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

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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.

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,

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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 desmokont 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 µmol photons m^{-2} 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).

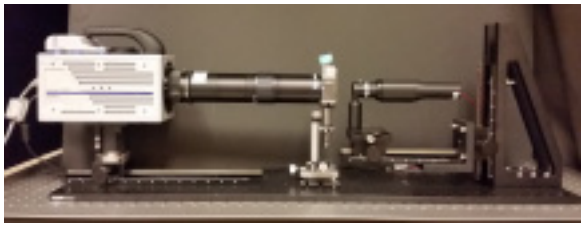

Fig. 1. The high-speed microscale imaging system (HSMIS).

(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_2SeO_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 × 10^5 cells mL^{-1}) *T. amphioxeia* was fed to 80 mL stocks of *M. rubrum* (~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 120 K 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 20×/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 (1600–2200 cells mL^{-1}) and one flask of ~20 mL *Mesodinium rubrum* culture (2300–3100 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