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Journal Article

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LETTER

Finding patches in a heterogeneous aquatic environment: pH-taxis by the dispersal stage of choanoflagellates

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Scientific Significance Statement

Microbial eukaryotes that feed on bacteria are critical links in aquatic food webs. Because the aquatic environment is often patchy at the microscale, feeding of microbial eukaryotes can depend strongly on their ability to find patches of concentrated prey. Choanoflagellates provide a system in which feeding performance can be studied in the same species for different life forms—slow swimmers, fast swimmers, and colonies—yet, it has remained unknown whether choanoflagellates are capable of directed motion ("taxis") to find prey patches. Here, we report for the first time that choanoflagellates are capable of taxis toward pH 6–7, which is characteristic of the acidification caused by concentrated patches of bacteria. This behavior is limited to the dispersal form of choanoflagellates—fast swimmers—and may allow them to exploit prey-rich microhabitats.

Abstract

Microbial eukaryotes that feed on bacteria are critical links in aquatic food webs. We used the choanoflagellate *Salpingoeca rosetta*, whose life history includes fast- and slow-swimming unicellular forms and multicellular colonies, to study performance of these different forms at finding and accumulating in patches of prey. Video microscopy of behavior in microfluidic experiments showed that only unicellular fast swimmers exhibited taxis into patches of attractant. Of the chemical cues tested, only pH affected behavior: fast swimmers moved toward patches of water that had pH in the range of 6–7 from water with higher or lower pH. Since bacteria can lower the pH of the water in their vicinity, we suggest that seeking regions with a moderately more acidic pH than average seawater helps the fast-swimming dispersal stage of choanoflagellates find particles or surfaces rich in prey.

Heterotrophic microbial eukaryotes that feed on bacteria are important components of food webs in marine (Azam et al. 1983; Worden et al. 2015) and freshwater systems (Tikhonenkov and Mazei 2008). The feeding performance of microbial eukaryotes depends not only on the rate at which they capture bacteria from the surrounding water, but also on their ability to find patches of prey. Choanoflagellates provide a system in which the consequences of different life forms on feeding performance can be studied in the same species. The life history of the best-studied choanoflagellate, *Salpingoeca rosetta*, includes at least three unicellular forms ("slow swimmers," "fast swimmers," "thecate cells") and two multicellular forms ("rosettes," "chains") (Dayel et al. 2011). While the ecological relevance of these life stages is not yet well understood, the fast swimmers are typically thought to be a dispersal form (Leadbeater 1983, 2015; Dayel et al. 2011). Yet, whether dispersal is used in conjunction with chemical sensing, and more generally whether choanoflagellates are capable of taxis to locate prey, has remained unknown.

The aquatic environments in which choanoflagellates live are far from homogeneous, with a plethora of nutrient-rich "hotspots" producing microscale heterogeneity (*see* Stocker 2012), including sinking marine particles (Kiørboe and Jackson 2001; Stocker et al. 2008), plumes of dissolved organic matter around phytoplankton cells (Smriga et al. 2016), and nutrient-rich filaments produced by turbulence (Crimaldi and Koseff 2001; Koehl et al. 2007; Taylor and Stocker 2012). Many microorganisms, such as bacteria, are able to utilize these nutrient hotspots by chemotaxis (Stocker et al. 2008). We hypothesize that choanoflagellates may also be capable of chemotaxis to exploit hotspots where bacterial density is high. The specific questions addressed by this study are: (1) Do choanoflagellates show taxis toward patches of high bacterial density?, (2) If so, which of their life stages show the response?, (3) What is the chemical cue for their taxis? Here, we address these questions by using quantitative image analysis of choanoflagellates in microfluidic experiments.

Methods

Growth media and solutions

Artificial seawater (ASW; 32.9 g L^{-1} of Tropic Marine sea salts in Milli-Q water) was prepared with pH of 8.0 ± 0.1 and salinity of 32 parts per thousand (King et al. 2009). Cereal grass media (CGM; 5 g L^{-1} of cereal grass pellets from Basic Sciences Supplies infused in ASW) was diluted in ASW (10% vol/vol) and used to grow rosettes (ATCC PRA-366) and a rosette-free culture (Dayel et al. 2011). Seawater complete medium (SWC; 24 g L^{-1} of Tropic Marine sea salts, 5 g L^{-1} peptone, 3 g L^{-1} yeast extract, 3 mL glycerol in Milli-Q water) was diluted in ASW (5% vol/vol) to grow *S. rosetta* fed with *Echinicola pacifica* (SrEpac, ATCC PRA-390) (Levin and King 2013).

2X phosphate buffered saline (PBS; diluted from a 10X stock, pH = 7.2 ± 0.1; Sigma-Aldrich) was used to wash bacteria and as a chemoattractant in the taxis experiments. Choanoflagellate-conditioned medium (CM) was obtained by centrifuging the choanoflagellate culture, then filtering with a 0.2 μm syringe filter (VWR, Radnor). To match the concentration of potassium in PBS, KCl was added to ASW to reach a potassium concentration of $\sim$ 10 mM (final pH = 8.2). Similarly, NaH_2PO_4 was added to ASW to match the concentration of PO_4 in PBS ($\sim$ 20 mM) and NaOH was used to maintain the pH at 8. Addition of NaOH and HCl was used to increase or decrease the pH of ASW, respectively (Supporting Information Fig. S2a).

Choanoflagellate cultures

Different cultures of *S. rosetta* were used to test taxis by different cell types (Dayel et al. 2011).

i. Rosettes: A monoxenic strain (ATCC PRA-366, *S. rosetta* and *Algoriphagus machipongonensis*) containing a high concentration of rosettes was maintained by diluting 1 mL of choanoflagellate suspension into 9 mL of fresh 10% CGM every 5–7 d at room temperature.

ii. Fast and slow swimmers: Two protocols were used to control the ratio of fast to slow swimmers in culture (Supporting Information Fig. S1):

a. ATCC PRA-390 (SrEpac) cultures produce high concentrations of slow swimmers (Levin et al. 2014) and they were maintained by diluting 1 mL of culture into 9 mL fresh 5% SWC medium every 4–5 d at room temperature. Experiments were performed after the SrEpac cultures reached stationary phase and cell concentrations were $(4 \pm 1) \times 10^6$ cells mL^{-1}. Choanoflagellate concentrations were determined using a hemocytometer (Bright-Line, Hausser Scientific).

b. Rosette-free cultures (Dayel et al. 2011) produce a higher percentage of fast swimmers from thecate cells. A flask containing thecate cells was washed twice with ASW and refilled with 15 mL of 10% CGM 36–48 h before experiments were conducted. In order to reach concentrations of $(1.5 \pm 0.5) \times 10^6$ cells mL^{-1}, 10 mL of culture was centrifuged for 10 min at $1000 \times g$ and the cell pellet was resuspended in 1 mL of CM. Thecate cell cultures were maintained by diluting 1 mL of a suspension of fast swimmers in 9 mL of 10% CGM every 4–5 d and incubated at room temperature.

Microinjector assay to measure taxis

With standard soft lithography, we fabricated microinjector channels (Seymour et al. 2008). This device consists of a 3 mm wide, 0.1 mm deep channel that injects the chemoattractant into a larger channel (Fig. 1a,b). Imposing a flow allows the formation of two regions within the channel: a central band (1/5 of the channel width) containing the
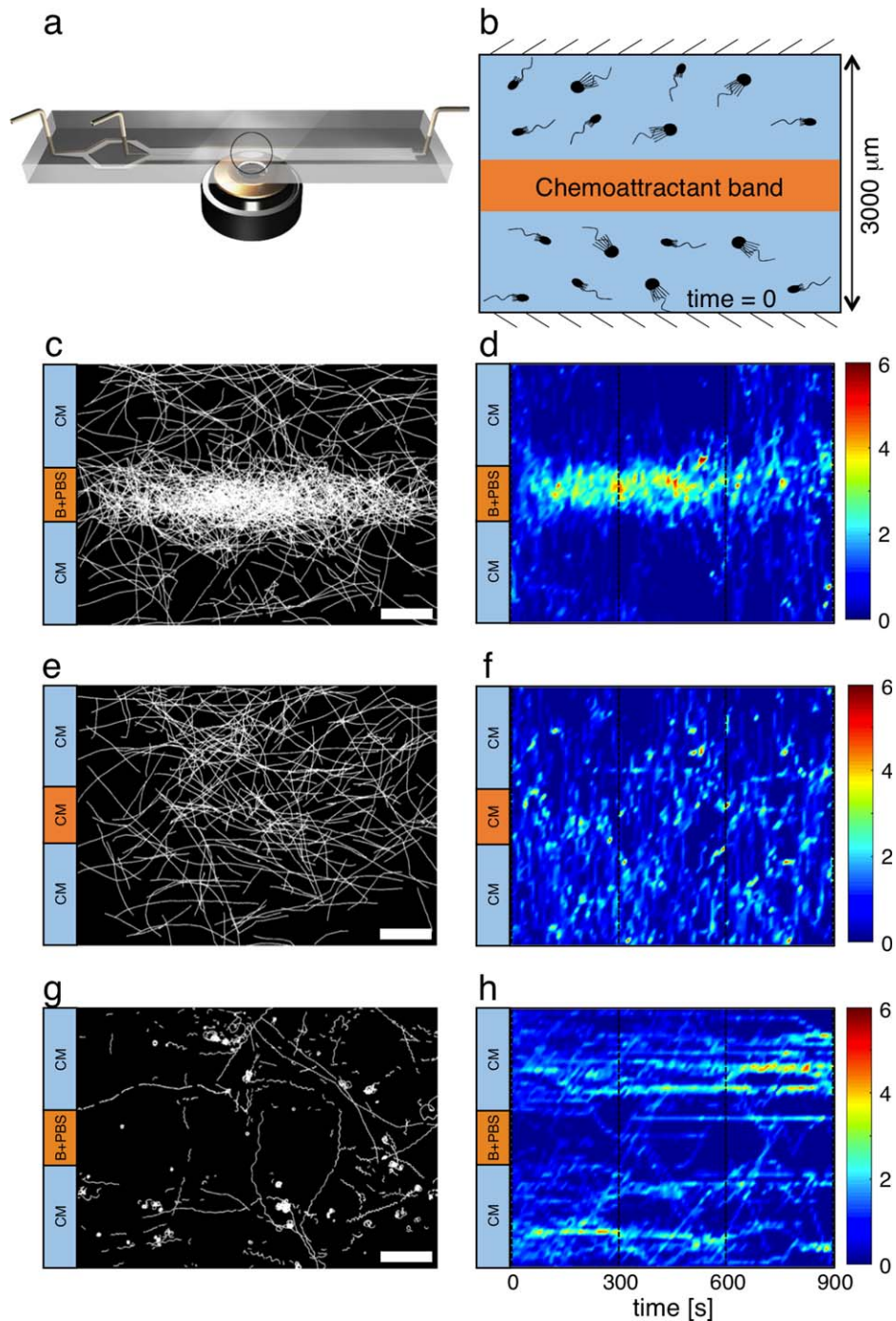
Fig. 1. Chemotaxis by the choanoflagellate *S. rosetta*. (a) Schematic view of the microfluidic chemotaxis assay, and (b) zoomed-in view of the observation region. The device consists of a microfluidic injector through which chemoattractant was injected as a 600-μm wide band (through the middle inlet in panel a), into a channel otherwise filled with a suspension of choanoflagellate cells (injected through the left-most inlet in panel (a)). In panel (b), cells are not drawn to scale (actual length $\sim$ 5 μm) and diagonal lines denote the sidewalls of the channel. (c) Trajectories of unicellular choanoflagellates exposed to a band of *A. machipongonensis* bacteria suspended in PBS ("B + PBS"), observed over 900 s after cells were exposed to the band. (d) Normalized distribution (color scale) across the width of the channel (vertical axis) of the cells from the experiment in panel (c), plotted as a function of time after exposure to the central band (in this case, PBS). Note the intense accumulation of choanoflagellates in the central band during the first 300 s, showing strong chemotaxis. At longer times, the chemoattractant band diffuses and cell accumulation ceases. (e) Control experiments showing trajectories of unicellular choanoflagellates exposed to a band of conditioned medium ("CM," prepared by filtration of a choanoflagellate culture; *see* Methods), as in the rest of the chamber, observed over 900 s as in panel (c). (f) Normalized distribution across the width of the channel (vertical axis) of the cells from the experiment in panel (e), plotted as a function of time. Note the absence of accumulation in the center, indicative of a lack of chemotaxis, for this control case. (g) Trajectories of rosettes exposed to a band of *A. machipongonensis* bacteria suspended in PBS, observed over 900 s after cells were exposed to the band. (h) Normalized distribution across the width of the channel (vertical axis) of the cells from the experiment in panel (g), plotted as a function of time. Note the absence of accumulation in the center, indicative of a lack of chemotaxis. Scale bar = 600 μm.
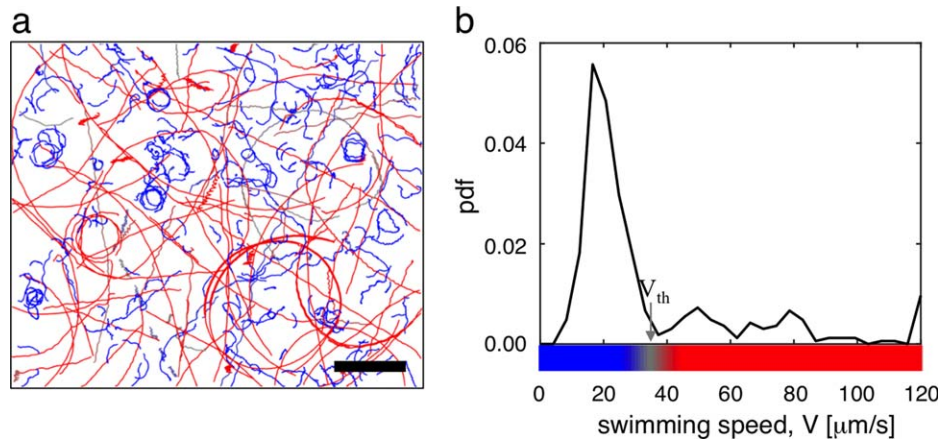
Fig. 2. Diversity of swimming speeds and trajectories in cultures of unicellular *S. rosetta*. (a) Trajectories of choanoflagellates swimming close to the bottom surface of the culture flask, collected at the beginning of stationary phase ($t = 120$ h), over 30 s. Each trajectory is colored by its mean speed (*see* x-axis of panel (b) for color-coding). Note that slow swimmers (blue) followed looping trajectories, whereas faster (red) cells follow slightly curved trajectories that approach linear. Scale bar $= 100$ μm. (b) Probability density function (pdf) of the swimming speed (a measure of the distribution of swimming speeds within the population) for the same culture ($n = 407$ trajectories).

chemical to be tested, and the region extending on both sides of the band containing the choanoflagellates.

Taxis experiments were performed in triplicate with choanoflagellate concentrations of $\sim 10^6$ cells mL^{-1}. In experiments where bacteria were tested as potential chemoattractants, overnight cultures of *A. machipongonensis* were grown in 10% CGM, centrifuged and washed twice in CM. Moderate variations in the initial cell concentration among experiments are unlikely to have affected results, because even at cell concentrations of $\sim 10^7$ cells mL^{-1} individual cells are ~ 45 μm (i.e., many body lengths) from each other, making cell-to-cell contact unlikely.

Imaging and motility characterization

An inverted microscope (Ti-Eclipse, Nikon) was used with a Neo sCMOS digital camera (Andor Technology) to image cell responses to chemoattractants. Image analysis and cell tracking were performed with ImageJ and TrackMate (NIH) and MATLAB. For chemotaxis characterization, image sequences were acquired at 10 frames s^{-1} for 300–900 s at 10 mm from the tip of the injector using dark-field microscopy and a 4× objective (field of view $= 3000 \times 4200$ μm^2). For motility characterization, image sequences were acquired at 20 frames s^{-1} for 30 s using bright-field microscopy and a 20× objective, near (but not directly on) the bottom of the culture flask. Spatiotemporal diagrams were computed by dividing the channel width into 60 bins and for each bin (area $= 50 \times 4200$ μm^2) the choanoflagellate numbers were averaged over 10-s windows.

The probability distribution function (pdf) of the swimming speed (i.e., the relative occurrence of different speeds within the population) indicated the presence of two types of swimmers, fast and slow (Fig. 2; $n = 407$ trajectories), corresponding to mean speeds of 19.1 ± 7.1 μm s^{-1} and

56.3 ± 41.8 μm s^{-1} (mean $\pm$ sd, determined by fitting the pdf with the superposition of two Gaussians). The transition between the two groups occurs at 30–40 μm s^{-1} and we therefore used a threshold of $V_{th} = 35$ μm s^{-1} to compute the fraction of slow and fast swimmers in each sample.

Results

S. rosetta performs directional migration

The chemotaxis experiments were set up in a microfluidic device that generates a narrow (~ 600 μm wide) band of a potential attractant along the midline of a channel 3000 μm wide and 100 μm deep (Seymour et al. 2008). This "band" is established by flowing the hypothesized attractant through a central microinjector and by flowing a suspension of choanoflagellates on either side of the band (Fig. 1a,b; Methods). At time zero, the flow is stopped, allowing the lateral diffusion of the attractant and the creation of attractant gradients to which choanoflagellates can respond by swimming toward the band (attraction) or away from it (repulsion). Chemotaxis was quantified by live imaging and image analysis, recording the positions of choanoflagellates at 10 frames s^{-1}.

Motivated by the fact that bacteria represent a primary nutrient source for choanoflagellates, we first tested whether a suspension of bacteria elicited directional migration by *S. rosetta*. We used a concentrated suspension of *A. machipongonensis*, washed and resuspended in PBS solution, then injected as the central band in the microdevice (Fig. 1b). Unicellular choanoflagellates exhibited strong directional migration into the bacterial suspension (Fig. 1c,d; Supporting Information Movie SV1), accumulating in the central band and indicating that unicellular swimmers of *S. rosetta* are capable of taxis. In contrast, rosettes showed no taxis in response to bacteria suspended in PBS (Fig. 1g,h).
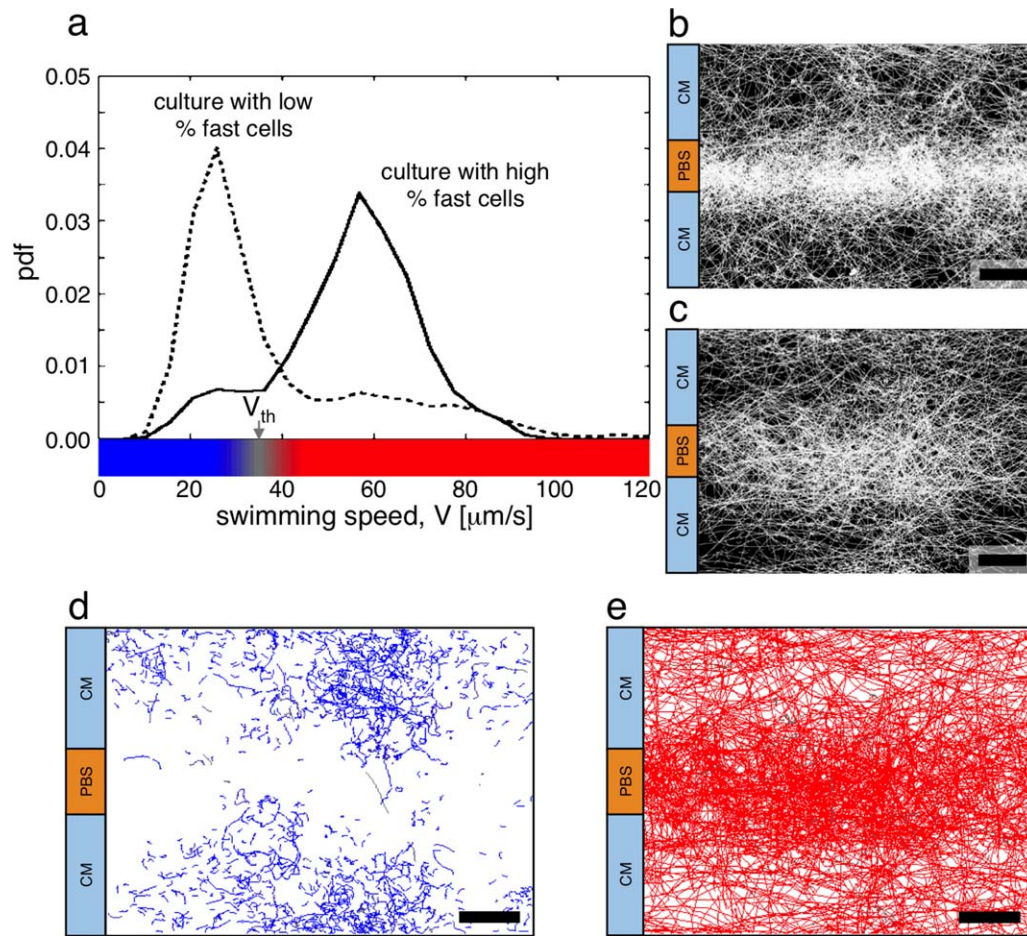
Fig. 3. Taxis is performed by unicellular fast swimmers, but not by slow swimmers. (a) Swimming speed distribution (probability density function, pdf) of individual cells in two cultures with different proportions of fast-swimming choanoflagellate cells, obtained with two different growth protocols (Methods). The arrow indicates the speed threshold, $V_{th} = 35$ μm s^{-1}, used to separate fast (red) from slow (blue) cells. In the first culture (dashed line), 11% of cells were fast swimmers, whereas in the second culture (solid line) the fast swimmers represented 87% of all cells. (b) Taxis experiment toward PBS performed with the choanoflagellate culture containing 87% fast swimmers (corresponding to the solid line in panel (a)), observed over 900 s after cells were exposed to the band. (c) Same as (b), but for the choanoflagellate culture containing 11% of fast swimmers (corresponding to the dashed line in panel (a)). Note the reduced accumulation of trajectories in the central band, relative to case (b). (d,e) When the trajectories of slow swimmers and fast swimmers in a culture composed of 11% fast swimmers (dashed line in panel (a)) are plotted separately, it becomes clear that slow swimmers ($V < V_{th}$; panel (d); in blue) fail to accumulate in the PBS band, whereas fast swimmers ($V > V_{th}$; panel (e); in red) swim toward and accumulate in the PBS band. Scale bar = 600 μm in panels (b–e).

To determine which component of the bacterial suspension induced the observed taxis response, we exposed unicellular swimmers to a band of *A. machipongonensis* washed and resuspended in the CM (*see* Methods). No taxis was observed (Supporting Information Fig. S3a,b), suggesting that the bacteria themselves were not the source of the cue. In contrast, when we exposed planktonic, unicellular *S. rosetta* to a band of PBS lacking bacteria, we observed strong taxis, resulting in accumulation of cells within a few tens of seconds (Supporting Information Fig. S4a,b). As a further control, we verified that CM did not cause taxis (Fig. 1e,f). Rosettes did not respond to PBS, to CM, or to bacteria in CM (Supporting Information Fig. S5). Together, these experiments revealed

that PBS is sufficient as a source of attraction for unicellular *S. rosetta*.

To quantify the taxis response, we computed the chemotaxis index, $I_C = \text{Cells}_{IN}/\text{Cells}_{OUT}$ (Stocker et al. 2008), which measures the ratio of the cell concentration within the central 600 μm band (Cells$_{IN}$) to that outside the band (Cells$_{OUT}$). Values of $I_C > 1$ denote accumulation of cells in the central band (attraction). The chemotaxis index (Supporting Information Fig. S4c) confirms the strength of the response to PBS, with the cells inside the central band reaching a concentration 5.4-fold higher than the average concentration in the channel (mean of I_C between 200 s and 400 s; Supporting Information Fig. S4c). For comparison, the chemotaxis

index of *Escherichia coli* did not exceed 2 under nearly optimal conditions (Stocker et al. 2008; Garren et al. 2014).

Fast swimmers, but not slow swimmers or rosettes, perform taxis

To determine which form(s) of unicellular *S. rosetta* perform taxis, we measured swimming speeds to distinguishing fast- from slow-swimming cells (Dayel et al. 2011). The distribution of swimming speeds within different *S. rosetta* cultures (Fig. 2; Methods) was found to display two peaks (Fig. 2b), one above and one below a speed of $V_{th} = 35$ μm s^{-1}, which we chose as the operational threshold speed to separate slow and fast swimmers. The considerable difference in the swimming patterns of cells swimming slower and faster than this threshold (Figs. 2a, 3d,e) further corroborates the distinction of two speed classes.

Two observations revealed that only fast swimmers performed taxis in response to PBS. First, a comparison of the accumulation of cells from two cultures, one with a high and one with a low percentage of fast swimmers (Fig. 3a; Supporting Information Fig. S1; Methods), revealed a considerably stronger accumulation in the former than the latter (Fig. 3b,c). Second, a comparison of the accumulation of slow ($V < V_{th}$) and fast swimmers ($V > V_{th}$) from the same experiment performed in post-processing revealed that slow swimmers did not migrate into the PBS band, even when given ample time (900 s), whereas fast swimmers did (Fig. 3d,e).

The observed taxis is a response to pH gradients and its direction is pH-dependent

A systematic search revealed that pH gradients were responsible for the taxis to PBS. We reached this conclusion by modifying the concentration of particular substances in ASW and using those solutions in the central band, while choanoflagellates suspended in CM were introduced into the rest of the channel. No accumulation was observed in response to ASW containing the same concentration of K$^+$ (10 mM) or PO$_4^{3-}$ (20 mM) as in PBS (Supporting Information Fig. S3c–f), ruling out K$^+$ and PO$_4^{3-}$. The pH of the choanoflagellate cultures (8.0; Methods) was higher than the pH of PBS (7.0), so we next investigated pH as the cue. When the pH of ASW (8.0) was adjusted by addition of hydrochloric acid (HCl) to 7.0 and used as the candidate attractant, *S. rosetta* accumulated in the central band to levels comparable to those elicited by PBS (Supporting Information Fig. S4). This is also apparent in the values of the chemotaxis index, which in both cases reached values considerably above 1, over a comparable time (100–200 s) (Supporting Information Fig. S4a,c). Although we cannot rule out that additional components of PBS also elicited taxis, these results indicate that pH differences are sufficient to elicit taxis. Such directional motion of cells along pH gradients has previously been observed for bacteria (Seymour and Doetsch 1973; Tsang et al. 1973; Croxen et al. 2006;

Zhuang et al. 2015), *Dictyostelium discoideum* (Bonner et al. 1985), and fungal and algal zoospores (Morris et al. 1995; Govorunova and Sineshchekov 2005).

The response of *S. rosetta* to different values of pH (from 5.2 to 9.0) was investigated by running experiments in which we changed the pH of the ASW band (Supporting Information Fig. S2a). Cells moved away from a band with pH = 5.2 and accumulated at the sidewalls (Fig. 4c), suggesting repulsion from very low pH. When the pH of the central band was between 6.0 and 7.0, cells swam from the CM (pH = 8.0) to the band (Fig. 4e,f). When the pH was 8.0 both outside the band (CM) and within the band (either CM, Fig. 4b; or ASW with pH = 8.0, Fig. 4g), no accumulation was observed. Moreover, when the pH of the central band was higher (pH = 9.0) than that of CM, no accumulation was observed (Fig. 4h). Together, these experiments indicate that *S. rosetta* fast swimmers swim towards a preferential pH range of 6–7, considerably lower than the typical pH of seawater (pH = 8.18 ± 0.15 [Marion et al. 2011]). Additional analysis revealed that the observed accumulations are a true case of taxis, rather than being caused for example by modulation in swimming speed, and are consistent with a strategy to seek the source of the pH gradient, rather than optimal physiological pH conditions per se.

The cell accumulation resulted from directional swimming, rather than from a local reduction in swimming speed, which would also cause accumulation (Schnitzer 1993). To show this, we measured choanoflagellate swimming speeds at different pH values, and found no change in speed even after 3 h (Fig. 5a, Supporting Information Fig. S2b). Instead, we directly observed directional swimming into the band. Cells swam toward the central band of PBS from both sides of the channel during the first $\sim$ 100 s (shown by blue in the area above the PBS band and by red in the area below it in Fig. 5b). The timescale of 100 s is consistent with the lifetime of the pH gradients. Using the diffusion coefficient of H$^+$ ions in seawater at 25°C ($D = 9.31 \times 10^{-9}$ m^2 s^{-1} [Yuan-Hui and Gregory 1974]), we solved the one-dimensional diffusion equation across the width of the microchannel to predict the evolution of the pH profile over time (Fig. 5c) (without taking into account the buffer capacity of ASW). This computation showed that after a timescale in the order of 100 s, pH gradients became small across the channel. Thus, the timescale of directional, inward swimming corresponds to the timescale over which pH gradients persisted.

Discussion

We hypothesized that seeking regions with a more acidic pH than average seawater helps choanoflagellates find patches rich in bacterial prey. Bacterial metabolic products can acidify the water when they grow in high concentrations (Anderson et al. 1920; Solé et al. 2000) and bacterial
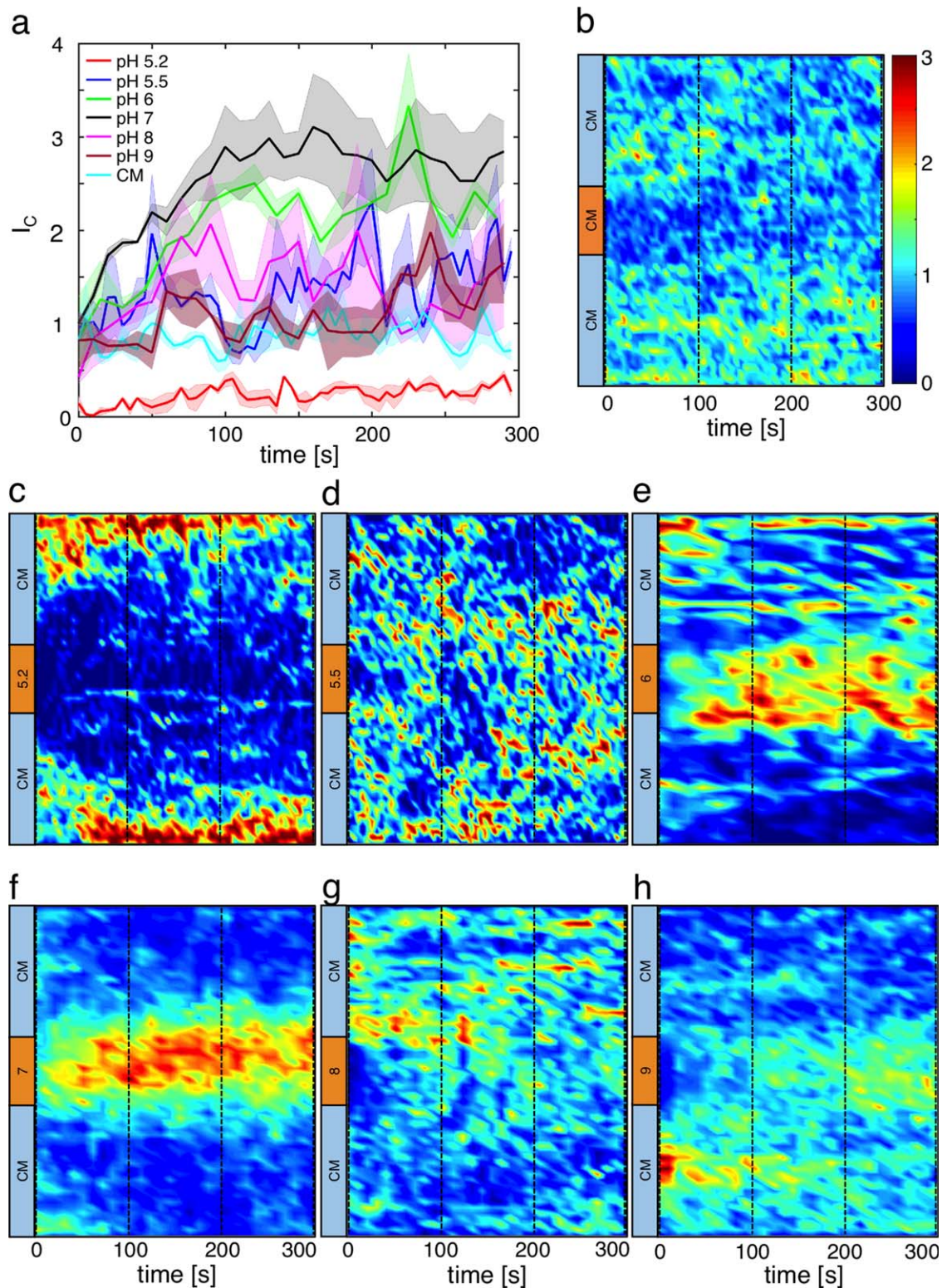
Fig. 4. Unicellular *S. rosetta* perform pH-taxis, a response to gradients in pH. (a) Chemotaxis index (I_C) plotted as a function of time from the onset of an experiment. The index I_C, a measure of the strength of taxis, is the ratio of the number of cells inside the central band (600 μm wide) and the number of cells in the rest of the channel width. $I_C = 1$ denotes no taxis (uniform cell distribution), $I_C > 1$ denotes positive taxis (attraction), and $I_C < 1$ denotes negative taxis (repulsion). Lines and shading represent the mean and SE of three replicate experiments. (b) Distribution of choanoflagellate cells over the channel width (vertical axis) in a control experiment with CM (pH = 8) in the central band. (c–h) Distribution of unicellular choanoflagellates over the channel width relative to a central band of ASW amended to pH 5.2 (c), pH 5.5 (d), pH 6 (e), pH 7 (f), pH 8 (g), and pH 9 (h). Note the strong positive taxis response to pH = 7, the somewhat weaker positive response for pH = 6, the negative response (repulsion) for pH = 5.2, and the absence of a response for other pH values. pH-adjusted ASW (rather than CM) was used in the experiments in panels (c–h) to precisely control pH, since CM may contain additional metabolites that affect pH.
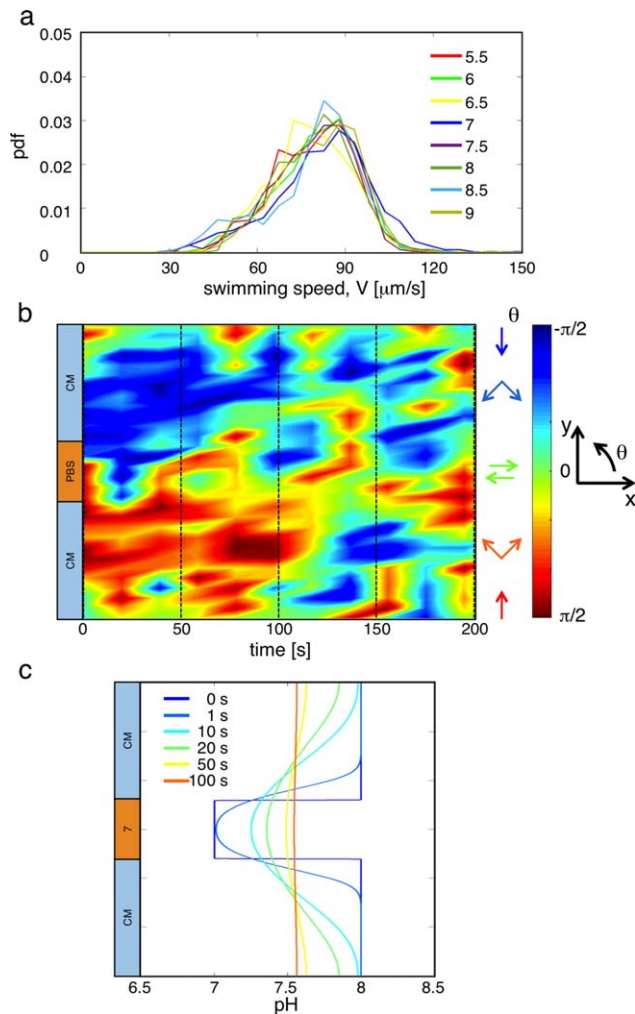
Fig. 5. The response of unicellular *S. rosetta* to pH is a true case of taxis. (a) *S. rosetta* shows no change in swimming speed when grown at a range of different pH values. The distribution of swimming speeds (probability density function, pdf) within a population obtained for fast swimmers in ASW with different pH values. (b) Swimming direction as a function of the position of cells across the width of the channel, plotted as a function of time. The central band contained PBS (corresponding to the experiment in Fig. 3b). Color denotes swimming orientation, as indicated by arrows next to the scale bar. The angle of the instantaneous swimming directions of all the cells at each time were measured relative to the along-channel direction, so that an angle of $\pi/2$ (red) corresponds to swimming upward in the plot and an angle of $-\pi/2$ (blue) corresponds to swimming downward in the plot. Over the initial 100 s there was directional swimming into the central band: upward from below and downward from above the band. (c) The temporal evolution of the pH gradient in the microchannel. The solution of the one-dimensional diffusion equation for H^+ ions across the channel width is shown at different times after the injection of the central band.

biofilms have lower pH than the surrounding water (Liermann et al. 2000; Hidalgo et al. 2009). The observation that low values of pH cause repulsion suggests that intermediate acidic conditions ($5 < pH < 7$) are most indicative of bacterial patches, which is consistent with observations of pH in

this range in bacterial biofilms (Hidalgo et al. 2009). In the environment, more extreme acidification may also be prevented by the diluting effect of fluid flow and diffusion, both on benthic surfaces and on marine particles. We thus suggest that the fast-swimming form of *S. rosetta* can pursue pH cues to move rapidly into microenvironments rich in bacterial prey.

Feeding performance of bacterivorous eukaryotes depends on two processes: finding prey-rich microhabitats and capturing prey. Our discovery that only the fast-swimming stage of *S. rosetta* uses taxis is consistent with the hypothesis that fast swimmers are dispersers that can find new prey patches. Rapid swimming is often a feature of chemotactic marine microorganisms (Stocker and Seymour 2012). However, *S. rosetta* fast swimmers have reduced collars and no doubt capture bacterial prey at a lower rate than the long-collared slow swimmers and rosette colonies. We suggest that there is a trade-off between the ability to swim rapidly to find new prey patches and the ability to capture bacteria at a high rate. Larger collars may themselves reduce swimming speed, as they cause more drag. These hypotheses suggest a division of labor between different life cycle stages in choanoflagellates.

In laboratory cultures of fast swimmers, it takes several hours for the long-collared thecate cells and slow swimmers to be produced (Dayel et al. 2011). Therefore, having separate searching and feeding life stages would not enable choanoflagellates to take advantage of ephemeral microscale hotspots in the water column that dissipate in minutes (Stocker 2012; Stocker and Seymour 2012). However, choanoflagellates settle onto marine snow particles and detritus (reviewed in Leadbeater 2015), and *S. rosetta* live in shallow estuaries where dense consortia of bacteria form on suspended particles and benthic surfaces. Having separate searching and feeding stages could be an effective strategy for utilizing particles and their wakes that are rich in bacteria (Kiørboe and Jackson 2001; Stocker et al. 2008), or for foraging near benthic surfaces that continuously shed filaments of resources in turbulent ambient water flow (Crimaldi and Koseff 2001; Koehl et al. 2007). Fast swimmers can attach rapidly to surfaces using filopodia, and then develop into long-collared thecate cells (Dayel et al. 2011). If fast swimmers can find and attach to particles or benthic surfaces, then the long-collared thecate cells would be in the prey-rich boundary layer of water next to these surfaces, and subsequent production of long-collared, slowly-swimming forms would place them in the resource-rich plumes shed by particles and substrata.

Directional responses to pH gradients may in general serve a range of functions, though in few cases has direct evidence for the functional role of pH-taxis been reported. Bacteria have been suggested to migrate away from regions of very low pH to avoid physiologically damaging conditions (Seymour and Doetsch 1973) or overcrowding (Tsang et al. 1973). Slime molds may migrate toward low pH because

acidic environments enhance fruiting (Bonner et al. 1985) and fungal zoospores may accumulate in a pH range that enhances encystment (Morris et al. 1995). While we cannot completely rule out that pH taxis in choanoflagellates serves a similar role, of allowing cells to reach physiologically optimal conditions, its occurrence only in the rapid swimming stage of choanoflagellates suggests a prey-seeking function of pH taxis.

Although *S. rosetta* were collected in a shallow muddy estuary, little else is known about their ecology. The colonization by *S. rosetta* of marine snow should be investigated, but studies of particles suggest that it would be feasible for fast swimming choanoflagellates to find them. The success of a chemotactic strategy for finding sinking marine particles depends on the competition between the timescale of availability of the chemical signal and the timescale of the chemotactic response. However, this does not simply reduce to a comparison of the sinking speed of particles (often $\sim$ 100–1000 μm s^{-1}) and the swimming speed of the organisms ($\sim$ 50 μm s^{-1}), because of the boundary layer around the particles. Indeed, considerable attachment to sinking particles was found in a model of bacteria swimming at speeds comparable to those observed here for *S. rosetta* (Kiørboe and Jackson 2001). The same model showed that these swimming speeds are sufficient to move from particle to particle under typical particle concentrations in the ocean. We thus propose that the chemotactic responses described here will be relevant under natural conditions and enable fast-swimming choanoflagellates to exploit pH gradients associated with biofilms, enabling the choanoflagellates to colonize food-rich marine particles and surfaces.

Studies of bacteria and of microscopic larvae using chemical cues to find benthic surfaces suggest that choanoflagellate fast swimmers might also be able to do so. For example, in still water, pathogenic bacteria that swim at speeds comparable to those of *S. rosetta* can successfully use chemotaxis to coral exudates to reach and infect their coral hosts (Garren et al. 2014). Even in turbulent flow across benthic surfaces that continuously release chemical cues, the frequency of encounters with filaments of cue is very high near the surfaces, and the behavior of weakly swimming microorganisms can bias how they are transported to the surfaces by the ambient flow (Koehl et al. 2007; Koehl and Cooper 2015). These hypotheses could be tested in experiments in which choanoflagellates are exposed to biofilms, preferably grown under controlled conditions, for example in microfluidic devices, and both the pH conditions (e.g., through optodes) and the choanoflagellate distribution are measured.

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