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Mass entrapment and lysis of *Mesodinium rubrum* cells in mucus threads observed in cultures with *Dinophysis*

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ABSTRACT

The entrapment and death of the ciliate *Mesodinium rubrum* in the mucus threads in cultures with *Dinophysis* is described and quantified. Feeding experiments with different concentrations and predator–prey ratios of *Dinophysis acuta*, *Dinophysis acuminata* and *M. rubrum* to study the motility loss and aggregate formation of the ciliates and the feeding behaviour of *Dinophysis* were carried out. In cultures of either *Dinophysis* species, the ciliates became entrapped in the mucus, which led to the formation of immobile aggregates of *M. rubrum* and subsequent cell lysis. The proportion of entrapped ciliates was influenced by the concentration of *Dinophysis* and the ratio of predator and prey in the cultures. At high cell concentrations of prey (136 cells mL^{-1}) and predator (100 cells mL^{-1}), a maximum of 17% of *M. rubrum* cells became immobile and went through cell lysis. Ciliates were observed trapped in the mucus even when a single *D. acuminata* cell was present in a 3.4 mL growth medium. Both *Dinophysis* species were able to detect immobile or partly immobile ciliates at a distance and circled around the prey prior to the capture with a stretched out peduncle. Relatively high entrapment and lysis of *M. rubrum* cells in the mucus threads indicates that under certain conditions *Dinophysis* might have a considerable impact on the population of *M. rubrum*.

1. Introduction

Species of the genus *Dinophysis* have a worldwide distribution and are well known producers of Diarrhetic Shellfish Toxins (DSTs), thereby causing a global health risk to shellfish consumers (Reguera et al., 2014). The toxins produced by *Dinophysis* include okadaic acid (OA), dinophysistoxin (DTX), and pectenotoxins (PTX), which may accumulate in mussels that feed on toxin-containing *Dinophysis* cells. The genus is globally distributed in marine environments over a broad range of salinities. In temperate waters, members of the "*Dinophysis acuminata* species complex", which also include the morphospecies *Dinophysis sacculus* and *Dinophysis ovum*, are the most widespread members of genus (Reguera et al., 2012). These species peak in abundance from late spring to early autumn, but can be found throughout the year and extend into estuaries with quite low salinity (Reguera et al., 2012). *Dinophysis acuta* Ehrenberg 1839 is another common species in temperate waters which tend to be common during summer and autumn periods (Escalera et al., 2006; Hällfors et al., 2011; Farrell et al., 2012).

Conventional peridinin-containing chloroplasts, so typical of phototrophic dinoflagellates, are lacking in *Dinophysis* species (*sensu stricto*). Instead they contain phycoerythrin-rich cryptophyte chloroplasts (Schnepf and Elbrächter, 1988; Meyer-Harms and Pollehne, 1998; Janson and Granéli, 2003). The eight species of the genus cultured so far all depend upon red *Mesodinium* spp. as their prey for long term growth; they are obligate mixotrophs (Kim et al., 2008; Riisgaard and Hansen, 2009; Hansen et al., 2013; Reguera et al., 2014). Recently, it has been documented that the chloroplasts of *Dinophysis acuta* and *Dinophysis caudata* Saville-Kent 1881 are indeed kleptochloroplasts, which are sequestered from the ciliate prey (e.g. Kim et al., 2012; Raho et al., 2014). It is presently unknown to what extent this is true for the remaining ~90 species of *Dinophysis*, and more research on this topic is required.

The ciliate *Mesodinium rubrum* (Lohmann, 1908) and other red forms of the genus are known for their characteristic backward jumps interrupted by periods where the cells remain non-motile (e.g. Fenchel and Hansen, 2006; Garcia-Cuetos et al., 2012). In this

Abbreviations: DTX, dinophysistoxin; OA, okadaic acid; PTX, pectenotoxins.

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non-motile stage, cirri are stretched out from the cell in all directions sensing changes in shear caused by approaching predators. This allows the ciliate to escape from being caught by predators (Jonsson and Tiselius, 1990). In comparison with *M. rubrum*, *Dinophysis* swims fairly slowly (Smayda, 2010), and very few studies are available that describe and document how *Dinophysis* spp. manage to catch the ciliates. Direct observations at low magnification indicate that *Dinophysis* cells can detect their prey from a certain distance and start to circle around the prey prior to capture. The exact capture mechanism is still unknown, but it has been reported that after circling around the prey for some time, the *Dinophysis* cells become connected with the prey. It has been proposed that similarly to many other phagotrophic dinoflagellates (e.g. Hansen and Calado, 1999) a capture filament may be involved (Hansen et al., 2013). It is important to emphasize that the previous observations have been carried out at low magnification with limited resolution and no photographic or video documentation of a capture filament has been published.

Nagai et al. (2008) and Nishitani et al. (2008) have observed *Mesodinium* cells forming aggregates with significantly altered mobility when mixed with *Dinophysis* spp. and have witnessed *Dinophysis* to feed on them. These authors have not tried to quantify this interaction, neither have they described the fate of the *Mesodinium* aggregates. In the present study the feeding behaviour of *Dinophysis acuminata* (Claparède & Lachmann, 1859) and *Dinophysis acuta* were looked into and the immobilization and aggregate formation of *Mesodinium rubrum* when exposed to its predator were described and quantified. It is hypothesized that the proportion of immobilized ciliates and the formation of aggregates are related to the cell concentrations of *Dinophysis* and *Mesodinium* and to the predator–prey ratios. To investigate this phenomenon, a mixture of short-term experiments and microscopic observations of these species using a variety of predator–prey ratios and cell concentrations were designed. Cell concentrations will most likely impact the amount of chemical substances released into the surroundings by either the predator or the prey. The amount of chemical substances may play a role in cell swimming or in prey capture efficiency.

2. Materials and methods

2.1. Cultures and culturing conditions

The cultures of the cryptophyte *Teleaulax amphioxeia* (K-1837; SCCAP) and the ciliate *Mesodinium rubrum* (MBL-DK2009) were established from single cells isolated from water samples collected from Helsingør Harbour in 2009. Cultures of *M. rubrum* were fed with *T. amphioxeia* at a predator–prey ratio of 1:5 once in every two weeks to enable mixotrophic growth. The culture of *Dinophysis acuta* (DANA-2010) was established in June 2010 from the North Sea (Nielsen et al., 2013) and *Dinophysis acuminata* (strain FR101009) was isolated from Little Belt, Denmark in October 2009 (Nielsen et al., 2012). All cultures were maintained on a glass table in autoclaved sterile-filtered f/2 medium (Guillard and Ryther, 1962) with a salinity of 35 at temperature of 15 °C. Illumination was provided from beneath by cool white fluorescent lights of 100 μmol photons m^{-2} s^{-1} on a 14:10 h light:dark cycle. All cultures were non-axenic.

Prior each experiment a new culture of *Dinophysis acuminata* and/or *Dinophysis acuta* was established onto a 24-well tissue culture plate by transferring a fraction from a culture starved for 5–7 days into newly filtered f/2 medium and subsequently fed with *Mesodinium rubrum* at a predator–prey ratio of ≈1:10. Prey was allowed to disappear from the wells during the next few days. Cell isolations of *D. acuminata* (experiments 2 and 3) were carried out instantly after pooling cells into a 65 mL tissue culture flask for homogenization and subsequent pipetting on a new multi-dish plate. Single cells were isolated using a drawn-out Pasteur micropipette and were thereafter allowed to acclimate 2–4 h before the start of experiments (also in experiment 1 after bulk pipetting both *D. acuminata* and *D. acuta*). To ensure that the cells were as healthy as possible, only swimming cells were selected; dividing cells have not been observed to feed on prey and were therefore not selected. In experiment 1 the two species of *Dinophysis* were used to see possible interspecies behavioural differences. In experiments 2 and 3 the focus was set on *D. acuminata* only.

2.2. Experiment 1. Behaviour of Dinophysis in absence and presence of M. rubrum

To give a general description of the changes in the behaviour of *Dinophysis acuminata* and *Dinophysis acuta* after addition of prey, cultures of cells starved before for 2–3 days were pooled into 65 mL (true capacity) tissue culture flasks (TPP, Switzerland) and cell suspensions of *D. acuminata* and *D. acuta* containing ca. 100, 300 and 700 cells (respectively for both species) were pipetted in triplicates into 1 mL of f/2 medium onto 24-well tissue culture plates. The cultures of *D. acuminata* at concentrations of 84, 194 and 307 cells mL^{-1} and the cultures of *D. acuta* at concentrations of 89, 217 and 368 cells mL^{-1} were allowed to acclimate for 2–3 h. After acclimatization, microscopic observations were carried out to describe the swimming behaviour of dinoflagellates in absence of *Mesodinium rubrum*. For further observations in the presence of ciliates, each *Dinophysis* cell suspension was mixed with *M. rubrum* cell suspension of 0.06, 0.180 and 0.42 mL (ca. 1000, 3000, and 7000 cells, respectively). Culture mixtures in each well were raised to the full capacity (to the rim) by adding f/2 medium and covered with a cover glass. Final concentrations for *D. acuminata* and *D. acuta* were 29, 88 and 206 cells mL^{-1} and for *M. rubrum* 290, 880 and 2060 cells mL^{-1}. The experiment was carried out over 8 days. Observations were documented as videos and pictures using an inverted microscope (Nikon Diaphot-TMD, Nikon Corporation, Japan) equipped with a digital camera (Canon EOS 5D Mark III, Canon, Japan).

2.3. Experiment 2. Effect of exposure time of D. acuminata on M. rubrum motility: different concentrations of Dinophysis and fixed concentrations of Mesodinium

A timed experiment was carried out to determine the effect of concentration and exposure time of *Dinophysis acuminata* on the motility of *Mesodinium rubrum*. The cells of *D. acuminata* were isolated with a drawn out Pasteur pipette in triplicates into 2 mL of f/2 medium in a 24-well tissue culture plate and were allowed to acclimate for 2–3 h. Thereafter the cultures were mixed with 0.28 mL *M. rubrum* cell suspension (2000 cells, final concentration 588 cells mL^{-1}), which marked the starting point of the experiment. Each culture mix was raised to 3.4 mL by adding f/2 medium and was covered with a cover glass for microscopic observations. The initial number of cells for *D. acuminata* treatments in 3.4 mL culture were 1, 2, 5, 17, 34, 70, 153, 306, 510, resulting in concentrations of 0.3, 0.6, 1.5, 5, 10, 20.6, 45, 90, 150 cells mL^{-1} and in predator–prey ratios of 0.0005, 0.001, 0.003, 0.009, 0.02, 0.04, 0.08, 0.2 and 0.3. The concentration levels chosen for *D. acuminata* corrrespond to common natural abundances of *Dinophysis*. For controls, three replicates of *M. rubrum* without *D. acuminata* were established at the same concentrations as in the treatment wells. The number of single *M. rubrum* cells that displayed abnormal swimming behaviour, immobilized cells, aggregates and cells in each aggregate were counted at the time steps of 0, 10, 20, 30, 40, 60, 90, 120, 180, 240 min. Single cells with altered motility were