Limnol. Oceanogr., 56(1), 2011, 371–378

doi:10.4319/lo.2011.56.1.0371

Avoidance, movement, and mortality: The interactions between a protistan grazer and *Heterosigma akashiwo*, a harmful algal bloom species

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Abstract

A reduction in predator-induced grazing pressure may be a mechanism that facilitates the formation and persistence of harmful algal blooms. Here, the hypothesis was tested that the heterotrophic ciliate *Favella ehrenbergii* would use avoidance behaviors to reduce encounters with the toxic bloom–forming alga, *Heterosigma akashiwo*. Using video and image-analysis, population distributions and three-dimensional movements of *F. ehrenbergii* and *H. akashiwo* were quantified in triplicate, hourly for 11 h, at nine horizons in a 1-liter experimental column. The salinity structure in the column was manipulated to include a halocline, resulting in layer formation by *H. akashiwo*. The ciliate's vertical distributions were restricted to high-salinity waters below the halocline, while *H. akashiwo* was broadly halo-tolerant and could occupy the whole water column. When observed together, *F. ehrenbergii* did not avoid layers of *H. akashiwo*. In the presence of *H. akashiwo*, *F. ehrenbergii* mortality rates were higher than in either no prey or beneficial prey controls. Swimming behaviors of *F. ehrenbergii* were erratic, in response to *H. akashiwo*, compared to aggregative movements in response to beneficial prey, indicating either a behavioral response or the effect of *H. akashiwo* toxicity on the ciliate. The inability of *F. ehrenbergii* to avoid *H. akashiwo* enhanced predator mortality and may contribute to the survival of the harmful algal bloom species, ultimately promoting the formation of *H. akashiwo* harmful algal blooms.

Harmful algal blooms (HABs) can be detrimental to marine ecosystems, human health, and fishing economies. Blooms of the toxic raphidophyte, *Heterosigma akashiwo* (Hada), have been found globally in temperate coastal waters (Smayda 1998). These blooms can be ichthyotoxic, causing mortality in both caged and naturally occurring fish populations (Honjo 1993; Khan et al. 1997). Sublethal effects, including the destabilization of cellular defense mechanisms in oysters (Keppler et al. 2005) and altered respiratory activity of mammalian cells (Twiner et al. 2004) have been observed upon exposure to *H. akashiwo*. Although there is no consensus on the mode of toxicity for *H. akashiwo*, possible modes include the production of reactive oxygen species (Twiner et al. 2001), mucus (Nakamura et al. 1998), and neurotoxins (Khan et al. 1997).

Phytoplankton population dynamics are driven by the relative rates of cell growth and loss; formation of an algal bloom can result when population growth rates exceed loss rates. It is unclear why many HAB species are highly successful at frequently forming mono-specific blooms. Hypotheses on the mechanisms of bloom formation include vertical migration (Smayda 2002), allelopathy (Granéli et al. 2008), eutrophication (Anderson et al. 2002), and pelagic-benthic life cycles (McGillicuddy et al. 2003). Another possibility is that HAB species have a greater grazer resistance than other phytoplankton species. Heterotrophic protists are the main consumers of marine phytoplankton biomass, consuming on average 50–60% of phytoplankton production (Calbet and Landry 2004). Low protist grazing pressure could, therefore, shift the population dynamics from net loss to rates of rapid accumulation. Low grazing pressure has been suggested as a possible mechanism of HAB formation (Strom et al. 2001; Tillmann 2004; Irigoien et al. 2005). Experimental evidence on the grazing response of heterotrophic protists to *H. akashiwo* is varied. Laboratory research has shown that the heterotrophic dinoflagellates *Oxyrrhis marina*, *Noctiluca scintillans*, and *Stoeckeria algicida* feed readily on *H. akashiwo* (Jeong et al. 2003; Clough and Strom 2005). However, other protists, such as large ciliates, die when they ingest *H. akashiwo* cells (Verity and Stoecker 1982; Clough and Strom 2005; Graham and Strom 2010). Thus, while some predators readily consume *H. akashiwo* others die or avoid feeding on this HAB species. In the field, *H. akashiwo* blooms have been associated with low heterotrophic protist grazing rates (Verity and Stoecker 1982; Kamiyama et al. 2000; Menden-Deuer et al. 2010). Moreover, heterotrophic protist grazer abundance was reduced during a *H. akashiwo* bloom (Kamiyama 1995). Therefore, both laboratory and field studies suggest that low grazing pressure may be an important contributor to *H. akashiwo* HAB formation.

The measurable, grazer-induced mortality rate is a community-level average of many individual-level predator–prey interactions; different types of interactions can result in similar population abundances. For example, low grazing pressure may be due to low predator abundance caused by mortality, avoidance behaviors, reduced ingestion, or a combination thereof. Moreover, not all predator–prey interactions result in consumption. Nonconsumptive effects on predator–prey population dynamics, including prey selectivity or prey avoidance, can have consequences that may change predator or prey behavior or growth (Lima 1998; Schmitz et al. 2004). Each type of interaction has different consequences for HAB population dynamics. Predator mortality or avoidance of the area reduces overall grazing pressure on all prey species. On the other hand, prey selectivity reduces predation pressure only on the avoided species and possibly reduces resource competition

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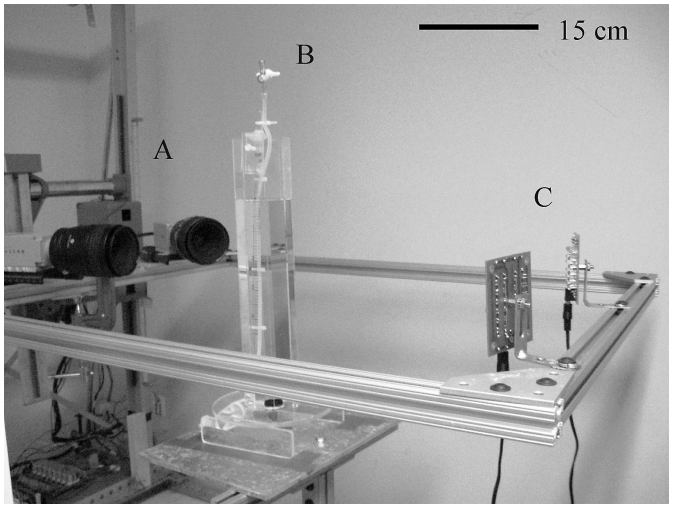

Fig. 1. Photo of the experimental set-up. (A) Stereo cameras monitor (B) the 30-cm-high tank that is illuminated by (C) two LED light banks. The entire camera and light bank platform moves vertically via computer control.

among algae, favoring the avoided species. Deciphering the nature of these cell–cell interactions provides the opportunity to understand the mechanisms driving average grazing rates and is necessary for predictions of HAB occurrence.

Here we investigated behavioral interactions between *H. akashiwo* and the ciliate predator, *Favella ehrenbergii* (Jörgensen). Specifically, we mimicked the patchy nature of *H. akashiwo* blooms, where bloom patches are bordered by low HAB abundances that could theoretically provide a spatial refuge for the predator. In the laboratory, we created a halocline structure that induced layer formation of *H. akashiwo* and measured *F. ehrenbergii's* population distribution and swimming behavior using high-resolution video analysis. We found no evidence that *F. ehrenbergii* avoided dense *H. akashiwo* layers, despite increased mortality of the ciliate in the presence of *H. akashiwo*.

Methods

Culture of microorganisms—A strain of *Heterosigma akashiwo* (CCMP 2809), known to be toxic to some heterotrophic protists (Graham and Strom 2010) was used for all behavior experiments. *Heterocapsa triquetra* (unknown origin) and *Isochrysis galbana* (CCMP 1323) were used to rear ciliate cultures. *Heterocapsa triquetra* was also used as a beneficial control prey in behavior experiments. We will refer to *H. triquetra* as beneficial prey and *H. akashiwo* as toxic prey; however, this does not imply that these are the only prey species that could promote the growth or death of the predator. All phytoplankton cultures were grown in 0.2-μm sterile-filtered autoclaved seawater (FSW), enriched with f/2 nutrients (Guillard 1975). The ciliate predator, *Favella ehrenbergii* (SPMC 133), was cultured in FSW only. All cultures were maintained on a 12 : 12 h light : dark cycle, at 15°C, salinity of 30, and a light intensity of 70–80 μmol photon m^{-2} s^{-1} for the phytoplankton cultures and 8–15 μmol photon m^{-2} s^{-1} for *F. ehrenbergii*. The cultures were not axenic. Phytoplankton cultures were transferred every 4–7 d to maintain exponential growth. Ciliate cultures were fed *H. triquetra* (final concentration of 200 cells mL^{-1}) and *I. galbana* (final concentration of 1500 cells mL^{-1}) twice a week. Throughout all experiments, cell concentrations of both predator and prey cultures were determined by microscope counts using samples fixed in 1% acid Lugol's solution.

Experimental set-up—To quantify population distributions and movement behaviors, a 30-cm-tall, 5.5-cm-wide, 800-mL, octagonal, acrylic observational chamber was used (Fig. 1). The chamber was filled with FSW using a peristaltic pump; this method allowed for the creation of defined salinity structures and eliminated fluid convection in the chamber (Bearon et al. 2006; Menden-Deuer and Grünbaum 2006). A halocline was created in the middle of the chamber between two weakly stratified linear salinity gradients, one from 8 to 10 (above) and another from 27 to 30 (below). This halocline was used to induce layer formation of *H. akashiwo* (Bearon et al. 2006). The same source water was used in all experiments and cultures.

Three treatments were used to quantify predator–prey interactions: (1) *F. ehrenbergii* alone, (2) *F. ehrenbergii* and *H. akashiwo* together, and (3) *F. ehrenbergii* and *H. triquetra* together. All treatments were run in triplicate. Using a syringe, organisms were introduced at the bottom of the tank through silicone tubing with an internal diameter of 1 mm. Cells were introduced slowly at a rate of 10 mL min^{-1} to reduce stress to cells as well as disturbance to the water column. Phytoplankton cells were introduced into the tank first, and allowed to acclimate for 10 min. *Heterosigma akashiwo* was added to the tank for an average final concentration of 180 cells mL^{-1}. In order to observe a final concentration of 50 *F. ehrenbergii* mL^{-1}, 2 liters of ciliate culture were gently condensed to 30 mL using a submerged, 20-μm Nitex mesh 15 min before introduction to the bottom of the experimental chamber. Cells were then added to the tank using the same silicone tubing as used for phytoplankton. An equivalent volume of water was added to the control tanks, so that volumetrically the treatments remained the same. To minimize prey-cell carryover and to maximize feeding motivation, *F. ehrenbergii* cultures were starved for 3 d prior to use in the experiment. The residual prey in the concentrated samples were enumerated using microscope counts; the concentration of *H. triquetra* was below the detection limit, and the carryover of *I. galbana* was < 2 cells mL^{-1}, or 5000 pg C of total *I. galbana* biomass in the entire tank, equivalent to the biomass of 1.7 *F. ehrenbergii* cells (Verity and Langdon 1984).

An equal abundance of the 12-μm *H. akashiwo* and 30-μm *H. triquetra* were offered in terms of carbon content, not cell concentration. Carbon content was determined from cell size (Menden-Deuer and Lessard 2000), the calculated carbon content for *H. akashiwo* was 134 pg C $cell^{-1}$ compared to 796 pg C $cell^{-1}$ for *H. triquetra*. Offering equivalent carbon abundances ensured that the encounter rates of *F. ehrenbergii* on *H. akashiwo* (2.2 cells

h^{-1} predator^{-1}) and *H. triquetra* (2.4 cells h^{-1} predator^{-1}) remained theoretically constant (Kiørboe 2008). Final concentration in the tank was ~ 31 *H. triquetra* cells mL^{-1}, lower than the 200 *H. triquetra* mL^{-1} that *F. ehrenbergii* were fed in maintenance cultures. Therefore, in *F. ehrenbergii* experiments with beneficial prey, prey concentrations were an order of magnitude lower than the concentrations used to sustain growth of the predator.

Video capture and analysis—Two infrared-sensitive cameras (Pixelink) with Nikon 60-mm Micro Nikkor lenses monitored a two-dimensional (2D) field of view of 1.5 cm × 1.3 cm × 3.3 cm each. The cameras were mounted at a 45° angle with maximally overlapping fields of view to enable reconstruction of three-dimensional (3D) movement behaviors. All filming was conducted with no visible light illumination, to eliminate the potential for light-mediated behavioral responses. In order to view the organisms, the chamber was illuminated with dark field infrared (960 nm) light-emitting diodes (LEDs). Filming occurred at nine, initially random horizons, vertically distributed evenly ~ 2–3 cm apart. This constituted ~ 36% of the viewable volume. To calculate the 3D volume captured, we used the Cartesian positions of a subset of cells and calculated the area of a convex hull to be 3.2 cm^3. Thus, the viewing volume was 3.2 mL per horizon. Each horizon was filmed for 1 min every hour for an 11-h period, resulting in a total of 108 1-min videos per treatment. Video was captured at 30 frames s^{-1}.

To determine the population distribution and swimming behaviors of both the ciliate and phytoplankton species, all videos were analyzed using the same protocol. The 2D position of each individual organism in each frame of the stereo cameras was determined, using automated ImageJ image-processing software to remove stationary background objects. The threshold was determined manually, so that background subtraction could be automated. The same threshold values were used for all videos. In mixed-species videos, those organisms that had an area of 20 ± 5 pixels were classified as *F. ehrenbergii*, and those with an area of 5 ± 3 pixels were classified as *H. akashiwo* or *H. triquetra* depending on the experiment. This size classification was established using the control videos. Abundances were determined by averaging the number of individuals per frame over the 1-min video (1800 frames). Due to the volume of data generated with each of these experiments, only the distributions of organisms at time (t) = 1 h, 5 h, and 11 h elapsed are shown graphically, representing the beginning, middle, and end of the observation period. The results reported here accurately represent the results observed between time points.

Three-dimensional swimming paths were determined by first assembling 2D trajectories from Cartesian coordinates of each organism in each stereo frame and then joining 2D tracks based on matching space–time occurrence in the two 2D segments. Trajectories from all treatments were determined using the exact same video analysis and trajectory assembly parameters; more details are reported in (Menden-Deuer and Grünbaum 2006). Swimming behaviors, including the x, y, and z velocity vectors and turning rates were calculated from 3D paths, subsampled at 0.25-s intervals. Turning rate is a measure of the magnitude of the directional change an organism undergoes over time. The vertical velocity component of the speed indicates the rate of vertical displacement of the cell, with negative values indicating downward movement and positive values upward movement. The upper and lower 0.5% of each frequency distribution were discarded before analysis to eliminate extreme outliers. Trajectories from each horizon and replicate were pooled by time point. For *F. ehrenbergii* videos, only time points that had ≥ 10 individuals in each replicate and cells tracked for a minimum duration of 5 s or longer were used to measure movement behaviors. Reduced abundance over time limited analysis of *F. ehrenbergii* swimming behavior in the latter half of the experiment, since greater data density was required to quantify *F. ehrenbergii* movement behaviors compared to population distributions. Thus, population distributions are reported for the entirety of the observation period, but movement behaviors could only be analyzed from the first 6 h.

Statistical analysis—The Kolmogorov–Smirnov test was used to determine significant differences among the abundance data, as well as among distributions of swimming behaviors. The abundance data are displayed graphically as percent population distribution to allow visual comparisons between time points and treatments. All statistical analyses were done on the absolute abundance data, not the percentages. Mortality rates of *F. ehrenbergii* were determined from the difference between initial and final cell abundance per time elapsed. A one-way ANOVA was used to test for significant differences in predator mortality rates among treatments. For all analyses the significance level was $p < 0.05$.

Results

Prey distributions—Both prey species, in the presence of *F. ehrenbergii*, rapidly formed an aggregation at the halocline horizon (Fig. 2). Approximately 36% of the *H. akashiwo* cells and 42% of the *H. triquetra* population were found at the halocline after 1 h. Prey cells were found at all horizons observed. While the abundance of cells of both prey species significantly increased below the halocline over time ($p < 0.001$), the largest proportion of the population was always found aggregated at the halocline horizon.

Favella ehrenbergii *distributions*—After 1 h, in the FSW control, 47% of the *Favella ehrenbergii* population were aggregated at the halocline (Fig. 3). There were no significant changes in *F. ehrenbergii* distribution over time (min. $p = 0.10$), with ≥ 40% of *F. ehrenbergii* consistently found at the halocline. All remaining cells were found below the halocline. No *F. ehrenbergii* cells were ever observed above the halocline, at salinities < 15.

In predator–prey exposure experiments, *F. ehrenbergii* did not avoid layers of *H. akashiwo.* The population distribution of *F. ehrenbergii* did not significantly change when exposed to a layer of *H. akashiwo* ($p = 0.12$) or beneficial prey ($p = 0.82$), in comparison to a FSW control.

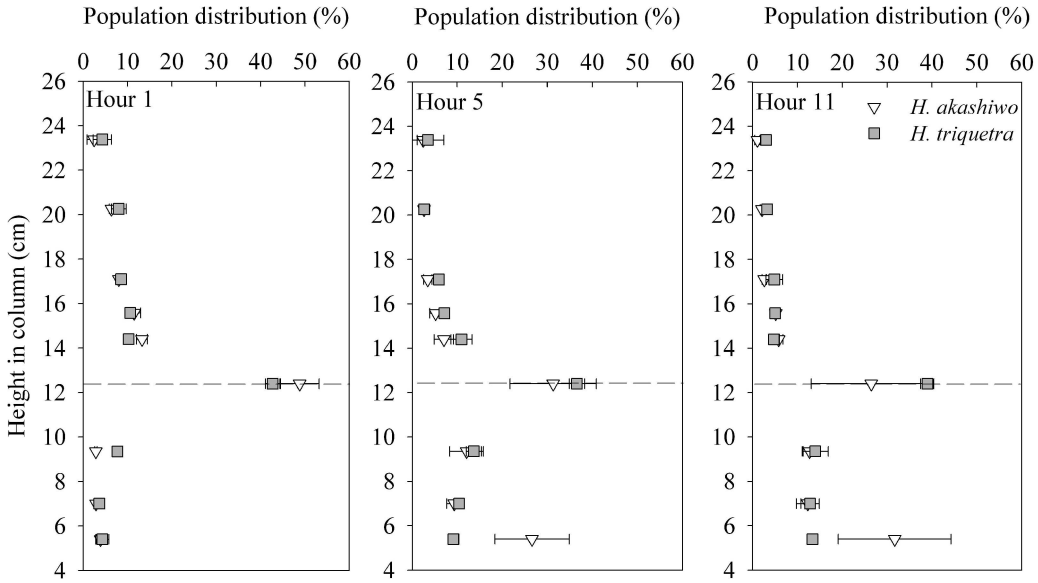

Fig. 2. Distributions of *H. akashiwo* (triangle) and *H. triquetra* (square) after 1 h, 5 h, and 11 h in the presence of *F. ehrenbergii*. For both prey species, the majority of the population remained at the halocline at all time points. Dashed line indicates the halocline. Error bars represent one standard error of the mean of triplicate incubations.

When *H. akashiwo* were present in the tank, ~ 55% of the *F. ehrenbergii* population was aggregated at the halocline. The population distribution of *F. ehrenbergii* in the presence of *H. akashiwo* did not change over time (min. $p = 0.95$; Fig. 3). Nearly identical to the other two treatments, 52% of *F. ehrenbergii* aggregated to the halocline in the presence of the beneficial prey *H. triquetra* and did not change over time (min. $p = 0.97$).

While there were no significant differences observed in the distributions of *F. ehrenbergii* in the three treatments, we observed significant differences in the abundance of *F. ehrenbergii* among treatments over time ($p = 0.009$; Fig. 4). *Favella ehrenbergii* showed significantly higher mortality rates when *H. akashiwo* were present in the tank (24.4 ± 2.8 *F. ehrenbergii* h^{-1}) vs. control (15.4 ± 2.5 *F. ehrenbergii* h^{-1}) or *H. triquetra* (6.2 ± 1.9 *F. ehrenbergii* h^{-1}) treatments, signifying enhanced mortality of *F. ehrenbergii* in response to *H. akashiwo*.

Favella ehrenbergii *swimming behaviors*—Swimming speeds of *F. ehrenbergii* were significantly different among all treatments (all $p < 0.001$). The magnitude of variability

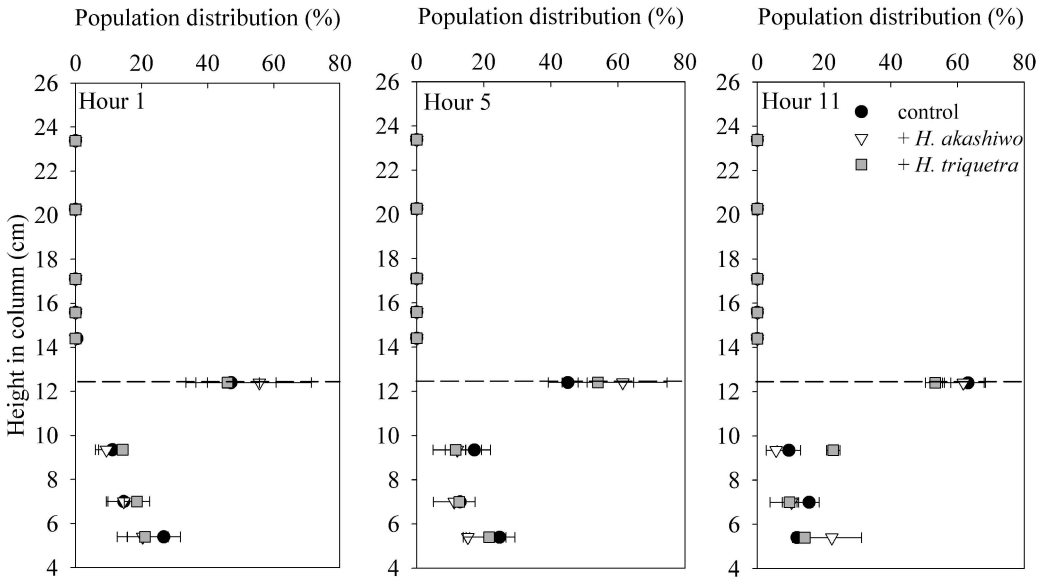

Fig. 3. Population distributions of *F. ehrenbergii* after 1 h, 5 h, 11 h with *H. akashiwo* (white triangles), in the FSW control (black circles), and with beneficial prey (gray squares). None of these distributions were significantly different from one another, either among treatments or across time. Dashed line indicates the position of the halocline. Error bars represent one standard error of the mean of triplicate incubations.

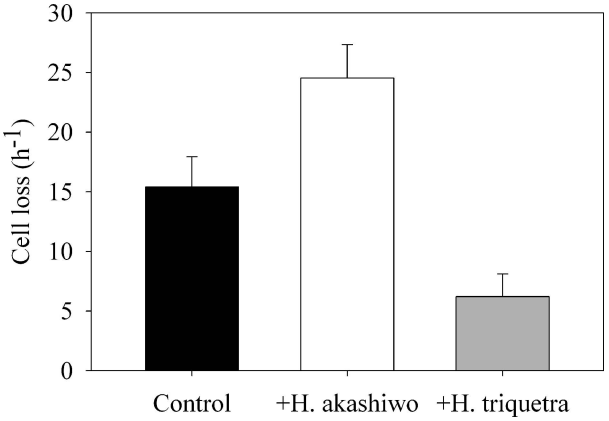

Fig. 4. Mortality rate (cell loss h^{-1}) of *F. ehrenbergii* in the total water column in the three different prey conditions, no prey (black), toxic prey (*H. akashiwo*; white), and beneficial prey (*H. triquetra*; gray). There was a significantly higher mortality rate of *F. ehrenbergii* in the presence of *H. akashiwo* than in either the control or beneficial prey treatments. Error bars are one standard error of the mean of triplicate incubations.

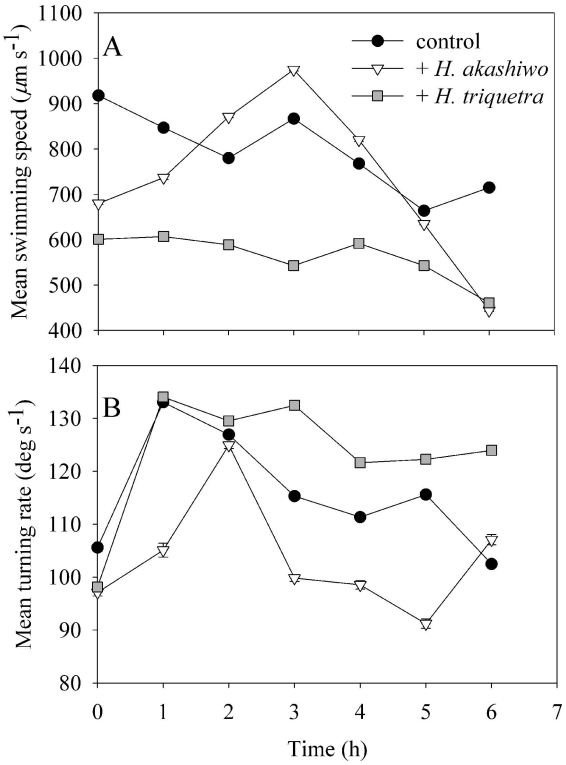

Fig. 5. (A) Mean swimming speed (μm s^{-1}) and (B) turning rate (degrees s^{-1}) over time for *F. ehrenbergii* cells in the control (black circle), with *H. akashiwo* (white triangle), and with beneficial prey (gray square). Turning rate of *F. ehrenbergii* was significantly slower in response to *H. akashiwo* than either of the controls. Swimming speed varied over time only in response to *H. akashiwo*, but decreased steadily in response to the two controls. Error bars represent one standard error of the mean and are largely contained within the symbols. Movement data are not shown after 6 h, because too few long swimming trajectories were observed due to *F. ehrenbergii* mortality.

was large, with the coefficient of variation (CV) ranging from 10% to 100%. *Favella ehrenbergii* swam slowest in response to the beneficial prey and initially the fastest in the FSW control (Fig. 5A). After 1 h of observation, the mean swimming speed of *F. ehrenbergii* cells when with *H. akashiwo* was 737 ± 460 μm s^{-1}, 17% faster than *F. ehrenbergii* with *H. triquetra* (607 ± 511 μm s^{-1}) but 12% slower than in the FSW control (847 ± 534 μm s^{-1}; Fig. 5A). For all treatments, swimming speeds of *F. ehrenbergii* decreased over time. On average, the mean swimming speed of *F. ehrenbergii* in the control was 26% slower than the swimming speed in the presence of *H. triquetra*, over the entire observation period. From 2 h to 6 h, the mean swimming speed in the FSW control and in presence of *H. triquetra,* while different from each other, remained relatively consistent from hour to hour. However, the mean swimming speed of *F. ehrenbergii* in the presence of *H. akashiwo* changed over time. Mean swimming speeds increased until peaking at hour three of observation (975 ± 496 μm s^{-1}). Mean speed then slowed, until it reached a low at hour six (444 ± 331 μm s^{-1}), which was 38% slower than the mean speed in the control and 4% slower than when *H. triquetra* were present.

Overall, *F. ehrenbergii* consistently had the highest turning rates in response to *H. triquetra* and the slowest turning rates in response to *H. akashiwo*, with turning rates in the FSW control intermediate between those measured in response to the two prey types. Despite the high variation of turning rates in all treatments (average CV > 100%), distributions of turning rates were significantly different among the treatments (all $p < 0.001$). After 1 h of observation, the mean turning rate of *F. ehrenbergii* when *H. akashiwo* were present was 105.1 ± 135.7 degrees s^{-1} (Fig. 5B). This was ~ 20% slower than the mean rate measured in either the control (133.0 ± 160.3 degrees s^{-1}) or when *H. triquetra* were present (134.0 ± 162.3 degrees s^{-1}, Fig. 5B). From 2 h to 6 h, *F. ehrenbergii* turned on average 7% faster in the presence of *H. triquetra* than in the filtered seawater control. In contrast, turning rates of *F. ehrenbergii* in the presence of *H. akashiwo* continued to be slower than in the presence of either the control (12%) or in the presence of *H. triquetra* (18%).

Discussion

The ability of *H. akashiwo* and other HAB species to frequently form dense, mono-specific blooms is puzzling and begs the question, what factors enhance the species' survival rate? A decrease in heterotrophic protist grazing pressure may contribute to *H. akashiwo*'s success. Observing the details of predator–prey interactions provides a mechanistic understanding of the resultant population abundances. To mimic the patchy conditions found in the coastal ocean, where *H. akashiwo* forms dense surface slicks, the experiments reported here were designed to include spatial structure that would afford the predator refuge from exposure to the toxic alga. *Heterosigma akashiwo* abundances varied by orders of magnitude across the tank, providing areas where *F. ehrenbergii* could have avoided interaction with the majority of the *H. akashiwo*

population. Our results show that *F. ehrenbergii* did not exploit a spatial refuge that could have reduced its rapid mortality in the presence of the HAB alga.

Montagnes et al. (2008) depicted six steps in protistan prey capture: searching, contact, capture, processing, ingestion, and digestion. Despite almost certain mortality as a result of *H. akashiwo* exposure, our results suggest the toxicity of *H. akashiwo* does not induce *F. ehrenbergii* to reduce contact with the toxic alga by avoidance. Over the course of the experiment, the vertical distribution of *F. ehrenbergii* did not change significantly, regardless of the presence of *H. akashiwo*, beneficial algal prey, or in the absence of potential prey all together. The inability of *F. ehrenbergii* to avoid layers of *H. akashiwo* suggests that *F. ehrenbergii* does not detect the toxicity of *H. akashiwo* prior to capture. It is known that *Favella* sp. will reject captured *H. akashiwo* cells (Taniguchi and Takeda 1988; Stoecker et al. 1995), and will discriminate against *H. akashiwo* in prey mixtures (Graham and Strom 2010). However, when *H. akashiwo* is ingested by *Favella* sp., mortality of the ciliate rapidly follows (Verity and Stoecker 1982; Clough and Strom 2005; Graham and Strom 2010).

The inability of *F. ehrenbergii* to avoid the toxic *H. akashiwo* has important consequences for our understanding and ability to predict HABs. Regardless of the lethality of *H. akashiwo* to *F. ehrenbergii*, our results show that the herbivore remains in the area occupied by *H. akashiwo*. If feeding were to continue, it is likely that feeding selectivity could remove other algae. Such selectivity would have a two-fold benefit to *H. akashiwo*: first, selective avoidance of *H. akashiwo* would promote further accumulation of *H. akashiwo* cells; second, because H. *akashiwo* has a relatively high nutrient requirement (Smayda 1998), the removal of co-occurring phytoplankton could decrease nutrient competition, further increasing *H. akashiwo* growth rates. Furthermore, we observed that the *F. ehrenbergii* population died rapidly (14.5% h^{-1} in the presence of *H. akashiwo*), which would result in a rapid reduction in grazing pressure over time. If *H. akashiwo* has a similar effect on other predator species, the HAB alga's net survival rate should increase relative to competing algae that do not negatively impact their respective predators. Thus, the toxicity of *H. akashiwo* provides this alga an effective defense against predation even when predators are not deterred from the immediate area. The inability of *F. ehrenbergii* to avoid *H. akashiwo* could, therefore, benefit HAB formation and persistence, through multiple mechanisms including mortality and feeding selectivity of the predator.

There is evidence that *Favella* sp. will consume low concentrations (< 200 cells mL^{-1}) of other toxic algae such as *Heterocapsa circularisquama* or *Alexandrium tamarense*, without significant changes in growth and survival rate from those observed with nontoxic species (Kamiyama 1997; Kamiyama et al. 2005). However, increases in abundance of *H. circularisquama* increased the mortality of *Favella* sp. (Kamiyama 1997). This positive correlation in toxic prey abundance and resultant predator mortality suggests that grazing can adversely affect HAB species biomass accumulation when algal abundances are low. Thus, as long as HAB abundances are below the predator mortality threshold, grazing could prevent HAB formation. The consequences of severe toxicity reported here may only apply to instances where algal abundances are high (> 1000 cells mL^{-1}). Investigating the transition from HAB concentrations that can be tolerated and grazed upon to concentrations that adversely affect protistan predators could provide insight into the mechanisms of *H. akashiwo* toxicity to protistan predators.

Favella ehrenbergii exhibited prey-specific changes in swimming behavior. In response to *H. triquetra*, the ciliate showed more aggregative swimming behaviors (e.g., slower speeds and faster turning rates) compared to the FSW control. Increasing aggregative behaviors is an effective mechanism for remaining in a resource patch (Davis et al. 1991; Visser and Thygesen 2003). Our results agree well with previous work where aggregative swimming was observed in *F. ehrenbergii* in the presence of beneficial prey (Buskey and Stoecker 1988). Despite these modifications in movements, an enhanced aggregation of *F. ehrenbergii* to the beneficial prey layer was not observed, which may have been due to the low abundance of *H. triquetra,* insufficient to sustain *F. ehrenbergii* growth.

The behavioral response to *H. akashiwo* was more complex, undergoing shifts, including dispersive and retentive swimming behaviors. Because *F. ehrenbergii* experienced significant mortality in the presence of *H. akashiwo*, we cannot determine if these changes were a behavioral response or a physiological consequence of toxicity. Previous work observed erratic swimming of *Favella* sp. in the presence of the toxic alga *Alexandrium tamarense* (Hansen 1989). The observed changes in *F. ehrenbergii* swimming behavior in response to the HAB species, whether voluntary or induced by toxicity, would ultimately decrease the encounter rate of predator and prey. Given the high mortality of *F. ehrenbergii*, it is questionable, however, whether these behavioral modifications were effective. These results support the idea that exposure to toxic phytoplankton species may lead to changes in heterotrophic protist movement behaviors, ultimately altering encounter rates.

The structuring function of salinity was a key aspect to our experiment, where *F. ehrenbergii* distributions were restricted to higher salinity waters below the halocline. In previous experiments, *F. ehrenbergii* was able to cross a halocline with a four-part difference in salinity (Jonsson 1989). Yet, our results showed that with a stronger halocline, *F. ehrenbergii* was excluded from lower salinity regions of the tank. This indicates either intolerance to low-salinity water or an inability to cross the salinity density gradient. Given this restricted distribution, *F. ehrenbergii* could not access a large portion of the tank that was significantly lower in abundance of the toxic prey species. This physiological restriction on the *F. ehrenbergii* distribution in the tank, coupled with the broad halo-tolerance of the HAB alga, enabled *H. akashiwo* to occupy depths void of the predator. Thus, strong haloclines could prevent *F. ehrenbergii* from co-locating in areas where toxic phytoplankton may exist. This physical barrier would further benefit survival of the toxic alga, and impact the success of HAB formation and persistence.

Exposure to light is also an important factor in influencing plankton behavior and vertical distribution. These experiments were deliberately conducted in the dark to eliminate light-mediated behaviors. Layers of *H. akashiwo* still form in the light, under similar salinity conditions (Bearon et al. 2006). Our own preliminary experiments showed no significant difference in *H. akashiwo* distribution in the presence or absence of light (data not shown). Therefore, *H. akashiwo* distributions would be unchanged in the light. If the ciliate were positively phototactic, increased aggregation of the ciliate at the halocline would result, further increasing its exposure to *H. akashiwo* and possibly ingestion rates (Strom 2001; Jakobsen and Strom 2004). Our results indicate that co-location of these two species led to the mortality of *F. ehrenbergii*. Thus, all currently available data suggest that our results would be qualitatively the same, if the presented experiments were done in visible light, and the conclusion that *F. ehrenbergii* was unable to avoid *H. akashiwo* would remain.

Our results highlight the importance of deciphering the underlying predator–prey interactions that mechanistically identify why certain population dynamics arise. Decreases in heterotrophic protist grazing rates on phytoplankton may facilitate the formation or enhance the duration of blooms. There are both lethal (toxicity) and nonlethal (avoidance) mechanisms that can lead to such decreases in grazing pressure. We hypothesized that *F. ehrenbergii* would avoid dense layers of *H. akashiwo* to avoid mortality induced by this toxic prey species. Instead, we found that *F. ehrenbergii* did not exploit a spatial refuge nor show avoidance behaviors toward the toxic alga, and died rapidly in the presence of *H. akashiwo*. The inability of *F. ehrenbergii* to utilize an avoidance behavior significantly enhanced its mortality rate. If these laboratory observations apply to conditions in the ocean and other predators and their potential HAB prey, predator–prey interactions may constitute an important factor in deciphering the success of harmful algal blooms.

Acknowledgments

We thank S. Strom for thoughtful advice with this research and provision of plankton cultures and W. Day for help with the automated filming protocol. Comments from two anonymous reviewers improved this manuscript.

Funding was provided by a Disease in Marine Organisms Fellowship through the US Department of Agriculture (2008-38420-18737 to M. Gomez-Chiarri), an Ecology of Harmful Algal Blooms grant (NA06NOS4780248 to S. Strom and S. Menden-Deuer), and the National Science Foundation (Biological-Oceanography Award 0826205 to S. Menden-Deuer).

References

Anderson, D. M., P. M. Gilbert, and J. M. Burkholder. 2002. Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. Estuar. Coasts **25:** 704–726, doi:10.1007/BF02804901

Bearon, R. N., D. Grünbaum, and R. A. Cattolico. 2006. Effects of salinity structure on swimming behavior and harmful algal bloom formation in *Heterosigma akashiwo*, a toxic raphidophyte. Mar. Ecol. Prog. Ser. **306:** 153–163, doi:10.3354/meps306153

Buskey, E. J., and D. K. Stoecker. 1988. Locomotory patterns of the planktonic ciliate *Favella* sp.: Adaptations for remaining in food patches. Bull. Mar. Sci. **43:** 783–796.

Calbet, A., and M. R. Landry. 2004. Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. Limnol. Oceanogr. **49:** 51–57, doi:10.4319/lo.2004.49.1.0051

Clough, J., and S. Strom. 2005. Effects of *Heterosigma akashiwo* (Raphidophyceae) on protist grazers: Laboratory experiments with ciliates and heterotrophic dinoflagellates. Aquat. Microb. Ecol. **39:** 121–134, doi:10.3354/ame039121

Davis, C., G. Flierl, P. Wiebe, and P. Franks. 1991. Micropatchiness, turbulence and recruitment in plankton. J. Mar. Res. **49:** 109–151, doi:10.1357/002224091784968602

Graham, S. L., and S. L. Strom. 2010. Growth and grazing of microzooplankton in response to the harmful alga *Heterosigma akashiwo* in prey mixtures. Aquat. Microb. Ecol. **59:** 111–124, doi:10.3354/ame01391

Granéli, E., M. Weberg, and P. S. Salomon. 2008. Harmful algal blooms of allelopathic microalgal species: The role of eutrophication. Harmful Algae **8:** 94–102, doi:10.1016/j.hal.2008.08.011

Guillard, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates, p. 29–60. *In* W. L. Smith and M. H. Chaney [eds.], Culture of marine invertebrate animals. Plenum Press.

Hansen, P. J. 1989. The red tide dinoflagellate *Alexandrium tamarense*: Effects on behaviour and growth of a tintinnid ciliate. Mar. Ecol. Prog. Ser. **53:** 105–116, doi:10.3354/meps053105

Honjo, T. 1993. Overview on bloom dynamics and physiological ecology of *Heterosigma akashiwo*, p. 33–41. *In* T. J. Smayda and Y. Shimizu [eds.], Toxic phytoplankton blooms in the sea. Elsevier.

Irigoien, X., K. J. Flynn, and R. P. Harris. 2005. Phytoplankton blooms: A 'loophole' in microzooplankton grazing impact? J. Plankton Res. **27:** 313–321, doi:10.1093/plankt/fbi011

Jakobsen, H., and S. Strom. 2004. Circadian cycles in growth and feeding rates of heterotrophic protist plankton. Limnol. Oceanogr. **49:** 1915–1922, doi:10.4319/lo.2004.49.6.1915

Jeong, H. J., and others. 2003. Feeding by the heterotrophic dinoflagellate *Oxyrrhis marina* on the red-tide raphidophyte *Heterosigma akashiwo*: A potential biological method to control red tides using mass-cultured grazers. J. Eukaryot. Microbiol. **50:** 274–282, doi:10.1111/j.1550-7408.2003.tb00134.x

Jonsson, P. R. 1989. Vertical distribution of planktonic ciliates—an experimental analysis of swimming behaviour. Mar. Ecol. Prog. Ser. **52:** 39–53, doi:10.3354/meps052039

Kamiyama, T. 1995. Change in the microzooplankton community during decay of a *Heterosigma akashiwo* bloom. J. Oceanogr. **51:** 279–287, doi:10.1007/BF02285166

———. 1997. Growth and grazing responses of tintinnid ciliates feeding on the toxic dinoflagellate *Heterocapsa circularisquama*. Mar. Biol. **128:** 509–515, doi:10.1007/s002270050117

———, S. Itakura, and K. Nagasaki. 2000. Changes in microbial loop components: Effects of a harmful algal bloom formation and decay. Aquat. Microb. Ecol. **21:** 21–30, doi:10.3354/ame021021

———, M. Tsujino, Y. Matsuyama, and T. Uchida. 2005. Growth and grazing rates of the tintinnid ciliate *Favella taraikaensis* on the toxic dinoflagellate *Alexandrium tamarense*. Mar. Biol. **147:** 989–997, doi:10.1007/s00227-005-1629-2

Keppler, C. J., J. Hoguet, K. Smith, A. H. Ringwood, and A. J. Lewitus. 2005. Sublethal effects of the toxic alga *Heterosigma akashiwo* on the southern oyster (*Crassostrea virginica*). Harmful Algae **4:** 275–285, doi:10.1016/j.hal.2004.05.002

Khan, S., O. Arakawa, and Y. Onoue. 1997. Neurotoxins in a toxic red tide of *Heterosigma akashiwo* (Raphidophyceae) in Kagoshima Bay, Japan. Aquat. Res. **28:** 9–14, doi:10.1046/j.1365-2109.1997.t01-1-00823.x

Kiørboe, T. 2008. A mechanistic approach to plankton ecology, 1st ed. Princeton.

Lima, S. L. 1998. Nonlethal effects in the ecology of predator–prey interactions. BioScience **48:** 25–34, doi:10.2307/1313225

McGillicuddy, D. J., R. P. Signell, C. A. Stock, B. A. Keafer, M. Keller, R. D. Hetland, and M. Anderson. 2003. A mechanism for offshore initiation of harmful algal blooms in the coastal Gulf of Maine. J. Plankton Res. **25:** 1131–1138, doi:10.1093/plankt/25.9.1131

Menden-Deuer, S., K. A. Fredrickson, and S. L. Strom. 2010. Formation and decline of a *Heterosigma akashiwo* layer in East Sound, Washington, USA, p. 98–103. *In* K. C. Ho, M. Y. Zhou, and Y. Z. Qi [eds.], Harmful algae 2008. International Society for the Study of Harmful Algae and Environmental, Hong Kong.

———, and D. Grünbaum. 2006. Individual foraging behavior and population distributions of a planktonic predator aggregating to phytoplankton thin layers. Limnol. Oceanogr. **51:** 109–116, doi:10.4319/lo.2006.51.1.0109

———, and E. J. Lessard. 2000. Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnol. Oceanogr. **45:** 569–579, doi:10.4319/lo.2000.45.3.0569

Montagnes, D. J. S., and others. 2008. Selective feeding behaviour of key free-living protists: Avenues for continued study. Aquat. Microb. Ecol. **53:** 83–98, doi:10.3354/ame01229

Nakamura, A., T. Okamoto, N. Komatsu, S. Ooka, T. Oda, A. Ishimatsu, and T. Muramatsu. 1998. Fish mucus stimulates the generation of superoxide anion by *Chattonella marina* and *Heterosigma akashiwo*. Fish. Sci. **64:** 866–869.

Schmitz, O. J., O. Ovadia, and V. Krivan. 2004. Trophic cascades: The primacy of trait-mediated interactions. Ecol. Lett. **7:** 153–163, doi:10.1111/j.1461-0248.2003.00560.x

Smayda, T. J. 1998. Ecophysiology and bloom dynamics of *Heterosigma akashiwo* (Raphidophyceae), p. 113–131. *In* D. M. Anderson, A. D. Cembella, and G. M. Hallegraeff [eds.], Physiological ecology of harmful algal blooms. NATO ASI Series. Springer-Verlag.

———. 2002. Turbulence, watermass stratification and harmful algal blooms: An alternative view and frontal zones as "pelagic seed beds". Harmful Algae **1:** 95–112, doi:10.1016/S1568-9883(02)00010-0

Stoecker, D. K., S. M. Gallager, C. J. Langdon, and L. H. Davis. 1995. Particle capture by *Favella* sp. (Ciliata, Tintinnina). J. Plankton Res. **17:** 1105–1124, doi:10.1093/plankt/17.5.1105

Strom, S. L. 2001. Light-aided digestion, grazing and growth in herbivorous protists. Aquat. Micro. Ecol. **23:** 253–261, doi:10.3354/ame023253

———, M. A. Brainard, J. L. Holmes, and M. B. Olson. 2001. Phytoplankton blooms are strongly impacted by microzooplankton grazing in coastal Pacific Northwest waters. Mar. Biol. **138:** 355–368, doi:10.1007/s002270000461

Taniguchi, A., and Y. Takeda. 1988. Feeding rate and behavior of the tintinnid ciliate *Favella taraikaensis*, observed with a high speed VTR system. Mar. Microb. Food Web **3:** 21–34.

Tillmann, U. 2004. Interactions between planktonic microalgae and protozoan grazers. J. Eukaryot. Microbiol. **51:** 156–168, doi:10.1111/j.1550-7408.2004.tb00540.x

Twiner, M. J. J., S. J. Dixon, and C. G. Trick. 2001. Toxic effects of *Heterosigma akashiwo* do not appear to be mediated by hydrogen peroxide. Limnol. Oceanogr. **46:** 1400–1405, doi:10.4319/lo.2001.46.6.1400

———, ———, and ———. 2004. Extracellular organics from specific cultures of *Heterosigma akashiwo* (Raphidophyceae) irreversibly alter respiratory activity in mammalian cells. Harmful Algae **3:** 173–182, doi:10.1016/j.hal.2003.10.003

Verity, P. G., and C. Langdon. 1984. Relationships between lorica volume, carbon, nitrogen, and ATP content of tintinnids in Narragansett Bay. J. Plankton Res. **66:** 859–868, doi:10.1093/plankt/6.5.859

———, and D. Stoecker. 1982. Effects of *Olisthodiscus luteus* on the growth and abundance of tinitinnids. Mar. Biol. **72:** 79–87, doi:10.1007/BF00393951

Visser, A. W., and U. H. Thygesen. 2003. Random motility of plankton: Diffusive and aggregative contributions. J. Plankton Res. **25:** 1157–1168, doi:10.1093/plankt/25.9.1157

Associate editor: Luc De Meester

Received: 01 June 2010
Accepted: 10 October 2010
Amended: 15 November 2010