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Behavioral and mechanistic characteristics of the predator-prey interaction between the dinoflagellate *Dinophysis acuminata* and the ciliate *Mesodinium*

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Abstract

Predator-prey interactions of planktonic protists are fundamental to plankton dynamics and include prey selection, detection, and capture as well as predator detection and avoidance. Propulsive, morphology-specific behaviors modulate these interactions and therefore bloom dynamics. Here, interactions between the mixotrophic, harmful algal bloom (HAB) dinoflagellate *Dinophysis acuminata* and its ciliate prey *Mesodinium rubrum* were investigated through quantitative microvideography using a high-speed microscale imaging system (HSMIS). The dinoflagellate *D. acuminata* is shown to detect its *M. rubrum* prey via chemoreception while *M. rubrum* is alerted to *D. acuminata* via mechanoreception at much shorter distances (89 ± 39 μm versus 41 ± 32 μm). On detection, *D. acuminata* approaches *M. rubrum* with reduced speed. The ciliate *M. rubrum* responds through escape jumps that are long enough to detach its chemical trail from its surface, thereby disorienting the predator. To prevail, *D. acuminata* uses capture filaments and/or releases mucus to slow and eventually immobilize *M. rubrum* cells for easier capture. Mechanistically, results support the notion that the desmokont flagellar arrangement of *D. acuminata* lends itself to phagotrophy. In particular, the longitudinal flagellum plays a dominant role in generating thrust for the cell to swim forward, while at other times, it beats to supply a tethering or anchoring force to aid the generation of a posteriorly-directed, cone-shaped scanning current by the transverse flagellum. The latter is strategically positioned to generate flow for enhanced chemoreception and hydrodynamic camouflage, such that *D. acuminata* can detect and stealthily approach resting *M. rubrum* cells in the water column.

Keywords

Dinophysis acuminata, *Mesodinium rubrum*; predator-prey interaction behavior; high-speed microscale imaging system (HSMIS); quantitative microvideography; diarrhetic shellfish poisoning (DSP)

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1. Introduction

Species of the marine dinoflagellate genus *Dinophysis* occur in coastal and oceanic waters throughout the world (Hallegraeff and Lucas, 1988; Maestrini, 1998; Reguera et al., 2014). Though typically present at concentrations <100 cells L^{-1} , under favorable conditions some species will form seasonal blooms that reach concentrations of up to 10^6 cells L^{-1} (Subba Rao et al., 1993; Dahl et al., 1996; Marcaillou et al., 2005; Reguera et al., 2012, 2014 and references therein). Some of these bloom-forming species cause diarrhetic shellfish poisoning (DSP) (Yasumoto et al., 1980; Lee et al., 1989; Hallegraeff, 1993; Reguera and Pizarro, 2008), a syndrome that threatens public health and shellfish fisheries in many areas around the world.

Park et al. (2006) successfully cultured a *Dinophysis* species (*D. acuminata*) by feeding it the ciliate *Mesodinium rubrum*, itself a kleptoplastic mixotroph that was fed the cryptophyte *Teleaulax* spp. Since then, a total of six *Dinophysis* species have been cultured via this chain of serial kleptoplasty, i.e., cryptophyte plastid acquisition from the *Teleaulax/Plagioselmis/Geminigera* clade to *M. rubrum*, which in turn provides plastids to *Dinophysis* (Tong et al., 2011, 2015b; Reguera et al., 2012, 2014; Hansen et al., 2013 and references therein). Still unclear has been whether the *Dinophysis* species must feed on *M. rubrum* for sustained growth or can survive through ingestion of other species in nature (Reguera et al., 2012; Hansen et al., 2013). Nevertheless, field observations have shown that populations of *D. acuminata* and *M. rubrum* co-occur in nature and that their population maxima overlap at times, resulting in predator-prey encounters and interactions (Velo-Suárez et al., 2008; González-Gil et al., 2010; Sjöqvist and Lindholm, 2011).

The ciliate *Mesodinium rubrum* is a cosmopolitan species that sometimes forms massive non-toxic “red-water” blooms in estuarine and coastal waters (Taylor et al., 1971; Lindholm, 1985; Crawford, 1989). Individual *M. rubrum* move via cycles of rest and fast jumps (Lindholm, 1985; Fenchel and Hansen, 2006). Across geographically diverse isolates, jumps are ~ 6 body lengths and are completed faster than the diffusion time scale defined by the ciliate’s body length (Jiang and Johnson, 2017). Thus, a typical jump completely detaches the chemical diffusive boundary layer that forms around the ciliate’s body during the resting period, thereby enhancing nutrient uptake and simultaneously disrupting chemical cues to chemoperceptive predators. Fast attenuation of flow fields produced by these jumps further limits detection of *M. rubrum* by predators that rely on mechanoperception (Jiang, 2011; Kiørboe et al., 2014).

Given the important role of *Mesodinium rubrum* for plastid acquisition by several HAB-forming *Dinophysis* species, a more complete understanding of predator-prey interactions between these species is needed. The dinoflagellate *D. acuminata* is a desmokyont species (Taylor, 1987), i.e., both of its flagella arising anteriorly or apically, and this flagellar arrangement has likely constrained its behavioral adaptations for prey capture. Through conventional inverted microscopy, Hansen et al. (2013) previously described the behavior of a *Dinophysis* cell upon detection of an *M. rubrum* cell. The *Dinophysis* cell first looped slowly around its prey before attaching a capture filament. Once the prey cell was captured, immobilized, and drawn close, the *Dinophysis* cell pierced and ingested its prey with a

peduncle (see also Park et al., 2006; Nishitani et al., 2008), i.e., tube feeding (Hansen and Calado, 1999). Besides using a capture filament to seize their prey, *Dinophysis* spp. may also release sticky mucus that immobilizes *M. rubrum* cells, prior to ingestion through tube feeding (Nishitani et al., 2008; Ojamäe et al., 2016; Papiol et al., 2016; Mafra et al., 2016). Despite these advances, significant research questions remain unanswered (see below).

In the present study, quantitative microvideography using a high-speed microscale imaging system (HSMIS) was conducted to document the behavioral characteristics of the predator-prey interaction between *Dinophysis acuminata* and *Mesodinium rubrum* in great detail. The study was conducted to shed light on several questions and aspects of this predator-prey interaction:

1. What mechanism, chemoreception or mechanoreception, does a *D. acuminata* cell use to detect and locate an *M. rubrum* cell, and at what distance? How does a *D. acuminata* predator capture an *M. rubrum* prey in the water column? Although previous work has suggested that a capture filament is involved, there is no published photo or video documentation to support this suggestion.
2. What mechanism, chemoreception or mechanoreception, does an *M. rubrum* prey use to detect an approaching *D. acuminata* predator, and at what distance? How does an *M. rubrum* prey jump to escape an approaching *D. acuminata* predator? Do escape jumps differ from spontaneous jumps that *M. rubrum* perform routinely for enhancing nutrient uptake?
3. What is the general strategy that *Dinophysis* spp. use to deal with fast-jumping *M. rubrum*? In other words, can a unified understanding be achieved for the two prey capture modes of *Dinophysis*, i.e., prey immobilization via capture filaments and mucus traps?

2. Material and methods

2.1. Culture maintenance

A clonal culture of *Dinophysis acuminata* (DAMV01) was established from a water sample collected off shore of Martha's Vineyard, Massachusetts, USA, in August 2008 (Fux et al., 2011; Tong et al., 2015a, b). The cultures of *Mesodinium rubrum* (JAMR) and the cryptophyte *Teleaulax amphioxeia* (JATA) were isolated from Inokushi Bay, Oita Prefecture, Japan, in February 2007 (Nishitani et al., 2008). All cultures were maintained at 15 °C with an irradiance of 65 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ on a 14 h light : 10 h dark photoperiod in medium prepared with sterilized 0.2 μm filtered Vineyard Sound seawater (32 psu). (The light phase began at 6 a.m. and ended at 8 p.m.) For the cryptophyte cultures, the seawater base was enriched with modified f/2-Si nutrients (Anderson et al., 1994) whereby H_2SeCO_3 was added and CuSO_4 was reduced to a concentration of 10^{-8} M each. One mL of this dense ($6.0 - 8.0 \times 10^5$ cells mL^{-1}) *T. amphioxeia* was fed to 80 mL stocks of *M. rubrum* ($\sim 10,000$ cells mL^{-1}) inoculated into 250 mL f/6-Si medium (1/3 strength of the f/2 stocks). After a period of ~ 14 days, 2 mL of 'clean' (cryptophyte free) *M. rubrum* cell suspension was fed to the *D. acuminata* maintained in 20 mL sterilized seawater on a weekly basis and these cultures were transferred to fresh sterilized seawater every four weeks.

The *Dinophysis* samples that were used in the present experiments were in late exponential to early stationary phase growth, approximately 20 – 30 days old. The *Mesodinium* samples used were also in late exponential to early stationary phase growth, approximately 7–14 days old. These samples were an aliquot of a larger culture and no additional medium was added.

2.2. The high-speed microscale imaging system (HSMIS)

The high-speed microscale imaging system (HSMIS; Fig. 1) was used to conduct quantitative microvideography of the motion behavior of *Dinophysis acuminata* and of *Mesodinium rubrum* and their predator-prey interaction. The HSMIS included a Photron FASTCAM SA3 120K monochrome video camera, which was controlled by a laptop computer and set to take images of 1024×1024 pixel resolution at 2000 frames per second (fps). The camera was mounted horizontally with a 180 mm FL objective lens plus a Zeiss LD Epiplan 20x/0.40 (7.3 mm WD) microscope objective to yield a field-of-view of a vertically oriented area of 761×761 μm . The culture was held in a 25 mL tissue culture flask of outer dimensions 26×44×82 mm. One of the flask's two faces with dimensions 44×82 mm was positioned very close to the tip of the microscope objective. By doing so, the field-of-view was focused far enough from the flask walls owing to the long working distance of the microscope objective. Thus, in view of small sizes of both *D. acuminata* and *M. rubrum* the wall effects of the vessel were minimal. If not otherwise specified, a 1 W red LED light source was collimated to provide backlit illumination in which light was shined toward the camera through the tissue culture flask containing the culture. Part of the collimated light beam was blocked to form a beam cross-sectional area only slightly larger than the field-of-view. Thus, the illumination introduced very limited heat and caused virtually no convection inside the observation vessel, thereby improving the accuracy of cell swimming speed measurements.

2.3. Behavioral observations

Five sets of experiments were conducted. The day of each set of experiments around 10 a.m., one flask of ~20 mL *Dinophysis acuminata* culture (1,600 – 2,200 cells mL^{-1}) and one flask of ~20 mL *Mesodinium rubrum* culture (2,300 – 3,100 cells mL^{-1}) were prepared. Temperature of these cultures was kept at ~18 °C in all experiments, slightly warmer than the 15 °C chambers they were maintained in between experimental sets.

A set of experiments began by putting the two flasks holding the cultures into the experimental room to allow the cells to acclimate for ~2 h. After acclimation, the swimming behavior of *Mesodinium rubrum* was observed by the HSMIS first without introduction of *Dinophysis acuminata*. Then, the *D. acuminata* culture was observed without the presence of *M. rubrum*. Each observation period lasted ~2 h. After observations alone, the *D. acuminata* and *M. rubrum* cultures were mixed in equal volumes into two fresh flasks, resulting in the ratio of predator to prey in the range of 0.6 – 0.8. Upon mixing, flasks were immediately placed on the HSMIS for ~1 h of observation (2 h total between the two mixed flasks). During these behavioral observations, high-speed digital videos of cells swimming within the HSMIS plane of focus were recorded (referred to as “events” hereafter).

2.4. Video and data analysis

High-speed digital videos were imported into ImageJ software for measurements of cell length (L_{Mr}) and width (W_{Mr}) for *Mesodinium rubrum* and cell length (L_{Da}), width (W_{Da}), and thickness (H_{Da}) for *Dinophysis acuminata*. Kinematics of cell movements and behavioral aspects of the predator-prey interaction were also measured. For events of *M. rubrum* jumping, measurements included jump duration (t_{jump}), maximum jump speed (U_{max}), and jump distance (d_{jump}). For events of *M. rubrum* swimming/zigzagging, measurements included swimming/zigzagging duration (t_{swim}), path length (l_{swim}), path-averaged speed ($U_{ave} = l_{swim} / t_{swim}$), and maximum path speed (U_{max}). For events of *D. acuminata* swimming, measurements included swimming duration (t_{swim}), path length (l_{swim}), path-averaged speed ($U_{ave} = l_{swim} / t_{swim}$), net to gross displacement ratio (NGDR; Buskey and Stoecker, 1988), and cell rotation speed (Ω). A *D. acuminata* cell usually rotates about its long axis while swimming along a helical path; since a *D. acuminata* cell is not axisymmetric about any cell axis, the time evolution of the projected area of the cell onto the vertically oriented plane of focus provides information of cell rotation speed, i.e., the peak-to-peak time is half rotation period.

For events of the predator-prey interaction between *Dinophysis acuminata* and *Mesodinium rubrum*, measurements included reaction distance of a *D. acuminata* predator to a detected *M. rubrum* prey (R_{da}) and reaction distance of an *M. rubrum* prey to an approaching *D. acuminata* predator (R_{mr}). The reaction distance R_{da} was measured by inspecting the video to determine the nearest surface-to-surface distance between the predator and the prey when the predator turned to approach the prey, and R_{mr} was the nearest surface-to-surface distance between the predator and the prey when the prey initiated an escape jump in response to the approaching predator.

3. Results

3.1. Motion behavior of *Mesodinium rubrum*

Cells of *Mesodinium rubrum* jump spontaneously and frequently over short or long distances (Event A and B of Supplementary Video Group S1). The cells also swim or zigzag or perform short-distance tumbles (Event C of Supplementary Video Group S1).

Furthermore, the *M. rubrum* cells were observed to swim or zigzag much less frequently than jumping spontaneously when not mixed with *Dinophysis acuminata*. Of the 96 observed events of *M. rubrum*, only six included swimming or zigzagging (Table 1).

3.2. Swimming behavior of *Dinophysis acuminata*

Cells of *Dinophysis acuminata* typically swim along left-handed helical paths and are propelled by movements of their longitudinal and transverse flagella. That is, a cell often translates while rotating counterclockwise around its long axis as viewed from its antapical or posterior end (Event A of Supplementary Video Group S2; see Table 2 for swimming kinematics). The smooth, longitudinal flagellum usually trails the cell, while the hairy, transverse flagellum encircles the apical or anterior end of the cell. When the cell swims forward, the longitudinal flagellum always beats as a wave that travels posteriorly from the flagellar base to tip, thereby providing a forward thrust to the cell (Fig. 2A). The wavy

longitudinal flagellum appears as a straight line when viewed from certain perspectives, indicating that the propulsive wave lies on a plane relative to the cell (Fig. 2B). Cell rotation about the long axis is always associated with movements of the transverse flagellum, which generates metachronal waves via fine hairs (called mastigonemes) along its length (Gaines and Taylor, 1985; Fenchel, 2001). These mastigonemes cause the cell to rotate in the same direction as waves propagating through the transverse flagellum (Event A of Supplementary Video Group S2).

In one event, a *Dinophysis acuminata* cell was observed hovering anterior upward with its cell body rotating counterclockwise as viewed antapically. The hovering cell generated a downward, cone-shaped scanning current that entrained a particle towards the anterior of the cell (Event B of Supplementary Video Group S2; Fig. 3A-C). By tracking the entrained particle, the speed of the scanning current was calculated to reach $110 \mu\text{m s}^{-1}$. This observation has provided unambiguous evidence that movements of the transverse flagellum induced a torque that caused the cell to rotate while simultaneously exerting a downward, distributed force along the outer edge of the transverse flagellum that drove the scanning current. The reaction force associated with the downward, distributed force was acting on the cell in the upward direction, tending to move the cell upward; however, the cell did not move upward because there was an opposing tethering force provided through the reverse beating of the longitudinal flagellum (Event B of Supplementary Video Group S2), i.e., the longitudinal flagellum beat reversely from the flagellar tip to base.

Cells of *Dinophysis acuminata* turn and reorient themselves by steering with their longitudinal flagella. For example, the above-mentioned hovering cell went on to turn and reorient itself by repositioning its longitudinal flagellum from posterior-pointing to anterior-pointing and then beating the flagellum to supply a steering force (Event B of Supplementary Video Group S2; Fig. 3D). During cell turning and reorienting, the transverse flagellum did not move and consequently the cell itself did not rotate about its long axis (see also Event D of Supplementary Video Group S5), thereby further confirming the dominant role played by the transverse flagellum in generating torque for cell rotation about the long axis. Once the turning and reorienting was finished, the cell retracted its longitudinal flagellum to point posteriorly and then beat the flagellum backward to swim forward (Fig. 3E, F).

3.3. Behavioral characteristics of the predator-prey interaction

In the water column, the predator-prey interaction between *Dinophysis acuminata* and *Mesodinium rubrum* roughly proceeds through four stages. In the first stage, *D. acuminata* remotely detects a resting *M. rubrum*, reorients itself by using its longitudinal flagellum, and then swims slowly toward the *M. rubrum* cell. Most often, the ciliate detects the approaching dinoflagellate and escapes via fast jumping along a zigzag path that disorients the *D. acuminata* (Event A of Supplementary Video Group S3). If the ciliate fails to detect the approaching dinoflagellate, then the predator-prey interaction proceeds to the second stage, where the dinoflagellate stealthily approaches the resting ciliate until it can link itself to the ciliate with a capture filament or peduncle, thereby constraining the motility of the ciliate (Event B of Supplementary Video Group S3; Fig. 4; It is suspected that the capture filament

is actually a peduncle.) Next, in the third stage, the dinoflagellate uses its peduncle to tow the ciliate around, while the ciliate tries to escape vigorously by pulling and stretching the peduncle (Event C of Supplementary Video Group S3). Because the ciliate's movement is restrained by the peduncle, other nearby dinoflagellate cells opportunistically try to capture the ciliate with their own peduncles finally in the fourth stage, the dinoflagellate conducts tube feeding while carrying the captured ciliate (Event D of Supplementary Video Group S3). In total, 40 events of the predator-prey interaction between *D. acuminata* and *M. rubrum* were recorded on videos. Of those, 26 events were for the first stage only, one for the second stage, one for the third stage, and 12 for the fourth stage.

Detection and reorientation toward prey by *Dinophysis acuminata* was only observed while *Mesodinium rubrum* cells were at rest. The reaction distance (R_{Mr} ; Table 3B) of a stationary *M. rubrum* prey to an approaching *D. acuminata* predator was significantly shorter than the reaction distance (R_{Da} ; Table 4B) of a *D. acuminata* predator to a stationary *M. rubrum* prey ($41 \pm 32 \mu\text{m}$ versus $89 \pm 39 \mu\text{m}$; Fig. 5; Student's *t*-test, $p < 0.0001$). In other words, *D. acuminata* detected *M. rubrum* before *M. rubrum* detected *D. acuminata*. Also, *D. acuminata* preferred to approach *M. rubrum* from below (19 out of 27 observed approach events).

3.4. Jumping kinematics of *Mesodinium rubrum*

Escape jumps by *Mesodinium rubrum* were observed differing both qualitatively and quantitatively from spontaneous jumps in terms of path trajectory and jumping kinematics (Tables 1A, 3A, 3B; Fig. 6); the spontaneous jumps included those performed both in the absence and presence of *Dinophysis acuminata*. Escape jumps were often along zigzag or curved paths (12 out of 26 observed events). By contrast, spontaneous jumps were overwhelmingly along straight trajectories. Escaping cells jumped at slightly lower maximum speeds than spontaneously jumping cells not exposed to *D. acuminata* ($180.2 \pm 46.3 L_{Mr} s^{-1}$ versus $194.6 \pm 43.9 L_{Mr} s^{-1}$; Fig. 6A; Student's *t*-test, $p = 0.17$); however, the duration of an escape jump was significantly longer than that of a spontaneous jump without *D. acuminata* present ($74.8 \pm 23.1 \text{ ms}$ versus $50.7 \pm 17.2 \text{ ms}$; Fig. 6B; Student's *t*-test, $p = 0.0013$). The escape jump distance was correspondingly significantly greater than that of a spontaneous jump without *D. acuminata* present ($7.8 \pm 4.5 L_{Mr}$ versus $5.1 \pm 2.9 L_{Mr}$; Fig. 6C; Student's *t*-test, $p = 0.039$). Within mixed cultures, escape jumps were significantly slower in maximum speed than spontaneous jumps ($180.2 \pm 46.3 L_{Mr} s^{-1}$ versus $211.4 \pm 67.6 L_{Mr} s^{-1}$; Fig. 6A; Student's *t*-test, $p = 0.031$), but longer in jump duration ($74.8 \pm 23.1 \text{ ms}$ versus $59.3 \pm 34.8 \text{ ms}$; Fig. 6B; Student's *t*-test, $p = 0.092$). As a result, escape jumps were greater in jump distance than spontaneous jumps; however, the difference was not statistically significant ($7.8 \pm 4.5 L_{Mr}$ versus $6.8 \pm 5.3 L_{Mr}$; Fig. 6C; Student's *t*-test, $p = 0.54$).

3.5. Swimming kinematics of *Dinophysis acuminata*

Cells of *Dinophysis acuminata* responded to the presence of *Mesodinium rubrum* cells by displaying quantitatively different swimming kinematics (Tables 2, 4; Fig. 7). When searching for *M. rubrum*, *D. acuminata* swam significantly faster when the prey were present than absent ($3.9 \pm 2.0 L_{Da} s^{-1}$ versus $3.0 \pm 1.5 L_{Da} s^{-1}$; Fig. 7; Student's *t*-test, $p = 0.0069$).

The dinoflagellate, however, slowed down significantly when approaching a targeted prey cell ($3.0 \pm 1.5 L_{Da} s^{-1}$ versus $3.9 \pm 2.0 L_{Da} s^{-1}$; Fig. 7; Student's *t*-test, $p = 0.043$).

The *Dinophysis acuminata* cells increased both their path-averaged swimming speeds and cell rotation speeds when *Mesodinium rubrum* cells were present (Fig. 8A, B). Also, path-averaged swimming speed and cell rotation speed were weakly positively correlated (Fig. 8C), and this correlation was weaker when *M. rubrum* cells were present (Pearson's correlation, $R = 0.331$, $n = 25$, $p = 0.11$) than absent ($R = 0.402$, $n = 62$, $p = 0.0012$).

3.6. Reduction of *Mesodinium rubrum* motility by *Dinophysis acuminata*

The dinoflagellate *Dinophysis acuminata* employs two methods to combat the remarkable capacity of the ciliate *Mesodinium rubrum* to escape through jumps. In the water column, *D. acuminata* discharges a capture filament or peduncle to connect with a targeted *M. rubrum* cell, thereby restraining the ciliate (Fig. 9A; Event C of Supplementary Video Group S3). At times the peduncle-linked ciliate can break free, but then a residual part of the broken peduncle often remains stuck fast to the ciliate, thereby slowing it (Fig. 9B; Event A of Supplementary Video Group S4). A broken peduncle can even adhere to several *M. rubrum* cells simultaneously, causing them all to reduce their jumping capability (Fig. 9C; Event B of Supplementary Video Group S4). Also, in the water column, individual *D. acuminata* cells can swim in a spinning fashion and cluster together to generate a clump of mucus (Fig. 10A; Event A of Supplementary Video Group S5); subsequently, the clump of mucus settles through the water column and drags and entangles *M. rubrum* cells along its way (Fig. 10B; Event B of Supplementary Video Group S5). When it finally reaches the bottom of the culture vessel, the clump of mucus has already entangled an array of *M. rubrum* cells whose motility has been more or less affected depending on the time order these cells are entangled (Fig. 10C; Event C of Supplementary Video Group S5). Then, *D. acuminata* cells swim to and feed on sufficiently immobilized prey (Event D of Supplementary Video Group S5). Previous studies observed cell lysis, loss of cilia, and cell swelling for mucus-trapped *M. rubrum* cells (Ojamäe et al., 2016; Papiol et al., 2016). The present study also observed that mucus-trapped *M. rubrum* cells started to lose their cilia and become round (Fig. 10C), but cell lysis was not observed, probably because of the short observation time.

The effectiveness of these two modes of *Dinophysis acuminata* prey capture has been demonstrated by the different distributions of maximum speed of individual *Mesodinium rubrum* cells before and after encountering *D. acuminata* cells. In the absence of *D. acuminata*, the *M. rubrum* population was dominated by fast spontaneous jumping cells with only a few swimming/zigzagging cells (Fig. 11A, B). By contrast, in the presence of *D. acuminata*, a significant portion of the *M. rubrum* population consisted of either entangled or swimming/zigzagging cells (Fig. 11C). If not entangled, *M. rubrum* cells were still prone to losing their jumping capability when exposed to *D. acuminata*, and became swimming/zigzagging cells that moved at much lower speeds. There were still spontaneously jumping cells and cells that jumped to escape approaching *D. acuminata*, but these only comprised a significantly reduced portion of the *M. rubrum* population (Fig. 11C). As a result, the mean swimming speed of the whole population was significantly reduced (Fig. 11C, D).

4. Discussion

4.1. Roles of the transverse and longitudinal flagella in *Dinophysis acuminata*

The HSMIS provides excellent resolution of flagellar beating, unambiguously demonstrating the respective functions of the transverse and longitudinal flagella in *Dinophysis* swimming behavior.

The transverse flagellum moves as a helix that circles over the anterior of the cell (Event A of Supplementary Video Group S2). The circling, axial component of the movement of the transverse flagellum exerts a torque on the surrounding water about the cell's long axis, and the reaction associated with this torque acts on and causes the cell to rotate. Simultaneously, the posteriorly-directed, tangential component of the movement of that same flagellum exerts a posteriorly-directed distributed force that acts on the water along the outer edge of the transverse flagellum, producing anteriorly-directed thrust. When this anteriorly-directed thrust is completely counterbalanced by a tethering force produced by reverse beating of the longitudinal flagellum, the cell remains immobile and all posteriorly-directed distributed force from the transverse flagellum generates a cone-shaped scanning current toward the apical surface of the cell (Fig. 3A-C; Event B of Supplementary Video Group S2). In effect, instead of moving forward due to the motion of the transverse flagellum, water is pulled towards the cell. When the anteriorly-directed thrust is only partially counterbalanced by a tethering force, part of the thrust will contribute to forward swimming of the cell and part to generation of a weaker scanning current. In an extreme case where no tethering force is supplied, the thrust will wholly contribute to forward swimming of the cell and no strong first-order scanning current is generated. Since the axial and tangential components of the helical movement of the transverse flagellum are linked, anteriorly-directed thrust generated by the transverse flagellum must be accompanied by cell rotation-inducing torque. In other words, the cell rotation in *Dinophysis acuminata* is likely an ancillary movement to the primary activity of generating a cone-shaped scanning current by the transverse flagellum.

The transverse flagellum can generate forward thrust, but the longitudinal flagellum plays the dominant role in generating thrust for the cell to swim forward (Fig. 2; Event A of Supplementary Video Group S2). At other times, the longitudinal flagellum beats reversely to supply a tethering or anchoring force to aid the generation of a posteriorly-directed, cone-shaped scanning current by the transverse flagellum (Fig. 3A-C; Event B of Supplementary Video Group S2). The versatile longitudinal flagellum also generates a steering force that allows the cell to turn and reorient itself (Fig. 3D; Event B of Supplementary Video Group S2).

The rotating torque and anteriorly-directed thrust of the transverse flagellum should be highly correlated because they are simultaneously generated by the linked axial and tangential components of the helical movement of the transverse flagellum; therefore, if anteriorly-directed thrust generated by the transverse flagellum were the dominant contributor to the total forward swimming thrust, the correlation between path-averaged swimming speed and cell rotation speed would be high. Such a correlation was, however, quite weak in the observations made here (Fig. 8C), suggesting that the longitudinal flagellum is the dominant source of forward swimming thrust. The data also show an even

lower correlation between path-averaged swimming speed and cell rotation speed when *Mesodinium rubrum* cells are present (Fig. 8C). When searching for prey, *Dinophysis acuminata* cells increase their swimming speeds presumably by beating their longitudinal flagella more rapidly (Fig. 7B; Fig. 8A). When approaching a targeted prey cell, *D. acuminata* slow down (Fig. 7C) and strengthen their scanning currents for enhanced chemoreception, presumably by beating their transverse flagella more rapidly (thereby faster cell rotation speed, Fig. 8B). Thus, the observed weak correlation between path-averaged swimming speed and cell rotation speed indicates that forward swimming and generation of a scanning current are two separate activities: the former depends mainly on the movement of the longitudinal flagellum, and the latter on the transverse flagellum.

4.2. Predator-prey interactions between *Dinophysis acuminata* and *Mesodinium rubrum*

The present observations using the HSMIS suggest that *Dinophysis acuminata* detects and locates *Mesodinium rubrum* via chemoreception. The observations further indicate that *M. rubrum* cells are only detected while at rest. An *M. rubrum* cell often stands still for 1 – 20 s between consecutive spontaneous jumps (Fenchel and Hansen, 2006). Compared to molecular diffusion time scale, these resting periods are sufficiently long for full development of a chemical diffusive boundary layer centered on the *M. rubrum* cell (Jiang and Johnson, 2017), detectable perhaps via cell exudates or nutrient deficits due to cell uptake. Scanning currents produced by *Dinophysis*' transverse flagellum enhance the transport of chemical cues to *D. acuminata* and increase detection range over simple diffusion. Also observed was frequent disorientation of *D. acuminata* following escape jumps by *M. rubrum*. Once again, this observation supports chemoreception by *D. acuminata*: The escape distance of *M. rubrum* is sufficiently long ($232 \pm 125 \mu\text{m}$; $7.8 \pm 4.5 L_{MT}$) such that the chemical diffusive boundary layer completely detaches from the cell surface (Jiang and Johnson, 2017). As a result, *D. acuminata* is unable to re-locate its prey immediately following an escape jump. The escape trajectory of *M. rubrum* is often a zigzag or curved path, which not only produces a complex pathway for *Dinophysis* to follow, but may also contribute to detaching the chemical diffusive boundary layer from the cell surface and/or help shed capture filaments.

The ciliate *Mesodinium rubrum* detects an approaching *Dinophysis acuminata* predator via mechanoreception, as also inferred from the present HSMIS observations. This is similar to previous reports of *M. rubrum* mechanoreception, e.g., *M. rubrum* jumping away from a copepod-generated feeding current, an approaching copepod (Jonsson and Tiselius, 1990), or an artificial siphon flow (Fenchel and Hansen, 2006). A resting *M. rubrum* cell detects and escapes from a *D. acuminata* cell when the latter approaches the former within a comparatively short distance [$41 \pm 32 \mu\text{m}$ (Mean $\pm$ SD), 2 – 134 μm (range), 27 (sample size)]. This is roughly 1.3 body lengths of *D. acuminata*.

When approaching a resting *Mesodinium rubrum* prey, *Dinophysis acuminata* not only beats its longitudinal flagellum to maintain a suitable approach speed but also moves its transverse flagellum to generate a cone-shaped scanning current that directs water toward its anterior. On the one hand, body and flagellar motions of *D. acuminata* impose hydrodynamic signals that the *M. rubrum* cell may detect via mechanoreception. On the other hand, the cone-

shaped scanning current, if positioned properly, may deceive the *M. rubrum* cell about the approaching motion of the *D. acuminata* cell, as the observed preference of *D. acuminata* to approach prey from below likely aids in its stealth. This is because *M. rubrum* is slightly negatively buoyant and usually sinks when not jumping. The downward scanning current produced by *D. acuminata* overlaps with the flow imposed by the sinking *M. rubrum* cell, likely providing *D. acuminata* a form of hydrodynamic camouflage and enabling its close range approach.

Combined with previous discussions on the propulsive roles of the transverse and longitudinal flagella, these observations support the notion that the desmokont flagellar arrangement in *Dinophysis acuminata* is well-suited for phagotrophy in the water column. In particular, the transverse flagellum is strategically positioned over the anterior of the cell. By moving in concert with the trailing longitudinal flagellum, the anterior transverse flagellum enables both detection and stealthy approach of resting, free-sinking *Mesodinium rubrum* prey.

The predator-prey interaction between *Dinophysis acuminata* and *Mesodinium rubrum* conforms to one of the two optimal scenarios predicted by the theoretical predator-prey encounter-rate model (Gerritsen and Strickler, 1977), namely, a cruising predator to prey upon a slow-moving or stationary prey. Despite being characterized as a fast-jumping ciliate, *M. rubrum* stands still for a long time between consecutive spontaneous jumps. It is this stationary behavior that makes *M. rubrum* susceptible to chemoreception-aided predation by *D. acuminata*. It has not yet been demonstrated that *D. acuminata* can feed on other prey species, but if so, such a species is unlikely to be a continuous-swimming species but instead one with substantial resting times in its motility pattern. Nevertheless, jumping is an effective predator avoidance behavior by *M. rubrum*. In the evolutionary arms race between predator and prey, *Dinophysis* species have developed strategies to cope with the difficulty caused by the fast-jumping behavior of *M. rubrum*. Species of *Dinophysis* have a stealthy detection and approach strategy, and then restrain their prey with capture filaments or peduncles (Figs. 4, 9) and create and deploy mucus traps (Nishitani et al., 2008; Ojamäe et al., 2016; Papiol et al., 2016; Mafra et al., 2016) to immobilize those prey for feeding. Species of *Dinophysis* may also produce toxins or bioactive chemicals that aid to immobilize their prey (Ojamäe et al., 2016; Papiol et al., 2016; Mafra et al., 2016). The present study observed that the maximum speed of escape jumping was statistically smaller than that of spontaneous jumping (Fig. 6A). This observation could indicate that *D. acuminata* targeted those *M. rubrum* cells with weakened jumping capabilities, which may have been caused either by exposure to *D. acuminata*-released toxins or by injuries due to previous *D. acuminata* attacks. The experimental cultures were 20 – 30 days old and therefore might have been rich in bioactive compounds that weakened the jumping capabilities of the prey. When encountering a *M. rubrum* bloom in the ocean, *Dinophysis* cells probably use the strategy of releasing mucus and/or toxins to immobilize *M. rubrum* cells before feeding on them. When there is no bloom, individual *Dinophysis* cells probably have to rely on chemoreception, the stealthy detection and approach strategy, and their capture filaments to encounter and capture individual *M. rubrum* cells.

4.3. The HSMIS quantitative microvideography approach for microplankton investigation

The HSMIS provides a novel and effective means for conducting quantitative microvideographic studies of microplankton behaviors and species interactions. Microplankton are small in size (20 – 200 μm), including flagellates, dinoflagellates, ciliates, copepod nauplii, and meroplanktonic larvae. They consist of photosynthetic, heterotrophic, and mixotrophic species. They are frequently the primary phytoplankton grazers and themselves are consumed by larger zooplankton such as copepods, thereby contributing dominantly to the recycling of particulate primary production in the ocean. Prominent among microplankton are HAB species that produce toxins that can kill fish, mammals, and birds, as well as human illness and mortality. Given ecological and societal impacts, it is of fundamental importance to investigate microplankton behaviors and species interactions in order to better understand ocean ecology and biogeochemistry, as well as human health and ecosystem impacts from toxin producing species. Because microplankton are small and can move rapidly relative to their body size, observing systems must provide micrometer spatial and millisecond temporal resolution. Traditional microscopy has been used, but is considered a qualitative technique because of wall effects associated with shorter focal length optics, confinement of observations to a horizontal field-of-view, and strong sample convection caused by intense illumination sources.

These limitations have been addressed through the development of the HSMIS. The HSMIS uses specially designed optics to provide a vertically oriented field-of-view with dimensions ranging from 0.1×0.1 to 5.0×5.0 mm. The vertically oriented field-of-view enables observation of vertically oriented behaviors like the downward scanning current (Fig. 3A-C; Event B of Supplementary Video Group S2) and upward prey interception behaviors of *Dinophysis acuminata* (Event A of Supplementary Video Group S3). Both are invisible by traditional microscopy.

The HSMIS is equipped with a high-resolution, high-speed camera that records submicrometer cell features at a frame rate up to 2000 frames per second. A great advantage of this high-resolution, high-frame rate microvideography is its ability to register rapid movements of both cells and their flagella or cilia in unambiguous detail. The HSMIS also makes use of long working distance optics that enable observations much further from cell chamber walls, reducing associated artifacts. The HSMIS has a more sophisticated optical setup than direct image projection using a microscope objective, and is able to form crisp images using illumination from very thin, collimated light. A custom-made collimator shapes a light beam that is just large enough to illuminate the field-of-view, thereby producing much less heat and dramatically reducing convective flows within observation vessels. As a result, accurate measurements of cell swimming speeds can be made directly without controlling for contributions from these flows.

The present study has demonstrated the capability of the HSMIS for observing microplankton behaviors and species interactions in great detail and has provided high-resolution data of cell motion kinematics. Ongoing investigations based on the HSMIS are unraveling a variety of other as yet poorly characterized aspects of microplankton behaviors and species interactions that are fundamental to microbial ecology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- *D. acuminata* detects its *M. rubrum* prey via chemoreception at 89 μm mean distance
- *M. rubrum* detects *D. acuminata* via mechanoreception at 41 μm mean distance
- On detection, *D. acuminata* approaches *M. rubrum* with reduced speed
- *M. rubrum* responds through long enough escape jumps to detach its chemical trail
- The desmokont flagellar arrangement of *D. acuminata* suits itself to phagotrophy

Fig. 1.
The high-speed microscale imaging system (HSMIS).

Fig. 2.

The composite of two frames from a recorded video of a swimming *Dinophysis acuminata* cell (Event A of Supplementary Video Group S2). Frame A is 1683 ms and Frame B is 359 ms after the beginning of the video. The two frames are superimposed along the cell's swimming path (the black line). The arrow in each frame points to the position of the longitudinal flagellum.

Fig. 3.

Time-course image sequence illustrating the swimming behavior of a *Dinophysis acuminata* cell (Event B of Supplementary Video Group S2). From 0 to 2870 ms, the cell hovers in the water column with its anterior pointing upward while rotating its body counterclockwise as viewed from the posterior end of the cell; the hovering cell generates a downward, cone-shaped scanning current (black line) that is evidenced by an entrained particle (A-C). From 2871 to ~3573 ms, the cell turns and reorients itself by repositioning its longitudinal flagellum from posterior-pointing to anterior-pointing (D), and then puts its longitudinal flagellum back to point posteriorly. From ~3574 to 5456 ms, the cell swims by beating its longitudinal flagellum backward (E, F) while rotating its body counterclockwise as viewed from the posterior end of the cell. The arrow in each frame points to the position of the longitudinal flagellum of the cell.

Fig. 4.

Time-course image sequence illustrating a *Dinophysis acuminata* cell capturing a *Mesodinium rubrum* cell (Event B of Supplementary Video Group S3): The *D. acuminata* cell approaches the *M. rubrum* cell stealthily (A, B), the *M. rubrum* cell jumps away (C), but the *D. acuminata* cell has already linked the *M. rubrum* cell with a capture filament (D, arrow). A 3 W IR LED light source was collimated to provide backlit illumination for this observation.

Fig. 5. The reaction distance (R_{Mr}) of a stationary *Mesodinium rubrum* prey to an approaching *Dinophysis acuminata* predator is significantly shorter than the reaction distance (R_{da}) of a *D. acuminata* predator to a stationary *M. rubrum* prey (Student's *t*-test, $p < 0.0001$). Error bars represent standard errors.

Fig. 6. Comparison of *Mesodinium rubrum* jumping kinematics: (A) maximum jump speed (U_{max}), (B) jump duration (t_{jump}), and (C) jump distance (D_{jump}) for three different situations, i.e., spontaneous jumping in the absence of *Dinophysis acuminata* (left columns), spontaneous jumping in the presence of *D. acuminata* (middle columns), and escape jumping (right columns). Error bars represent standard errors.

Fig. 7.

Comparison of *Dinophysis acuminata* path-averaged swimming speed (U_{ave}) for four different situations: (A) *D. acuminata* swimming in the absence of *Mesodinium rubrum*; (B) *D. acuminata* swimming in the presence of *M. rubrum*; (C) *D. acuminata* swimming to approach a detected and targeted *M. rubrum* cell; and (D) *D. acuminata* swimming while carrying a captured *M. rubrum* cell. Mean of B was significantly higher than mean of A (Student's *t*-test, $p = 0.0069$), and mean of C was significantly lower than mean of B (Student's *t*-test, $p = 0.043$). Error bars represent standard errors.

Fig. 8.

Comparison of *Dinophysis acuminata* swimming kinematics: (A) path-averaged swimming speed (U_{ave}) and (B) cell rotation speed (Ω) for two different situations, i.e., swimming in the absence of the prey *Mesodinium rubrum* (left columns) and swimming in the presence of *M. rubrum* (right columns), where error bars represent standard errors. (C) Ω vs. U_{ave} , where symbols show raw data and the solid lines represent linear regressions, plotted for the above-described two situations.

Fig. 9.

Image frames extracted from videos illustrating: (A) a *Dinophysis acuminata* cell deploys a peduncle to connect with a *Mesodinium rubrum* cell that tries vigorously to escape (Event C of Supplementary Video Group S3), (B) an *M. rubrum* cell swims abnormally with a broken *Dinophysis* peduncle attached to its body (Event A of Supplementary Video Group S4), and (C) four *M. rubrum* cells swim abnormally while all are attached to a same broken *Dinophysis* peduncle (Event B of Supplementary Video Group S4). The arrow in each image points to the peduncle.

Fig. 10.

Image frames extracted from videos illustrating: (A) *Dinophysis acuminata* cells swim around a clump of mucus that presumably has been generated by them (Event A of Supplementary Video Group S5; the arrow points to the mucus clump), (B) several *M. rubrum* cells are entangled by a clump of mucus, sink, and drag a few additional *M. rubrum* cells with them (Event B of Supplementary Video Group S5; the arrow points to a dragged cell), and (C) the motility of several *M. rubrum* cells is differentially affected by a clump of mucus sitting on the bottom of the culture vessel (Event C of Supplementary Video Group S5); ciliate #1 approaches and quickly escapes, ciliate #2 vigorously tries to escape but is dragged back by the viscous material, ciliate #3, along with a few nearby ciliates, has been entangled but still has its cilia, whereas ciliate #4 and a few neighboring cells have already lost their cilia and motility.

Fig. 11.

Maximum speed (U_{max}) of *Mesodinium rubrum* motion behavior. (A) Stacked histogram and (B) statistics of U_{max} according to two motion behavior categories, i.e., spontaneous jumping and swimming/zigzagging, which were the two behaviors displayed by *M. rubrum* in the absence of *D. acuminata*. (C) Stacked histogram and (D) statistics of U_{max} according to three motion behavior categories, i.e., spontaneous jumping, escape jumping, swimming/zigzagging, and being tangled by broken peduncles or released mucus by *D. acuminata*,

which were the four behaviors displayed by *M. rubrum* in the presence of *D. acuminata*. In (B) and (D), error bars represent standard errors.

Table 1.

Analysis summary of motion behavior of the ciliate *Mesodinium rubrum* in the absence of the dinoflagellate *Dinophysis acuminata*.

A. Spontaneous jumping.							
	Cell length (L_{Mr})	Cell width (W_{Mr})	Jump duration (t_{jump})	Maximum jump speed (U_{max})		Jump distance (t_{jump})	
	(μm)	(μm)	(ms)	(mm s^{-1})	($L_{Mr} \text{ s}^{-1}$)	(μm)	(L_{Mr})
Mean $\pm$ SD	34.6 $\pm$ 5.3	27.1 $\pm$ 4.7	50.7 $\pm$ 17.2	6.7 $\pm$ 1.5	194.6 $\pm$ 43.9	178 $\pm$ 108	5.1 $\pm$ 2.9
Range	21.4 – 49.9	17.9 – 41.1	28.5 – 102.0	3.0 – 10.2	93.2 – 291.9	35 – 642	1.1 – 13.1
Sample size	90	90	84	90	90	84	84

B. Swimming/zigzagging.								
	Cell length (L_{Mr})	Cell width (W_{Mr})	Duration (t_{swim})	Path length (t_{swim})	Path-averaged speed (U_{ave})		Maximum path speed (U_{max})	
	(μm)	(μm)	(ms)	(μm)	(mins^{-1}) ($L_{Mr} \text{ s}^{-1}$)	($L_{Mr} \text{ s}^{-1}$)	(μm)	(L_{Mr})
Mean $\pm$ SD	33.5 $\pm$ 4.0	25.8 $\pm$ 4.5	267.9 $\pm$ 419.1	274 $\pm$ 100	2.4 $\pm$ 1.4	87.4 $\pm$ 35.3	4.3 $\pm$ 2.0	154.5 $\pm$ 40.4
Range	29.0 – 40.1	19.6 – 31.7	65.5 – 1122.0	151 – 416	0.23 – 4.2	49.4 – 144.7	0.48 – 6.1	107.8 – 211.4
Sample size	5	6	6	6	6	5	6	5

Table 2.

Analysis summary of swimming behavior of the dinoflagellate *Dinophysis acuminata* in the absence of the ciliate *Mesodinium rubrum*.

	Cell length (L_{Da})	Cell width (W_{Da})	Cell thickness (H_{Da})	Duration (t_{swim})	Path length (l_{swim})	Path-averaged speed (U_{ave})		Rotation speed (Ω)	NGDR
	(μm)	(μm)	(μm)	(ms)	(μm)	($\mu\text{m s}^{-1}$)	($L_d \text{ s}^{-1}$)	(rad s^{-1})	
Mean $\pm$ SD	48.4 $\pm$ 5.3	33.2 $\pm$ 5.1	19.1 $\pm$ 3.8	2219.5 $\pm$ 1302.2	288 $\pm$ 148	145 $\pm$ 67	3.0 $\pm$ 1.5	2.31 $\pm$ 0.98	0.813 $\pm$ 0.182
Range	36.7 – 60.5	22.1 – 44.6	11.1 – 32.0	264.0 – 10912.0	32 – 829	38 – 452	0.7 – 9.8	0.83 – 5.18	0.233 – 0.999
Sample size	138	119	121	138	138	138	138	62	138

Table 3.

Analysis summary of motion behavior of the ciliate *Mesodinium rubrum* in the presence of the dinoflagellate *Dinophysis acuminata*.

A. Spontaneous jumping (not to escape).								
	Cell length (L_{Mr})	Cell width (W_{Mr})	Jump duration (t_{jump})	Maximum jump speed (U_{max})		Jump distance (d_{jump})		
	(μm)	(μm)	(ms)	(mm s^{-1})	($L_{Mr} \text{ s}^{-1}$)	(μm)	(L $_{Mr}$)	
Mean $\pm$ SD	30.8 $\pm$ 6.1	24.6 $\pm$ 5.6	59.3 $\pm$ 34.8	4.1 $\pm$ 3.0	211.4 $\pm$ 67.6	201 $\pm$ 151	6.8 $\pm$ 5.3	
Range	16.7 – 46.3	12.8 – 39.9	31.0 – 210.5	2.6 – 10.7	103.6 – 380.3	48 – 643	1.5 – 26.3	
Sample size	40	40	27	40	40	27	27	

B. Escape jumping.								
	Cell length (L_{Mr})	Cell width Jump (W_{Mr})	Jump duration (t_{jump})	Maximum jump speed (U_{max})		Reaction distance to <i>D. acuminata</i> (R_{mr})	Jump distance (d_{jump})	
	(μm)	(μm)	(ms)	(mm s^{-1})	($L_{Mr} \text{ s}^{-1}$)	(μm)	(μm)	(L $_{Mr}$)
Mean $\pm$ SD	32.1 $\pm$ 6.4	25.9 $\pm$ 5.2	74.8 $\pm$ 23.1	5.7 $\pm$ 1.6	180.2 $\pm$ 46.3	41 $\pm$ 32	232 $\pm$ 125	7.8 $\pm$ 4.5
Range	22.0 – 45.6	14.3 – 37.2	35 – 105	3.2 – 9.6	76.4 – 279.6	2 – 134	51 – 524	1.6 – 18.1
Sample size	26	26	15	25	25	27	16	16

C. Swimming/zigzagging.								
	Cell length (L_{Mr})	Cell width (W_{Mr})	Duration (t_{swim})	Path length (l_{swim})	Path-averaged speed (U_{ave})		Maximum path speed (U_{max})	
	(μm)	(μm)	(ms)	(μm)	(mm s^{-1})	(L $_{mr} \text{ s}^{-1}$)	(μm)	(L $_{Mr}$)
Mean $\pm$ SD	27.6 $\pm$ 5.0	21.6 $\pm$ 3.3	333.8 $\pm$ 429.7	345 $\pm$ 275	2.0 $\pm$ 1.3	72.6 $\pm$ 48.1	4.1 $\pm$ 1.8	150.7 $\pm$ 65.7
Range	19.5 – 37.3	14.6 – 29.5	31.5 – 1947.0	96 – 1386	0.4 – 4.3	11.6 – 162.4	0.8 – 8.6	34.8 – 292.7
Sample size	27	27	27	27	27	27	27	27

D. Being tangled by broken peduncles or released mucus by <i>D. acuminata</i>.								
	Cell length (L_{Mr})	Cell width (W_{Mr})	Duration (t_{swim})	Path length (l_{swim})	Path-averaged speed (U_{ave})		Maximum path speed (U_{max})	
	(μm)	(μm)	(ms)	(μm)	(mm s^{-1})	(L $_{Mr} \text{ s}^{-1}$)	(μm)	(L $_{Mr}$)
Mean $\pm$ SD	36.2 $\pm$ 4.1	29.7 $\pm$ 4.0	1067.8 $\pm$ 790.9	572 $\pm$ 187	1.0 $\pm$ 0.8	28.2 $\pm$ 23.4	3.7 $\pm$ 1.7	104.7 $\pm$ 46.7
Range	28.3 – 44.4	22.4 – 36.6	130.5 – 2728.0	274 – 913	0.19 – 2.9	5.1 – 75.6	1.7 – 8.5	42.9 – 222.4
Sample size	16	16	16	16	16	16	16	16

Table 4.

Analysis summary of swimming behavior of the dinoflagellate *Dinophysis acuminata* the presence of the ciliate *Mesodinium rubrum*.

A. Swimming and searching.									
	Cell length (L_{Da})	Cell width (W_{Da})	Cell thickness (H_{Da})	Duration (t_{swim})	Path length (l_{swim})	Path-averaged speed (U_{ave})		Rotation speed (Ω)	NGDR
	(μm)	(μm)	(μm)	(ms)	(μm)	($\mu\text{m s}^{-1}$)	($L_{Da} \text{ s}^{-1}$)	(rad s^{-1})	
Mean $\pm$ SD	44.7 $\pm$ 5.7	31.2 $\pm$ 5.0	16.6 $\pm$ 3.4	1857.9 $\pm$ 739.8	295 $\pm$ 163	170 $\pm$ 83	3.9 $\pm$ 2.0	3.18 $\pm$ 1.32	0.849 $\pm$ 0.185
Range	33.2 – 57.3	17.5 – 41.1	9.4 – 26.8	136.5 – 2728.0	33 – 714	67 – 420	1.6 – 9.1	1.37 – 7.74	0.275 – 0.997
Sample size	54	45	44	54	54	54	54	25	138

B. Swimming to approach a resting ciliate <i>M. rubrum</i>.									
	Cell length (L_{Da})	Cell width (W_{Da})	Cell thickness (H_{Da})	Duration (t_{swim})	Path length (l_{swim})	Path-averaged speed (U_{ave})		Reaction distance to <i>M. rubrum</i> (R_{Da})	NGDR
	(μm)	(μm)	(μm)	(ms)	(μm)	($\mu\text{m s}^{-1}$)	($L_{Da} \text{ s}^{-1}$)	(μm)	
Mean $\pm$ SD	46.8 $\pm$ 5.5	30.9 $\pm$ 5.8	17.8 $\pm$ 4.0	1447.5 $\pm$ 710.1	188 $\pm$ 121	137 $\pm$ 61	3.0 $\pm$ 1.5	89 $\pm$ 39	0.737 $\pm$ 0.267
Range	35.9 – 59.6	20.8 – 46.2	10.0 – 26.8	163.5 – 2728.0	23 – 537	36 – 285	0.6 – 7.0	48 – 224	0.112 – 0.993
Sample size	26	21	20	26	26	26	26	26	26

C. Swimming while carrying a captured ciliate <i>M. rubrum</i>.						
	Cell length (L_{Da})	Duration (t_{swim})	Path length (l_{swim})	Path-averaged speed (U_{ave})		NGDR
	(μm)	(ms)	(μm)	($\mu\text{m s}^{-1}$)	($L_{Da} \text{ s}^{-1}$)	
Mean $\pm$ SD	48.5 $\pm$ 6.3	1801.9 $\pm$ 730.4	294 $\pm$ 111	185 $\pm$ 76	3.9 $\pm$ 1.7	0.814 $\pm$ 0.248
Range	31.9 – 54.5	253.0 – 2728.0	96 – 484	89 – 381	1.6 – 7.5	0.304 – 0.991
Sample size	12	11	11	11	11	11