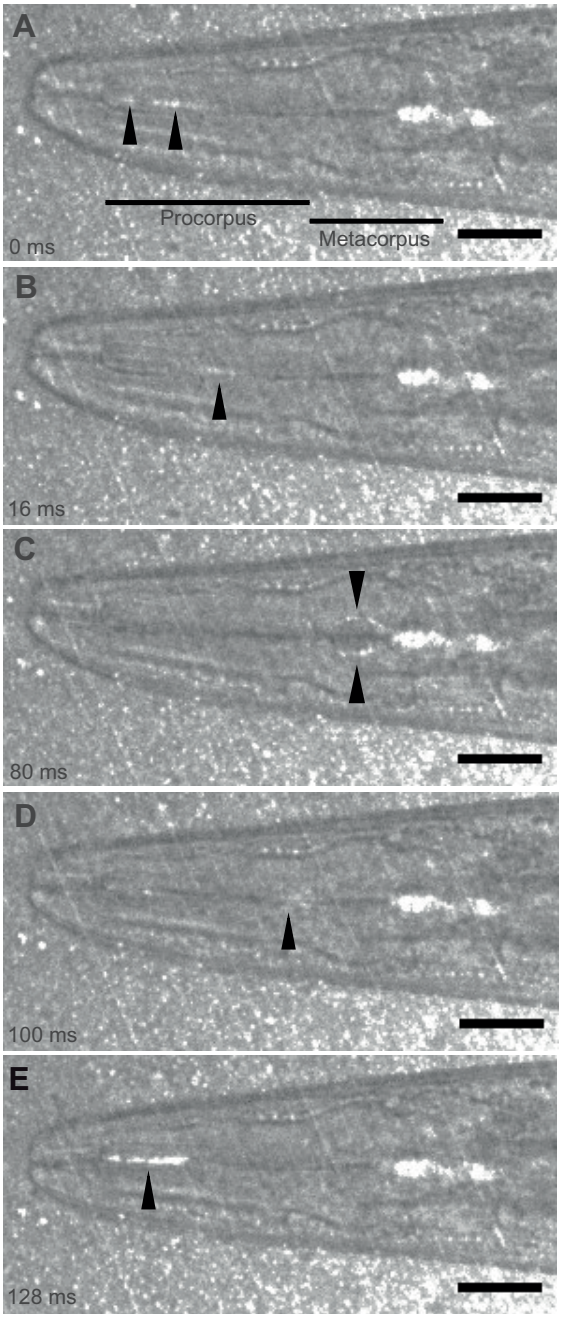


**Fig. A1. Time course of ingestion of fluorescent particles into the pharynx.** (A–E). The particles appear as white dots under the confocal microscope. The black arrowheads show the location of the particles within the pharynx. Time is shown in the lower left corner of each image. Scale bars: 20 µm.

velocity of the particles during intake and expulsion. We found a significant correlation ($P$=7.4×10$^{-4}$ and 8.42×10$^{-6}$, respectively, Kruskal–Wallis $H$-test). This shows that the flow velocity was not zero even at the highest viscosity, but did decrease with increases in viscosity. In nature, the viscosity of rotting materials, such as fruits, stems and compost or biofilms containing many bacteria, can be 10$^{2}$–10$^{4}$ mPa s (Picioreanu et al., 2000; Zhang et al., 2008; Peterson et al., 2015a; Borrell, 2006; Gómez-Díaz et al., 2009). These results suggest that *C. elegans* worms can maintain the function of the pharyngeal pump even in highly viscous habitats.

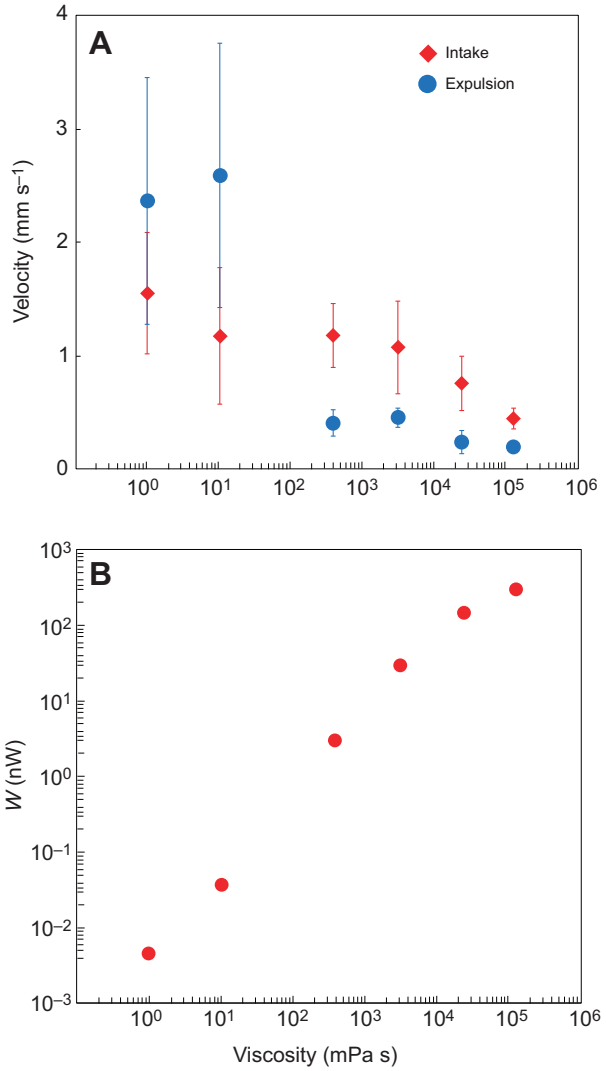


**Fig. A2. Effect of viscosity on flow velocity and pump power.** (A) Flow velocity in the posterior procorpus during intake (drawing) or expulsion of the suspension. Data are means±s.d., $n$≥5. (B) Pump power $W$ during intake of the suspension estimated from Eqn A4.

Finally, we estimated the pump power of the pharynx and the energy of feeding using the flow velocity and simple Hagen–Poiseuille's law. To calculate the pump power, we assumed the pharynx is a straight tube with circular cross-section of the lumen, where the flow can be assumed as Hagen–Poiseuille flow. We note, however, that the shape of the pharyngeal pump has to be measured in detail to accurately estimate the pressure loss in the pharyngeal pump as previously reported by Avery and Shtonda (2003). Otherwise, the scaling may overestimate the pressure, which can even be negative inside the pharyngeal pump.

The pump power $W$ is defined by applying Darcy–Weisbach's formula:

$$W = PQ, \tag{A1}$$

where $P$ is the pressure loss in the pharynx and $Q$ is the flow rate. $P$ can be expressed as:

$$P = \frac{64}{Re}\frac{L}{D}\frac{\rho U^2}{2} = \frac{32\mu LU}{D^2}, \tag{A2}$$

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where $Re$ is Reynolds number, $L$ is the length of the pharynx, $D$ is the representative inner diameter of the pharynx, $U$ is the flow velocity in the pharynx during corpus expansion or contraction, ρ is the density of an ambient suspension and μ is the viscosity coefficient of the ambient suspension. $Q$ can be written as:

$$Q = \frac{\pi}{4} D^2 U. \quad \text{(A3)}$$

Hence, Eqn A1 can be rewritten as:

$$W = 8\pi\mu L U^2. \quad \text{(A4)}$$

Fig. A2B shows a correlation between the viscosity of the suspension and the pump power during drawing of the suspension. Moreover, we calculated the energy of feeding, which represents the energy the pharyngeal pump consumes, using the values of the pump power and compared the energy with the number of calories the worms need per day. The energy the pharyngeal pump consumed in 24 h was $3.12\times10^{-3}$ J day$^{-1}$ at most. The number of calories needed per day was 761 J day$^{-1}$ (see Discussion). These values are not on the same scale. This shows that the energy the pharyngeal pump consumes is much less than the number of calories needed to survive.

### Estimation of the optimal food concentration

We calculated the optimal bacteria volume fraction of various viscous suspensions for the pharyngeal pump based on a previous study (Kim et al., 2011). We assumed that the apparent viscosity of suspensions containing bacterial cells is dependent only on the volume fraction of the cells. The apparent viscosity (μ′) of suspensions of nanoparticles is given by the following equation proposed by Pal (2016):

$$\mu' = \mu\left\{1 - \left[1 + \left(\frac{1-\phi_m}{\phi_m^2}\right)\phi\right]\phi\right\}^{-2.5}, \quad \text{(A5)}$$

where μ is the viscosity of the solvent, $\phi_m$ is the maximum packing volume fraction of suspended bacterial cells and ϕ is the volume fraction of bacterial cells. Eqn A5 is valid for $0<\phi<\phi_m$. In this study, $\phi_m$ was assumed to be 0.58 for a colloidal glassy state.

As shown in Fig. A3A, we plotted the volumetric flow rate of suspensions $Q$ as a function of the apparent viscosity of suspensions μ′ on a log scale. The fitted curve was obtained as:

$$Q = 21.4\mu'^{-0.161}. \quad \text{(A6)}$$

The energy intake rate for bacteria ingested by the pharyngeal pump is given by:

$$\dot{E} = cQ\rho\phi \propto \phi\mu'^{-0.161} = \phi\left[\mu\left\{1 - \left[1 + \left(\frac{1-\phi_m}{\phi_m^2}\right)\phi\right]\phi\right\}^{-2.5}\right]^{-0.161}, \quad \text{(A7)}$$

where $c$ is the product of the energy content per unit mass of bacteria and ρ is the density of bacteria. Finally, we can derive the energy intake rate of ingested bacteria $\dot{E}$ as a function of bacteria volume fraction ϕ (Fig. A3B), where the optimal bacteria volume fraction for the pharyngeal pump is 0.42. In this study, the highest food volume fraction was 0.02 and the lowest viscosity of the solvent was 1.0 mPa s, which were the conditions that maximized the amount of ingested food (Fig. 5J). From these results, the optimal condition of the food suspensions for the pharyngeal pump would be a bacteria volume fraction close to 0.42 and a solvent viscosity close 1.0 mPa s.

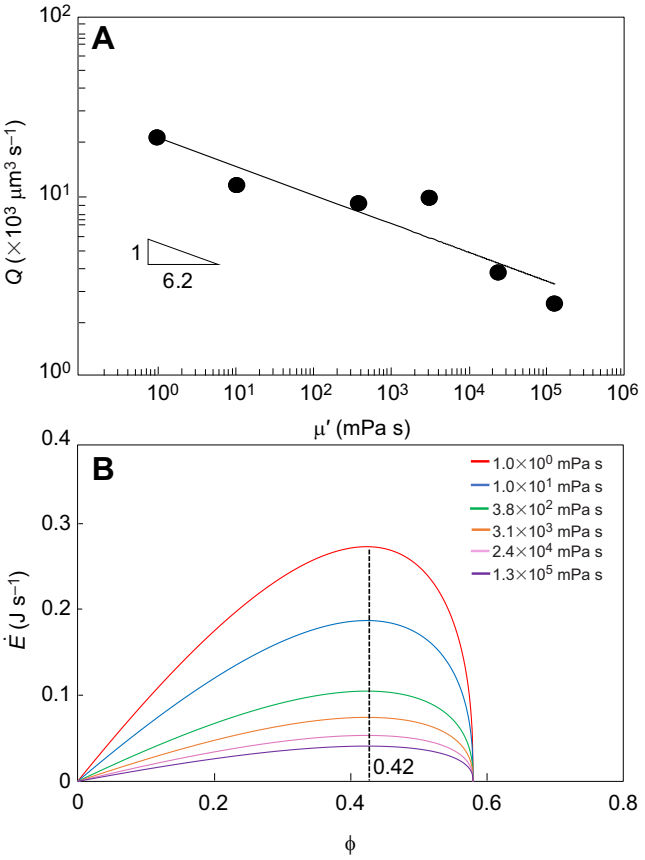


**Fig. A3. Effect of apparent viscosity on volumetric flow rate and the optimal bacteria concentration for the pharyngeal pump.** (A) Correlation between the volumetric flow rate ($Q$) and the apparent viscosity (μ′) of suspensions. Data were fitted with the function $Q=21.4\mu'^{-0.161}$ with coefficient determination $R^2$=0.866. (B) Correlation between the energy intake rate ($\dot{E}$) and the volume fraction (ϕ) of ingested bacterial cells.

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: K.K., T.I.; Methodology: Y.S., K.N.-T.; Validation: T.I.; Formal analysis: Y.S.; Investigation: Y.S.; Data curation: Y.S.; Writing - original draft: Y.S.; Writing - review & editing: K.K., T.I.; Visualization: Y.S., K.K.; Supervision: K.K., T.I.; Project administration: K.K., T.I.; Funding acquisition: K.K., T.I.

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#### Supplementary information

Supplementary information available online at
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