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The influence of viscosity on the motility and sensory ability of the dinoflagellate *Heterocapsa triquetra*.

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Abstract:

Seawater viscosity is influenced by temperature as well as through excretion of exopolymers by some plankton. We examined the role of viscosity on the movement patterns and sensory abilities of the dinoflagellate *Heterocapsa triquetra*, manipulating the viscosity of seawater to simulate a 10 ±1.5 °C temperature change. In a second treatment, we seeded the water with microbeads to examine swimming behaviours in the presence of a mechanical stimulus. Increased viscosity reduced distances between conspecifics 4.7 fold and increased distances between protists and microbeads by 3.4 fold. Increased viscosity also affected other aspects of motility, with an overall reduction in swimming speed of 2.0 and 7.0 fold for treatments with and without mechanical stimuli. Higher viscosities were associated with upward vertical migration, in both the presence and absence of microbeads. Cells were highly sensitive to disturbances to the velocity field, by as little as 1.5%, and different approach distances of *H. triquetra* to conspecifics over mechanical stimuli suggests sensory capacity to distinguish types of particles. Mediation of motility and migratory behaviours through viscosity implies ramifications for the distribution of protists and their encounters with resources, predators and conspecifics triggered by events such as temperature changes and phytoplankton bloom events.

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Introduction

Planktonic organisms are constantly moving, either passively via entrainment in the surrounding (usually turbulent) fluid, or through active propulsion. Through the act of locomotion and associated fluid movement, a plankter leaves behind chemical compounds and fluid disturbances in its wake that can be sensed and followed by conspecifics and predators, or avoided by prey, using chemo- or mechanosensory abilities (Yen 2000; Weissburg 2000; Codling et al. 2010).

Due to their small sizes, planktonic organisms generally operate at low Reynolds numbers (Re), where the fluid dynamics governing their actions are non-intuitive and the effects of viscosity dominate those of inertia. Although there have been studies investigating the influence of viscosity on fish larvae (Johnson et al. 1998), zooplankton (Podolsky and Emlet 1993; Podolsky 1994) and bacteria (Chen and Berg 2000), the influence of viscosity on behaviours in protists is poorly understood. Viscosity affects the transmission of fluid perturbations through the water such that for a given power output, the strength of the resulting fluid perturbation some distance away will be inversely related to viscosity. Viscosity might also affect signal generation (e.g. through qualitative changes in flagellar motion (Qin et al. 2015)), as well as signal detection (e.g. varying the ability to detect a given perturbation strength at the sensor location). Viscosity changes in nature are most commonly driven by changes in temperature, with the two being negatively correlated (Podolsky and Emlet 1993). The viscosity and viscoelastic properties of seawater are also influenced by the presence of biopolymers, proteins and macromolecules (Qin et al. 2015), and some phytoplankton are able to alter the local viscosity of seawater through the excretion of mucous exopolymers (Prairie et al. 2012; Seuront et al. 2007; Seuront et al. 2010).

Protistan motility not only transmits information via fluid disturbances, but also has a direct relationship to encounter rates with particles such as predators, prey, and conspecifics. Animal movement patterns are often classified as either diffusive or ballistic (Codling et al. 2008), or may lie between these two ideals: At a sufficiently small scale, organism movement appears straight and highly correlated (ballistic), but the accumulated effects of random turns or reorientations typically cause trajectories to appear convoluted at larger scales, akin to diffusive transport of molecules. The fundamental differences between diffusive and ballistic movements lead to predicted encounter rates between organisms moving in the ballistic regime that are much higher than those in the diffusive regime. This is due to displacement increasing linearly with time in the ballistic case (as opposed to non-linear displacement in the diffusive case), with no back-tracking of previously transited space (Visser and Kiørboe 2006). Taylor's equation (Taylor 1921; Codling et al. 2008) can be used to describe the transition between these two movement regimes in a particle such as a dinoflagellate undergoing a continuous random walk, with the transition occurring at what are termed the correlation time- and length-scales. For an application of these theoretical considerations see Schuech and Menden-Deuer (Schuech and Menden-Deuer 2014).

Plankton generate fluid disturbances by moving through water (Jiang and Paffenhöfer 2008; Jiang and Osborn 2003; Kiørboe and Visser 1999; Kiørboe 2013; Svensen and Kiørboe 2000). If a plankter becomes entrained in an external fluid flow, such as the flow field generated by a feeding copepod, then at a large distance from the copepod, the 'perception' of acceleration and deformation to its own flow field falls below a threshold value and elicits no escape response (Jiang and Osborn 2003). Only when the plankter is sufficiently close to the copepod is a sensory threshold for flow field perturbation reached (Jakobsen 2001). This sensing of flow perturbations could theoretically be bidirectional such that a motionless ambush predator might detect swimming plankters, and a swimming plankter might detect a motionless ambush predator (Zaret 1980; Bundy et al. 1998; Visser 2001). These ideas have been supported by experiments: the free-swimming copepod *Diaptomus sicilis* (length 1.2 mm) was observed to detect and ‘attack’ polystyrene beads suspended in the water body when within a distance of approximately one body length (1 mm) (Bundy et al. 1998), and swimming dinoflagellates (*Heterocapsa rotundata, Gymnodinium simplex* and *Rhodomonas salina*) approaching the neutrally buoyant and passively drifting ciliate *Mesodinium pulex* were able to detect the ciliate and escape capture in response to hydromechanical cues (Jakobsen 2001). Nonetheless, a mechanistic understanding of sensory capabilities of plankters has been hindered by lack of experimental data.

While hydrodynamic disturbances due to conspecifics might be identified as being from non-threatening moving bodies of similar size, the inert microbeads in our experiments might be perceived as, stationary ambush predators (Jakobsen 2001; Titelman 2001; Jakobsen et al. 2006; Gemmell et al. 2013; Kiørboe 2013). Here we investigate the influence of both viscosity and microbeads on the swimming behaviour of the mixotrophic marine dinoflagellate *Heterocapsa triquetra* (diameter 25 μm) at a range of manipulated viscosities corresponding to a 10° C temperature change. We manipulate viscosity using the polymer Ficoll, which has been shown to increase viscosity with no significant effect on fluid density or protist behaviour and can be modelled with Newtonian dynamics (Beveridge et al. 2010). The use of Ficoll gives a uniform polymer mesh size and allows for shear tress vs shear rate curves to be described with Newtonian hydrodynamics (Martinez et al. 2014). Although plankton are typically embedded in background shear flow in the environment, in the interest of distinguishing organism autonomous motility from advection here we only address swimming in still water. We predict that lower swimming speeds and lower turn rates should be associated with higher viscosity due to the increased energy costs associated with swimming (Dusenbery 2009). We use 3D video capture to determine the effect of changes in viscosity on sensory ability (estimated using nearest neighbour analysis) and other motility parameters, in both the presence and absence of large inert microbeads (63 to 75 μm in diameter). Specifically, we make predictions of biological sensitivity to flow perturbations from a model that has been shown to be reasonable in cases of copepods detecting microbeads (Visser 2001). Although the fluid surrounding the protist behaves in a simple linear way versus viscosity, the mechanical behaviour of the cell membrane or sensory organs may not behave in a simple way as viscosity changes. For example, if viscosity increases and the cell moves at the same speed as before, the hydrodynamic forces on it increase proportionally, but properties of the cell such as mechanical stiffness might change in a nonlinear way, resulting in nonlinear changes in sensitivity when viscosity changes. In addition to nearest neighbour distance (nearest approach to objects), we quantify changes in other protist motility parameters (e.g. swimming speed, vertical migration rate, correlation time and length scales) with viscosity and the presence of microbeads.

Methods

Culturing

Cultures of *Heterocapsa triquetra* (Stein, 1883) were reared in sterile f/2 (Guillard 1975) nutrient-enriched filtered sea water (FSW). The strain was derived from Narragansett Bay, RI, USA and has the internal strain designation PA160310. Water for culturing and experimentation was collected from Narragansett Bay (Rhode Island, USA), filtered with a 0.2 µm column filter, and autoclaved to remove particulate matter. Cultures were refreshed twice weekly with fresh culture medium. Cell density was determined every 12 hours using a Coulter Counter (Kim and Menden-Deuer 2013), with only cultures in exponential growth phase used for experiments. Cultures for all experiments were maintained in a 12:12h light/dark cycle at 15°C and a salinity of 31.6‰.

Experimental set up

All experiments were undertaken in transparent, octagonal, acrylic tanks, 30 cm tall, 5 cm wide, each with an internal volume of 1 L. The stereoscopic filming apparatus was calibrated using submerged grid plates, following Harvey and Menden-Deuer (Harvey and Menden-Deuer 2012; Harvey and Menden-Deuer 2011). All video footage was recorded in the horizontal centre of the filming tank to minimise possible wall effects. Five equally spaced, vertical horizons were filmed for three minutes every hour, for a six hour period, to provide 30 three-minute videos per replicate. Three replicates were performed for each treatment, resulting in a total of 90 three-minute videos per treatment. To ensure a sufficient number of organisms to allow robust statistical analysis of behaviours to be carried out, a density of approximately 500 (range 492 to 523) cells ml^{-1} was used in all treatments.

Control replicates were performed using FSW at an ambient temperature of 24 °C ± 1.5 °C and 0.99 centipoise (cP). We used the nontoxic non-ionic sucrose polymer Ficoll (Sigma-Aldrich) molecular weight 400k ± 100k, to artificially manipulate the viscosity of FSW at 24 °C ± 1.5 °C to reflect that of 20 °C (1.04 cP) and 15 °C (1.182 cP) seawater, an increase of 9.2% low to medium viscosity and 23.6% low to high viscosity independent of any temperature change. To ensure changes to viscosity were temperature independent, temperature in the room was maintained at 24 +- 1.5 °C. This resulted in three treatment viscosities that we define for brevity as low, medium and high viscosity (equivalent to seawater at 25 °C, 20 °C and 15 °C respectively) both with (WS) and without (NS) mechanical stimuli, giving a total of six treatments. Viscosity was measured using an Ubbelohde type viscometer (Cannon C457) with a 2.5% positive difference between the measured viscosity and the tabulated (Kennish 1989), viscosity of seawater at each of our reference temperatures. Treatments were then repeated with the inclusion of chemically inert, neutrally buoyant plastic microbeads (Cospheric, Santa Barbra, USA), 63 to 75 μm in diameter, at a concentration of approximately 500 beads ml^{-1}, to assess behavioural changes in the presence of passive mechanical stimuli.

Video capture and analysis

Filming was carried out in a manner similar to Menden-Deuer and Grünbaum (Menden-Deuer and Grünbaum 2006), and Harvey and Menden-Deuer (Harvey and Menden-Deuer 2012; Harvey and Menden-Deuer 2011), in a dark room to minimise light disturbance and possible light-mediated behaviours. Due to logistic constraints the experiments were commenced between 13:00-17:00 during the latter half of the light phase of the cultures. Illumination of the experimental tanks was provided by infrared LEDs (960nm) mounted in a bank, giving dark field illumination of the protists within the tanks. Two HD cameras (Allied Vision Technologies, AVT 1394), attached to Nikon 60-mm Micro Nikkon lenses, gave an overlapping field of view. The cameras were used to capture 2D video images (15 fps), which were later assembled to yield 3D movement paths and associated movement statistics using custom Python and MATLAB (Version: 7.14.0.739) scripts.

Protists and microbeads were identified, distinguished from each other and tracked, based on pixel intensity, with an algorithm similar to that used by Harvey and Menden-Deuer (Harvey and Menden-Deuer 2012; Harvey and Menden-Deuer 2011). Identical parameters (e.g. tracking settings, background extraction method) were applied to all of the video footage. To ensure individual behaviour was representative of the population, only videos with more than 50 tracks were analysed. Swimming behaviours were calculated from cubic splines fitted to the 3D paths, subsampled at 0.07s intervals to match the frame rate of the original recorded video. These behaviours included estimates of instantaneous swimming speed and vertical migration speed (μms^{-1}), turning rate (rad cm^{-1}), and nearest neighbour distances (μm) between protists as well as between protists and the mechanical stimulus, at each time point for the duration of each recorded trajectory. Nearest neighbour distance was determined by a K-nearest neighbour algorithm.

Following Schuech and Menden-Deuer (Schuech and Menden-Deuer 2014), horizontal and vertical swimming kinematics were analysed separately due to a clear vertical bias in movement.

Correlation time (τ_{xy}) and length (λ_{xy}) scales for horizontal movement components were obtained using curve fits of our data to Taylor's equation (Taylor 1921; Schuech & Menden-Deuer 2013). Effective swimming speed in the x-y plane (V_{xy}), was also taken from the fitting of Taylor's equation. Horizontal diffusivity was then calculated as $D_{xy} = V_{xy}^2 \tau_{xy}/2$ and a large-scale diffusive migration range in the x-y plane over time interval *t* estimated as $H_{mr} = \sqrt{4D_{xy}t}$. However, vertical correlation scales τ_z and λ_z could not be calculated from fitting Taylor's equation as movements in the z direction remained ballistic for as long as the tracks were recorded. Therefore, we present minimum possible bounds on τ_z and λ_z, and average instantaneous vertical swimming velocity V_z directly calculated from the swimming trajectories. These average vertical speeds can provide an estimate of large-scale ballistic migration ranges over a time interval t: $V_{mr} = V_z t$. Similarly to previous studies (Heuschele and Kiørboe 2012; Schuech and Menden-Deuer 2014), Taylor's model allowed us to simultaneously assess the effects of swimming speed and correlation length scale on large-scale effective diffusivity in each of our treatments.

Velocity field sensitivity

To estimate the hypothesized velocity field sensitivity to microbeads in each manipulated viscosity, we used an equation from Visser (Visser 2001) (eqn 31, p15) describing a situation where passive particles were approached by a self-propelled predator:

$$R^3(R-a)^2 = 15a^2b^3\frac{U}{s} \quad [1]$$

where *a* is the predator (protist) radius, *b* is the radius of the stationary object the protist is swimming towards, *U* is the swimming speed of the protist, *s* (µm/s) is the threshold velocity perturbation required for detection by the protist and *R* is the distance at which detection occurs. We take *R* as the closest approach distance to a microbead over the duration of each track. By rearranging equation 1, we predicted an average threshold velocity perturbation by substituting our empirical values for average sizes, approach distances and speeds in the differing treatments:

$$s = \frac{15a^2b^3U}{R^3(R^2-2Ra+a^2)} \quad [2]$$

Statistical analysis

Comparisons of approach distances, components of swimming velocity in the horizontal plane and vertical direction (V_{xy}, V_z), and turning rates across all horizons, over all time points, and between replicates were carried out using a Kruskal-Wallis test followed by a posthoc test (Kruskal-Wallis pairwise comparison of means). If no significant differences in behaviours were found between time points or horizons between replicates of the same treatment, the datasets for each treatment were combined and the same statistical analysis was rerun with the aggregated dataset to compare for differences between treatments. Nonparametric tests were used due to the non-normal distribution of the data. This approach allowed us to compare nearest neighbour distances and movement parameters between all treatments and tanks in relation to manipulated viscosity and the presence/absence of the mechanical stimuli.

For all treatments, the sample size was the number of protists with sufficiently long tracks (> 5 s). All data processing was undertaken in MATLAB (v7.14), while statistical analyses were carried out in R (Version 2.15.1, 2012).

Results

Significance of time and vertical horizon

Filming horizon did not significantly affect the recorded approach distances between individual protists either in the presence ($\chi^2_{4,\ 5130} = 35.5$, $p > 0.05$), or absence ($\chi^2_{4,\ 6845} = 72.33$, $p > 0.05$) of microbeads. Likewise, approach distances between protists and microbeads were not affected by horizon ($\chi^2_{4,\ 3671} = 459.49$, $p > 0.05$). There were no significant differences in approach distances between protists in the presence ($\chi^2_{5,\ 5130} = 9.6$, $p > 0.05$) or absence ($\chi^2_{5,\ 6845} = 7.91$, $p > 0.05$) of microbeads across the duration of the experiments. There were also no significant differences in minimum approach distances to microbeads ($\chi^2_{5,\ 3671} = 7.05$, $p > 0.05$) over the duration of the experiments among replicates. Turning rate (T_r) varied significantly between treatments, with the number of radians per micron, τ (time between direction decorrelation) and λ (distance travelled between turning events) varying over time, in both in relation to changing viscosity, and the presence (with stimulus, WS) or absence (no stimulus, NS) (Table 1). Viscosity and the elapsed time during the experiment, had a significant effect on velocity in the x-y orientation (V_{xy}), again in both treatments (NS, WS). Example 3D swimming trajectories of *H. triquetra* in all treatments is shown in Fig 1.

Swimming speed

X-Y plane

Swimming speed in the x-y plane (V_{xy}) exhibited non-monotonic trends with viscosity, with a relative minimum at medium viscosity for NS treatments, but a relative maximum at the same viscosity for WS treatments. Overall, a decrease from low to high viscosity occurred in NS and WS treatments: V_{xy} decreased by 51% overall from 176 μm s^{-1} to 86 μm s^{-1} NS, and by 67 % overall from 78 μm s^{-1} to 25 μm s^{-1} WS (Fig 2, Table 1).

The presence of microbeads resulted in lower V_{xy} at the low and high viscosities, but resulted in a peak V_{xy} at the medium viscosity (Fig 2, Table 1). The non-monotonic behaviour of V_{xy}, both in regards to viscosity and the presence/absence of microbeads, was much larger in relative magnitude than the non-monotonicity of nearest approach distance.

Z-dimension

In all cases, mean V_z was in excess of 300 µm s^{-1} (Fig 3, Table 2) histograms of V_z in all treatments is presented in Fig 4. V_z increased substantially at the highest viscosity tested, both in the absence and presence of mechanical stimuli, which appeared to have almost no effect on vertical swimming speed at any viscosity.

Nearest approach distances

Nearest approach distances between individual protists were negatively correlated with increasing viscosity ($\chi^2_{2,\ 6845}$ = 647.32, $p < 0.01$). Distances between individual protists in filtered seawater lacking microbeads decreased by 12% (40 µm) between the low and medium viscosity treatments, and by 75% (204 µm) between the medium and high viscosity treatments, yielding an overall reduction of 78% (244 µm) between the lowest and highest viscosity treatments (Table 3). From trajectories we determined the density distributions of approach distances for protists in each treatment (Fig 5).

The presence of mechanical stimuli/beads significantly reduced nearest approach distances between protists compared to the no-stimuli cases, for the low and medium viscosity treatments ($\chi^2_{2,\ 5130}$ = 724.45, $p < 0.05$) (Table 3). Nearest approach distances decreased by 261 µm (from 309 to 48 µm, a reduction of 84%) and 234 µm (from 269 to 35 µm, a reduction of 86%) for the low and medium viscosity treatments respectively. However, in the high viscosity treatment, distances between protists increased by 24 µm (from 65 to 89 µm, an increase of 36%).

Nearest approach distances between individual protists and microbeads decreased slightly (5%) from low to medium viscosity (122 to 116 µm), but increased by 52% overall (122 to 186 µm, an increase of approximately 4 body lengths) between the lowest and highest viscosity treatments (Table 3).

For all viscosity treatments, distances to microbeads were consistently greater than those to other protists by at least a factor of two. In all cases, nearest approach distances were much less than would be predicted if protists and microbeads were uniformly arranged on a rectangular lattice throughout the tank volume; in that instance, the distance between particles for NS treatments would be 1241 μm, and 973 μm for WS treatments.

Velocity field sensitivity

Sensitivity values for velocity flow field perturbations, estimated from nearest approach distances between protists and microbeads according to equation 2, again behaved non-monotonically with increasing viscosity, initially increasing by more than a factor of three but with an overall decrease of 97% (Table 4).

Turning rate

Turning rate (T_r) was significantly different between viscosity treatments. In the absence of beads (NS) it showed a strong maximum at intermediate viscosity, while in the presence of beads (WS) T_r was much higher at medium and high viscosity than in low viscosity (Fig 6). The presence of microbeads significantly increased T_r at medium and high viscosities, but did not have a significant effect at low viscosity (Fig 6). In the absence of microbeads, λ_{xy} decreased by 40 % overall from low to high viscosity (668 to 396), while T_r increased by 389 % (0.037 to 0.181 radians per μm), so these trends were consistent, with both indicating more convoluted paths at higher viscosity. However, in the presence of microbeads, T_r still increased with viscosity by an order of magnitude (0.035 to 0.39 radians per μm), while λ_{xy} displayed non-monotonic behaviour with a minimum at medium viscosity.

Correlation scales

Correlation time (τ_{xy}) and length (λ_{xy}) scales in the horizontal plane displayed non-monotonic trends with viscosity. For NS treatments, τ_{xy} increased by 21% (3.79 to 4.58) while λ_{xy} decreased by 41% overall (668 to 396) (Table 1). This was concomitant with an overall 51% decrease in horizontal swimming speed V_{xy} (176 to 86 μm/s), indicating that the cells swam both slower and in a more convoluted pattern at the highest viscosity compared to the lowest. However, in the presence of mechanical stimuli, both τ_{xy} and λ_{xy} were positively correlated with viscosity, with τ_{xy} increasing by 232% (6.86 to 22.83), and λ_{xy} increasing by 6% (538 to 573), while V_{xy} again decreased (by 68% (78.4 to 25.12)) overall from the low to high viscosity treatments. Therefore, at high viscosity and in the presence of microbeads, cells swam slower, but along paths of approximately the same tortuosity compared to the lowest viscosity treatment.

Movement in the vertical (z) direction was ballistic for as long as our observations lasted in all treatments, so only minimum bounds on τ_z and λ_z are reported (Table 2). Interestingly, substantial fractions of upward and downward swimming individuals existed at the same locations and times during each experiment, as seen in (Schuech and Menden-Deuer 2014). However, vertical swimming direction of the population did exhibit a large change from mostly downward to mostly upward, between low and high viscosity conditions (Table 2).

Extrapolated large scale behaviour

Diffusivity in the horizontal plane (D_{xy}) was overall negatively correlated with viscosity (Table 1), decreasing by 71% in NS treatments (from 5.88×10^{-4} to 1.7×10^{-4} cm^2 s^{-1}), and by 66% (from 2.1×10^{-4} to 7.2×10^{-5} cm^2 s^{-1}) in the presence of microbeads (Table 1). In the absence of microbeads, the decrease in D_{xy} was due to both increased track tortuosity (indicated by lower λ_{xy}) as well as lower swimming speeds (V_{xy}). However, in the presence of microbeads, decreased diffusivity at high viscosity was almost entirely due to three fold reduced horizontal swimming speeds.

While horizontal displacement (H_{mr}) via diffusive movement would only be expected to cover several cm over 12 hours (Table 5), ballistic vertical migration range (V_{mr}) of *H. triquetra* over the same time period is predicted to range from 14 to 17 m depending on viscosity and presence/absence of microbeads (Table 5). Increased viscosity had a much stronger influence on *direction* of vertical migration, with 77% of cells swimming downwards at low viscosity, but 78% of cells swimming upwards at high viscosity in the absence of microbeads, with similar changes in frequency of upward/downward swimming cells in the presence of microbeads. At the end of all experiments, large numbers of cells were observed to be aggregating at the surface. Due to the limited extent of our observation volume, it is unknown whether all the cells in the tank displayed the same qualitative behaviour at a given time, or if e.g. there was a circulation of cells swimming up in the centre and down near the walls.

Discussion

Swimming speed

XY- plane

Swimming speed in the horizontal plane (V_{xy}), displayed non-monotonic behaviour with viscosity, with a relative minimum at medium viscosity with NS and a relative maximum with stimuli. V_{xy} were less than the observed average swimming speeds (xy) of 370 µm/s, and below the maximum 496 µm/s reported for *H. triquetra* by Jeong *et al.* (Jeong et al. 2002). Higher resolution microscopic observations are required to determine whether this trend is robust and due to varying flagellar beat frequency, beat pattern, or both. For instance, flagellar movements of the alga *Chlamydomonas reinhardtii* are influenced by changes in viscosity, but while beat frequency is positively correlated with increasing viscosity, net swimming speeds are negatively correlated (Johnson et al. 1998). Flagellar movement of *C. reinhardtii* close to the cell body was severely restricted when swimming in viscoelastic fluids, with movements displaying 'spatio-temporal' changes over a range of viscosities (Johnson et al. 1998). In the dinoflagellate *Prorocentrum minimum* beat frequency of the transverse and longitudinal flagella decrease with increasing viscosity with a subsequent reduction in swimming speeds (Sohn et al. 2013). Hence, both beat frequency and beat pattern can simultaneously change with viscosity in a range of organisms from sperm (Brokaw 1975; Rikmenspoel 1984; Smith et al. 2009) to bacteria (Atsumi et al. 1996) and dinoflagellates (Sohn et al. 2013) with the overall effect on swimming speed impossible to predict without detailed observations of the flagella (for more information see review by Brennen and Winet (1977), and Qin *et al.,* (2015)).

As with the non-monotonic trend of V_{xy} with viscosity in the NS treatments, the addition of microbeads decreased V_{xy} at low and high viscosity but increased V_{xy} at medium viscosity. Cell concentration during filming was 500 cells ml^{-1}, and the introduction of microbeads increased total particle density in the filming tank to 1000 particles ml^{-1}. It is logical to expect that swimming speeds would decrease with more particles in the water, as suggested by Zaret (Zaret 1980) or with increases in the energy required to swim at higher viscosites (Schneider and Doetsch 1974; Sleigh 1991; Humphries 2013), and this is indeed what we found at low and high viscosity. However, the opposite effect of microbeads at medium viscosity may be consistent with observations of copepods (Zaret 1980), where swimming speeds first increased and then decreased with increasing population density. The observed density-induced swimming speed increases in *H. triquetra* might reduce competition for light or nutrients by allowing individuals to disperse and avoid localised competition, while the reduction in swimming speeds at higher densities could suggest behaviours to avoid collisions at higher population densities that could potentially cause cells to stick together or entangle their flagella.

Z-dimension

Vertical swimming velocity (V_z) was positively correlated with increasing viscosity. Maximum recorded V_z was 30% higher than the maximum 2D swimming speed of 273 µm s^{-1} reported by Jakobsen (Jakobsen 2005), but within 5% of the 370 (± 14) µm s^{-1} reported by Jeong *et al*., (Jeong et al. 2002).The increase in V_z with viscosity led to an increase in calculated vertical migration range (V_{mr}) to a maximum of 17 meters in the high viscosity treatments.

It is possible that the positive correlation between V_z and viscosity is consistent with the hypothesis put forward by Machemer (Machemer 1996) that microorganisms can modulate swimming speed as a function of spatial orientation. It is also possible that during cold periods (in temperate regions during winter with shorter light periods), adaptation to the increased viscosity of seawater might induce higher vertical swimming speeds and upward migration, thus increasing time spent in the surface layers for photosynthesis. Although, it should be noted that movement would be moderated by physiological changes within the cell due to changes in environmental temperatures (Podolsky & Emlet 1993). Finally, increases in viscosity may be indicative of high rates of photosynthesis and exudate production and might signal increased nutrient competition and thus induce cells to increase their vertical migration to access depths with lower levels of competition from photoauthotrophs. A competition-induced, pH-mediated alteration of phytoplankton vertical migration has been suggested previously for the raphidophyte *Heterosigma akashiwo* (Kim et al. 2013).

Approach distances

Our observations suggest a complex relationship between viscosity and putative sensory responses, with increasing viscosity reducing the approach distances between protists but increasing approach distance between protists and microbeads. In addition, approach distances between protists and microbeads were always larger (2-fold) than approach distances among protists regardless of viscosity, suggesting some method of differentiation between the hydromechanical signals produced by conspecifics and the signals produced by a physical object (microbeads). This is likely due, at least in part, to the active swimming of the protists, which would act to decrease the closest approach distance achieved over a given time period relative to a stationary arrangement. Although trends in nearest approach distance versus viscosity with microbeads present were non-monotonic, we suspect that the relatively small changes in approach distances (approximately ¼ of a body length) between low and medium viscosity are likely to be biologically insignificant. This approach distance of 6 μm is substantially less than the 1000 μm over which copepods have been observed to attack stationary beads (Bundy et al. 1998; Bundy and Vanderploeg 2002). Overall this result suggests that this dinoflagellate species is able to sense particles at several body lengths away and distinguish among types of particles as well. This sensory capacity allows *H. triquetra,* and likely other species to navigate its environment with information about surrounding abiotic and biotic particles.

The ability to recognise differences in the signal profile between conspecifics and predators over a range of viscosities may be important over seasonal changes. While vertically migrating phytoplankton can experience temperature changes of only a few degrees C (resulting in relatively small changes in viscosity) over vertical migration ranges (V_{mr}) (see below) in coastal waters during warm periods, during colder seasons the change in viscosity with depth might be more pronounced. Thus, an ability to determine the hydromechanical signals of approaching predators over a range of viscosities would be advantageous. In addition, planktonic communities have been shown to increase the viscosity of surrounding seawater by up to 85% in sub-surface waters through the excretion of viscous photosynthate (Seuront et al. 2010) which may help maintain blooms by reducing turbulence through exoploymer excretion (Prairie et al. 2012, Seuront and Vincent 2008; Seuront et al. 2007), much higher than the 19% increase in viscosity used in this study. Thus, the development or dispersal of a phytoplankton bloom may well result in large viscosity changes that a cells' sensory systems must cope with.

Velocity field sensitivity

The calculated velocity threshold sensitivities based on nearest approach distances to microbeads suggest that *H. triquetra* can detect differences in flow fields of less than 2% of their own swimming speed. Some unicellular organisms (specifically diatoms) are able to sense changes in osmotic conditions as well as those of nutrients (Fe) and fluid motion (Falciatore et al. 2000), as witnessed by changes in the concentration changes in their second messenger, cytosolic Ca^{++}. We note that in unicellular organisms such as ciliates and dinoflagellates, a response occurs above a critical fluid deformation rate (Jakobsen 2001; Maldonado & Latz 2007), equivalent to a threshold velocity difference across the cell. However, sensitivities measured in our study seem quite high for a mixotrophic organism with no obvious mechanosensory structures analogous to copepod antenna, but we do note that some cruising copepods appear to have sensitivity values around 1% of their swimming speeds (Visser 2001). It is possible that *H. triquetra* can sense and respond to shear stress through deformation of the cell wall in a similar manner as suggested for the dinoflagellate *Pyrocystis lunula* (Tesson and Latz 2015). This species has been shown to respond to high levels of shear stress (e.g. the interactions between boundary layers of swimming animals) causing deformation of the cell body as well as through direct mechanical interactions (applied force), again causing deformation of the cell body (Tesson and Latz 2015).

Our results further indicate that average velocity sensitivity decreases (i.e. detection precision increases) over the manipulated viscosity range from low to high. Enhanced remote detection of microbeads might allow plankton that increase local viscosity during blooms (Prairie et al. 2012, Seuront et al. 2007; Jenkinson and Biddanda 1995) to detect ambush predators over a larger distance, thereby reducing individual predation levels and maintaining the bloom for a longer time. *H. triquetra* is known to form dense blooms (Jeong et al. 2005) with cultures able to grow in a range of pH conditions with cell densities up to 7 x10^4 cells mL^{-1} (Havskum & Hansen 2006). Other bloom forming protists that secrete mucus do change viscosity (Jenkinson 1986; Jenkinson & Sun 2010), including within blooms (Seuront and Vincent 2008; Seuront et al. 2007; Jenkinson and Biddanda 1995). The ability to increase localised viscosity which reduces turbulence and helps maintain the bloom for a longer duration might also allow for detection of predators over a larger distance and increase the survival rates of individual protists and duration of blooms overall. Any *H. triquetra* and other flagellates present are likely to be influenced by and respond to such viscosity changes.

Turning rate

Trends in turning rate (T_r) can naturally be compared with the trends in horizontal correlation length scale $\lambda_{xy,}$ since both parameters are measures of path tortuosity. One would intuitively expect an inverse relationship between the two, since a large λ_{xy} is indicative of a straight path with low turning rate.

We found a non-monotonic trend in turning rate (T_r) with an overall increase in T_r in relation to increased viscosity, however, maximum Tr was recorded in the medium viscosity treatment with no mechanical stimuli present. We also found a non-monotonic effect of microbeads (an increase in turning rate in the presence of microbeads at medium and high viscosity but no effect at low viscosity). T_r in our treatments increased by 4.8 fold between lowest and highest viscosity in treatments with NS (0.037 to 0.181 turns per micron), while in treatments WS present, T_r increased by 10.2 fold (0.035 to 0.359 turns per micron). The increase in T_r between the lowest and highest viscosity NS treatments is consistent with the reduction in λ_{xy}, with both describing an increase in path tortuosity at high viscosity. In the treatments with no mechanical stimulus the relative maximum in T_r at medium viscosity is inconsistent with the relative in maximum in λ_{xy} at medium viscosity. In addition the trend in T_r with stimuli is inconsistent with the non-monotonic trend in λ_{xy} with viscosity in treatments with stimuli. One possible explanation for these apparent inconsistencies is the fact that T_r is a three-dimensional parameter, while λ_{xy} describes only horizontal components of movement. Perhaps more importantly, T_r is affected by any turns along the path regardless of spatial scale, while λ_{xy}, by definition, should not be affected by very small-scale turns, such as those making up the helical paths that many microorganisms display. For example, it is possible that in the absence of microbeads, cells travelled along the straightest paths in a large-scale sense at medium viscosity, even though the most tightly coiled helical paths also occurred at the highest viscosity.

The rotation rates of swimming paramecium (*Paramecium caudatum*) decrease with both reduced swimming speeds and increased viscosity (Jung et al. 2014) and the straightening of trajectories seen in our experiments (changes in τ_{xy}, λ_{xy} and radians per μm) were also coupled with reduced swimming speeds, diffusion and increased viscosity. It is likely that the reduction in swimming speeds associated with increased viscosity similar to that observed by Qin *et al.* (Qin et al. 2015) affected the number of turns and helical turn magnitude in our study. We note that paths such as helices can be ballistic and convoluted (Bianco et al. 2014), however, we use the term ballistic here as described in the context of Taylor's equation (Taylor 1921), which we apply to movement at a scale much larger than the fine details of the path.

Correlation scales and dispersal rates

Our reported values for τ_{xy}, λ_{xy} (Fig 7 and Fig 8), and D_{xy} are within a factor of ten of those previously published for a different strain of the same species *H. triquetra* CCMP 448 (Schuech and Menden-Deuer 2014). Intra-specific variability in movement parameters among clones of the same species have previously been observed and thus are to be expected (Menden-Deuer & Montalbano 2015). As with swimming speeds, we found a non-monotonic trend between each of these parameters and viscosity (Table 1), with the exception of D_{xy} in WS treatments. In NS treatments, both τ_{xy} and λ_{xy} were largest at medium viscosity, leading to D_{xy} being largest there as well, despite V_{xy} being smallest at medium viscosity. This pattern indicates a qualitative change in flagellar movements (Qin et al. 2015) as swimming speeds decreased, but cells swam along straighter paths. If the effect of increased viscosity were to simply slow down all movements, no changes in λ_{xy} would be expected and similar trajectories would be observed for all treatments. However, the largest λ_{xy} found at medium viscosity suggests a flagellar beating pattern that may have been more regular than at low or high viscosity, resulting in fewer random turns. Thus, our data again suggest cells actively modulate flagellar beating patterns in response to viscosity, rather than a passive effect of viscosity on movement pattern.

Movement is inherently risky as it increases the probability of encounters with predators. However, it can also increase the probability of encounters with potential mates and resources (Kiørboe 2013). It has even been suggested that the use of ballistic movement and adjustments to the timing of relocations may be common mechanisms to efficient random search strategies (Kiørboe and Visser 1999). Many plankters are simultaneously both predator and prey and consequently the motility behaviours of these organisms might have evolved to maximize encounters with prey and potential mates, while minimizing encounters with predators (Visser 2007; Kiørboe 2013). This might be accomplished by maintaining a correlation length scale λ_{xy} larger than the detection distance for resources, but smaller than the encounter radius of a typical predator, so that encounters with resources are in the efficient ballistic regime but encounters with predators are in the inefficient diffusive regime (Visser 2007). The protists studied here have high λ_{xy} of approximately 100 to 760 µm which is quite risky and essentially identical to values reported for copepods of typically between 100 to 500 µm (Visser 2001). Hence, the species studied here does not appear to be following a simple, hypothetically optimal movement strategy of modulating motility to minimize predator and maximize resource encounters. At the observed lambda and sub xy, encounters with zooplankton grazers are likely to be in the transitional region between fully ballistic and diffusive encounter regimes. However, predicted encounter rates with copepods would still be lower than if the protists had λ_{xy} much larger than that of copepods.

The presence of microbeads influenced correlation length and time scales λ_{xy} and τ_{xy} in a complex fashion, leading to both increases and decreases, depending on viscosity. However, the addition of microbeads always lead to a decrease in D_{xy}, indicating a robust decrease in overall horizontal dispersal rate. Since the presence of microbeads had little effect on vertical swimming patterns, we suggest that *H. triquetra* might decrease horizontal diffusivity to avoid lethal encounters but maintain the vertically ballistic behaviour necessary to traverse critical gradients in light and nutrients, despite the higher encounter rates associated with ballistic movement. This kind of strategy seems likely, given the prevalence of this type of behaviour amongst microplankton (Schuech and Menden-Deuer 2012).

While the horizontal component of swimming was always diffusive (with a minimum at medium viscosity), movement in the z direction was always ballistic regardless of viscosity. Using our measured vertical speeds, the V_{mr} range of *H. triquetra* over a 12 hour period would be approximately 14 m in the lower viscosity treatments (0.99 to 1.04 cP), increasing to 17 m in the highest viscosity treatment (1.18 cP). These predicted vertical distances are 140 to 480 times larger than diffusive horizontal dispersal (6.02 to 10.8 cm) over 12 hours. The large difference in ranges covered between the xy plane and z direction is likely to be adaptive since the marine environment is often vertically stratified and horizontal movements are not likely to offer significant improvements in resource availability, while vertical movements result in dramatic changes in light exposure and increase the chances of encountering a potentially nutrient rich environment (reviewed in Stocker & Durham 2009), although this motility pattern could also increase encounters with predators compared with isotropic diffusive movement (Seuront and Strutton 2004).

Conclusion

Due to the drag forces on beating appendages such as cilia and flagella, microorganism motility and performance are directly linked to the viscosity of the surrounding fluid (Humphries 2013). Manipulation of viscosity without alteration of seawater density allowed us to disentangle the role of viscosity and temperature on protist swimming behaviours. Our data indicate that viscosity has a significant and often complex effect on the behaviour of the photosynthetic dinoflagellate *Heterocapsa triquetra*. The non-monotonic responses observed are likely due to organism mediated alteration of flagellar beat frequency and patterns that overcome constraints by changes in viscosity. Our results clearly show that this dinoflagellate species has the sensory capacity for sophisticated distinction among different particle types in the surrounding fluid, which suggests that organisms can not only respond to external resource or internal cellular conditions (Grünbaum 2001), but also modulate their movements in response to the presence and types of particles.

Overall, both swimming speeds and diffusive dispersal ranges in the horizontal plane were negatively correlated with viscosity. While movements in the vertical direction were always ballistic for the duration of the experiments, high viscosity was also associated with a switch from downward to upward migration. Hence, at low viscosity (e.g. higher temperature, low phytoplankton exudate concentration), cells would tend to migrate downward and diffuse more horizontally, while at high viscosity (e.g. lower temperature, deeper water, or alternately, bloom conditions), cells of this species would tend to migrate upward and cover less horizontal area. The increase in upward movement during bloom conditions might increase the amount of light available to *H. triquetra* and as such increase photosynthetic output. Future research should attempt to determine whether viscosity might act as a proxy for sensing depth or population density of conspecifics, and more generally, further investigate the influence of viscosity on encounter rates between predators and prey in marine environments (Beveridge et al. 2010).

While the presence of microbeads had a strong and treatment-specific effect on horizontal swimming behaviour, protistan movements in the vertical direction were relatively unaffected by the presence of microbeads. This result suggests that vertical migration may be robustly maintained, even at the cost of an elevated predation risk due to ballistic movement. However, this risk may be partially mitigated by the lower horizontal diffusivities that occurred in the presence of microbeads at all viscosities. The effects of elastic liquids on flagella movement (Qin et al, 2015) and the role this has on mediating behaviour and interactions in moving microorganisms is open for further investigation. If beat frequency changes, one would expect changes in any swimming parameters that depend on time, but not entirely spatial parameters such as λxy. However, if the beating pattern qualitatively changes (e.g. changing amplitude), all swimming parameters could theoretically be affected. Since both beat frequency and beat pattern could change simultaneously at different viscosities, future work must include high resolution observations of flagellar movement if these two aspects of swimming kinematics are to be disentangled.

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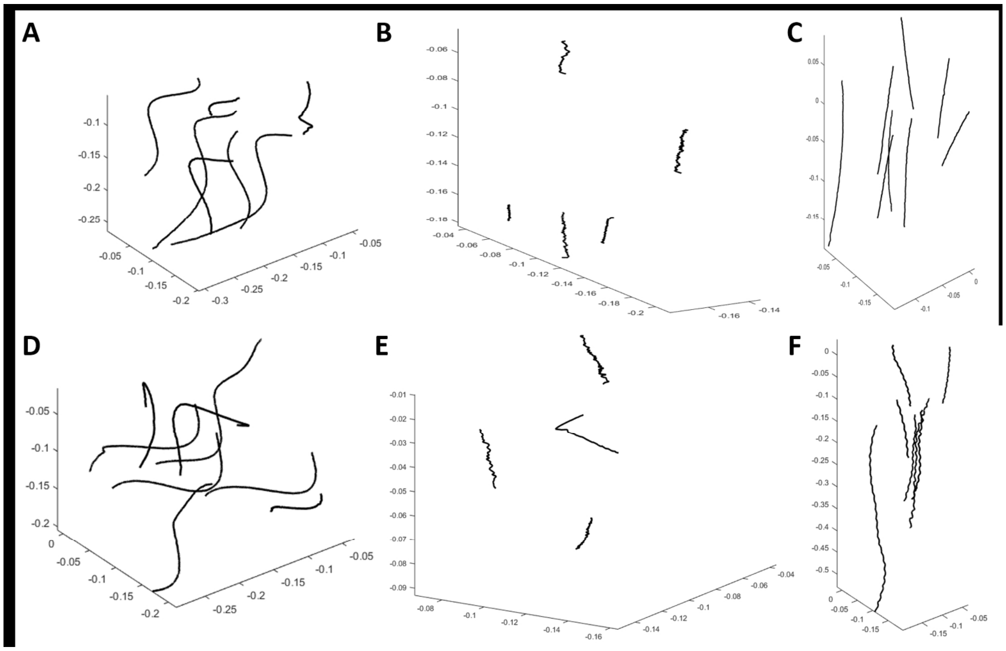

Fig 1: 3D swimming trajectoreis of H. triquetra in experimetnal treatments A-C, low, medium and high viscosity treatments without microbeads present, D-F, low, medium and high viscosity treatments with microbeads present.
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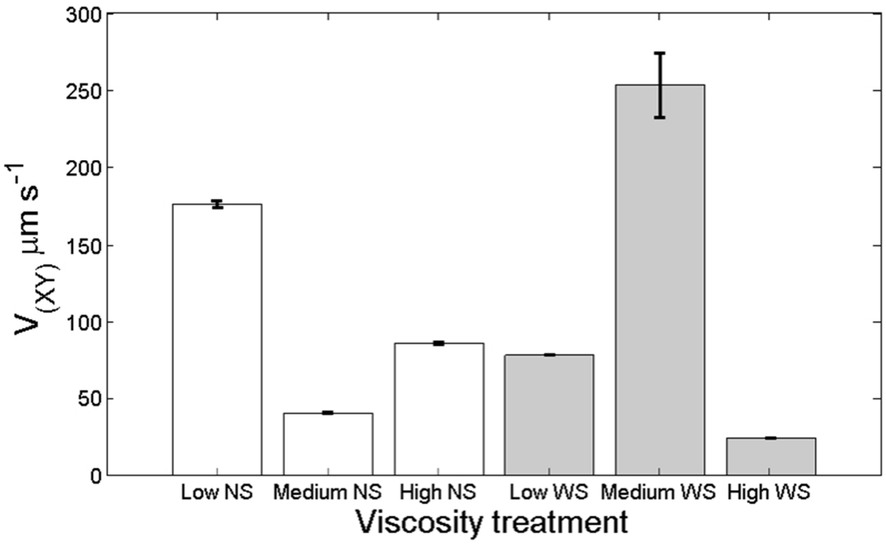

Fig 2: Swimming velocity in x-y plane (µm s-1) Heterocapsa triquetra; without (white bars, NS) and with microbeads present (grey bars, WS). Error bars represent the standard errors. Sample size N = 1800 to 2200 depending on treatment. All treatments were significantly different from each other at the 95 %.
237x135mm (96 x 96 DPI)

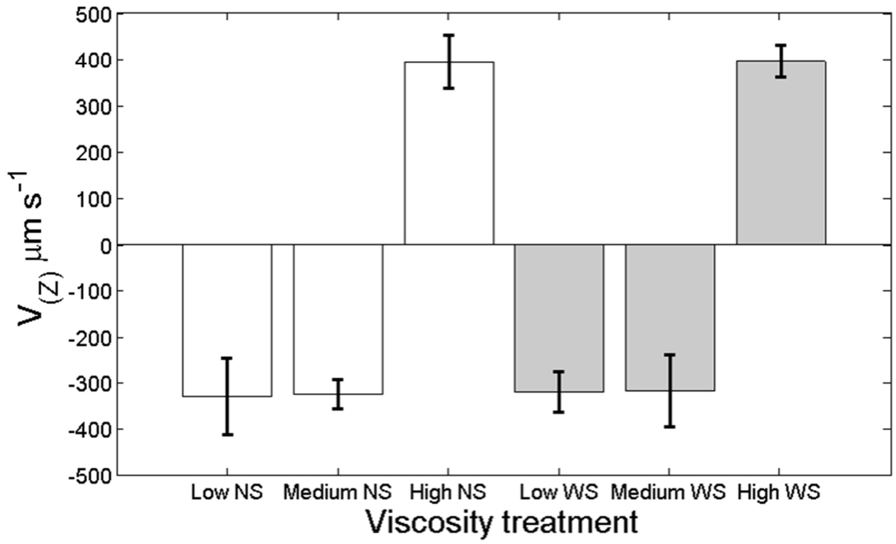

Fig 3: Vertical swimming velocity (Vz) (µm s-1) Heterocapsa triquetra; without (white bars, NS) and with microbeads present (grey bars, WS). Error bars represent the standard errors. Sample size N = 1800 to 2200 depending on treatment. Negative values indicate downward swimming. Upward and downward velocities were significantly different from each other at the 95% (indicated with *), however, no significant difference was found between populations moving in the same orientation (e.g. high viscosity treatment without microbeads compared against high viscosity treatment with microbeads).
237x135mm (96 x 96 DPI)

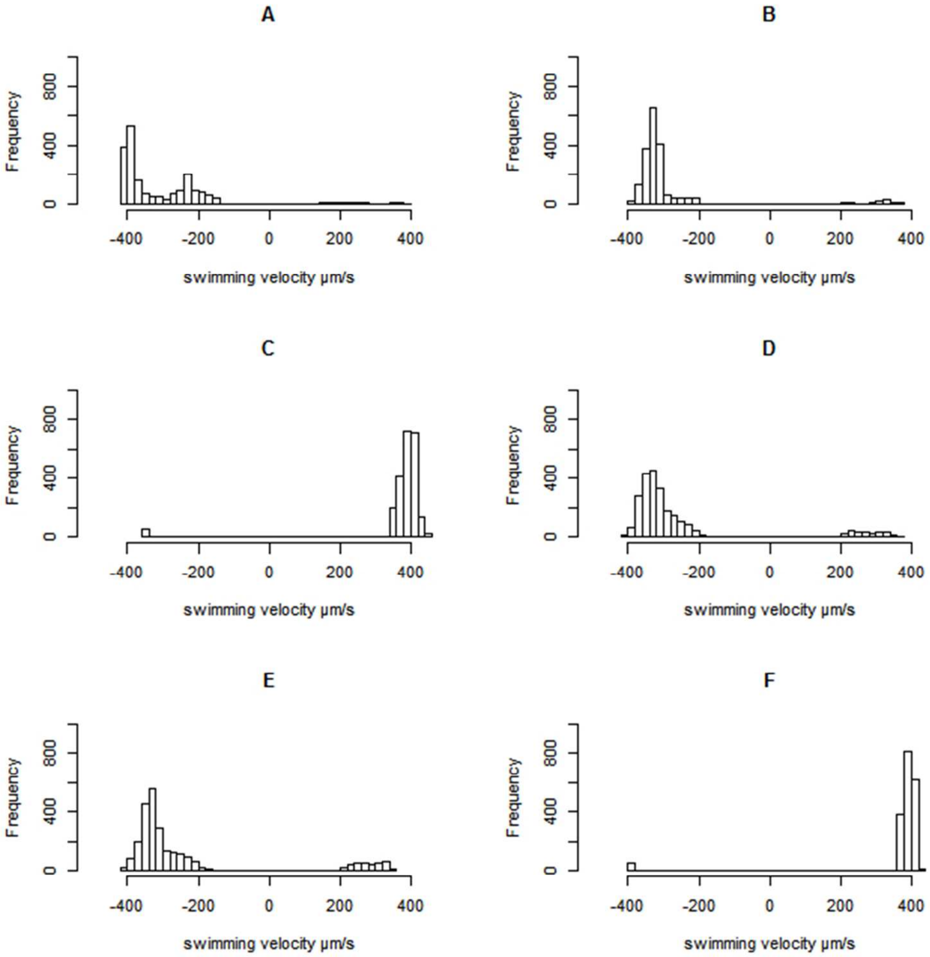

Fig 4: Frequency histogram of Vz for H. triquetra in all treatments, A – C, low, medium and high viscosity with no mechanical stimuli present, D – F, low, medium and high viscosity with mechanical stimuli present. Sample size N = 1800 to 2200 depending on treatment. Negative values indicate downward swimming.
234x234mm (72 x 72 DPI)

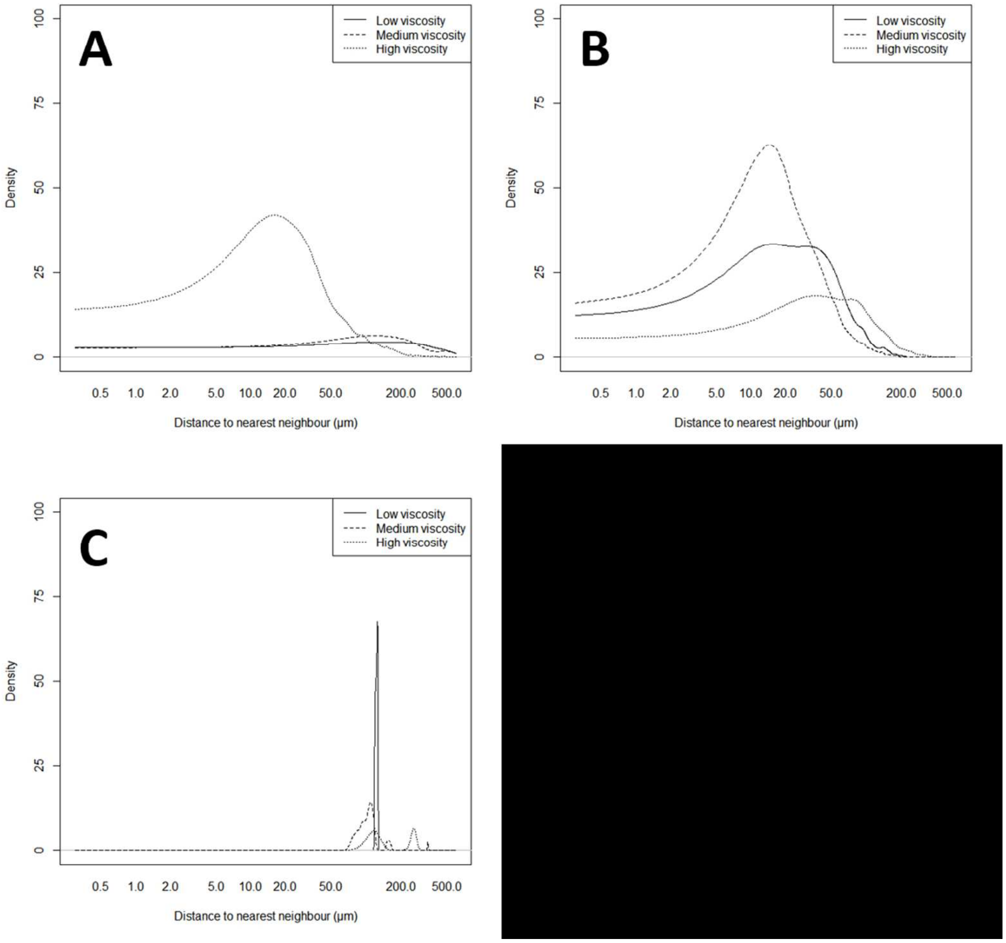

Fig 5: Density distribution plots of approach distances in all treatments, A, approach distances between protists with no microbeads in the water column, B, approach distances between protists with microbeads in the water column, C, approach distance between protists and microbeads.
200x198mm (150 x 150 DPI)

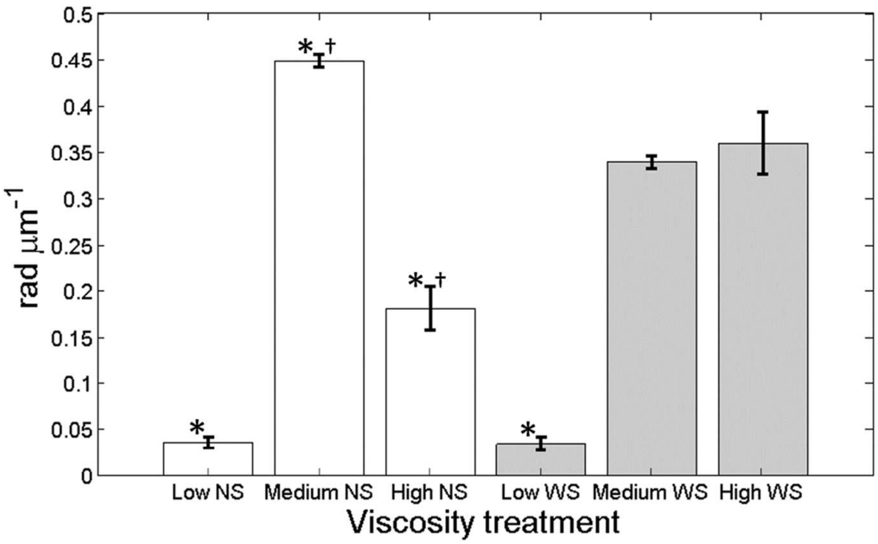

Fig 6: Turning rate (radians per micron) both without (NS, white bars) and with microbeads present (WS, gray bars). Error bars represent standard error. N = 1800 to 2200 depending on treatment. * denotes significant differences at the 95 % level for comparisons between treatments of manipulated viscosity but same stimuli level (eg. NS), † denotes significant differences at the 95% level for comparisons between treatments of same viscosity but differing stimuli levels.
211x120mm (150 x 150 DPI)

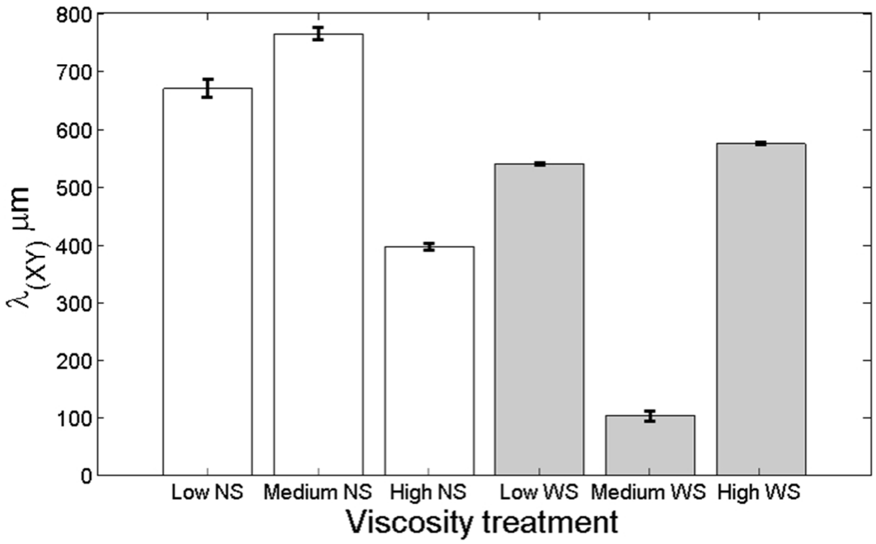

Fig 7: Distance between turning events (λ) x-y plane in µm both without (NS, white bars) and with microbeads present (WS, gray bars). Error bars represent one standard error of the mean. N = 1800 to 2200 depending on treatment. All treatments were significantly different from each other at the 95 % level for comparison.
237x135mm (96 x 96 DPI)

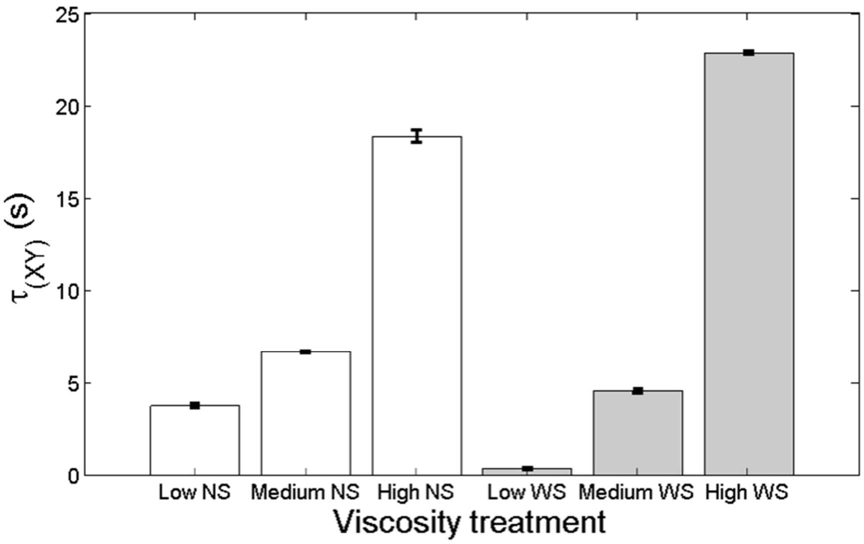

Fig 8: Average time (s) between turning events in x-y plane both without (NS, white bars) and with microbeads present (WS, gray bars). Error bars represent standard error. N = 1800 to 2200 depending on treatment. All treatments were significantly different from each other at the 95 % level for comparison.
237x135mm (96 x 96 DPI)

1 Swimming metrics for xy-plane

Viscosity treatment	**τ_{xy} (s) ± se**	**λ_{xy} (µm) ± se**	**Swimming speed V_{xy} (µm s^{-1}) ± se**	**D_{xy} (cm^2 s^{-1})**
Low NS	3.7 ± 0.1	668.2 ± 15	176.1 ± 1.9	5.8×10^{-4}
Low WS	6.8 ± 0.0	538.1 ± 2.3	78.4 ± 0.2	2.1×10^{-4}
Medium NS	18.3 ± 0.3	763.3 ± 10	41.6 ± 0.2	$1.5 \text{ x } 10^{-4}$
Medium WS	0.4 ± 0.16	104.0 ± 8.7	253.5 ± 21.0	$1.3 \text{ x } 10^{-4}$
High NS	4.5 ± 0.1	396.8 ± 5.8	86.6 ± 5.8	$1.7 \text{ x } 10^{-4}$
High WS	22.8 ± 0.1	573.7 ± 2	25.1 ± 0.0	$7.2 \text{ x } 10^{-5}$

2 Swimming metrics for z-dimension

Viscosity treatment	Minimum τ_z (s)	Minimum λ_z (µm)	V_z (µm/s) ± se	Upward moving fraction (%)
Low NS	120	1000	329.3 ± 82	33
Low WS	100	3000	319.4 ± 43	29
Medium NS	120	2600	324.8 ± 31	20
Medium WS	40	2000	317.6 ± 47	36
High NS	100	1200	394.2 ± 36	78
High WS	40	1000	395.6 ± 35	83

3 Empirically measured approach distances

Treatment	**Approach distance (µm) ± 95% confidence bounds**	
	conspecifics	**microbeads**
Low NS	309 (271, 350)	N/A
Low WS	48 (35, 63)	122 (121, 122)
Medium NS	269 (238, 300)	N/A
Medium WS	35 (22, 50)	116 (110, 121)
High NS	65 (52, 79)	N/A
High WS	89 (83, 96)	186 (179, 192)

4 Calculated velocity field sensitivity

Viscosity treatment	Sensitivity to velocity field perturbation (µm s^{-1})	Sensitivity as % of average swimming speed
Low NS	0.90	0.51
Medium NS	3.82	1.50
High NS	0.03	0.12

5 Calculated horizontal migration range over 12 hour period

Viscosity treatment	V_{mr} (m)	H_{mr} (m) over 12 hour period
Low NS	14.2	0.10
Low WS	13.8	0.06
Medium NS	13.9	0.05
Medium WS	13.7	0.04
High NS	17.0	0.05
High WS	17.0	0.03