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INVESTIGATING THE INTRASPECIFIC EFFECT OF CELL CONCENTRATION IN MEDIATING OXYRRHIS MARINA SWIMMING BEHAVIORS

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INVESTIGATING THE INTRASPECIFIC EFFECT OF CELL CONCENTRATION IN MEDIATING *OXYRRHIS MARINA* SWIMMING BEHAVIORS

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ABSTRACT

Heterotrophic protists are known to respond to a multitude of abiotic and biotic stimuli which confers a strong selective advantage in marine environments that are frequently dilute and heterogeneously distributed. In this laboratory study, we investigated the role of intraspecific signals in mediating *Oxyrrhis marina* swimming behavior that could be utilized to enhance dispersive behaviors and reduce competition between intraspecific predators. Using video and image analysis, three-dimensional movement behaviors of *O. marina* (on scales of micrometers and seconds) were simultaneously quantified with population-scale vertical distributions (on scales of centimeters and hours) and used in dispersal and encounter rate estimates. Three different concentrations of *O. marina* were filmed in both the absence and presence of the prey alga species, *Isochrysis galbana*, in at least triplicate films every 30 minutes for three hours at five horizons in 1-L experimental tanks. We found that the cell-cell interactions in the absence of prey cells resulted in modified swim behaviors that increased model estimates of encounter rates by 9%; however, individual swim behaviors between treatments were not significantly different in the presence of prey cells. Also, the relative proportion of the population near the top of the tank significantly decreased by 22% and 16% in both the absence and presence of prey cells, respectively, from low to high *O. marina* concentrations. These results suggest that *O. marina* can respond to the intraspecific cell concentration in the absence of competing signals which can ultimately result in significant changes to distributions, growth and grazing rates.

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INTRODUCTION

Heterotrophic protists are single-celled microzooplankton that are ubiquitous in the global ocean and are highly diverse in terms of size, taxonomy and feeding behavior (Sherr & Sherr, 1994). They play a vital role as consumers of primary production and dominate trophic interactions at the base of marine food webs, accounting for 60-70% of daily phytoplankton consumption (Calbet & Landry, 2004). By collecting minute prey into larger consumable particles and serving as prey items for larger macrozooplankton, such as copepods, microzooplankton contribute to the availability of food to higher trophic level organisms which ultimately affect the rate of export production (Calbet & Saiz, 2005; Caron & Hutchins, 2013). Therefore, it is important to identify the factors controlling protistan growth and grazing which are key carbon cycle processes that influence primary production, atmospheric carbon exchange and carbon sequestration of dissolved organic carbon to the deep ocean and sediments (Seymour, et al., 2009; Davidson, et al., 2011). There have been many studies that have identified a number of biotic and abiotic factors such as prey cells (Menden-Deuer & Grünbaum, 2006; Martel, 2006), light (Jakobsen & Strom, 2004; Hartz, et al., 2011), and nutrients (Breckels, et al., 2010) that influence population level growth and grazing rates, but there are very few quantitative studies that have focused on the influential factors mediating microzooplankton swimming behaviors. We are just beginning to understand the pace at which microorganisms can respond to changes in environmental conditions and the

associated impacts to the food web structure (Kim, et al., 2011; Caron, et al., 2012).

On the microscale level, grazing doesn't result from passive physical encounters between predator and prey, as these microorganisms operate in low Reynolds number environments where viscous forces are dominant and molecular diffusion of particles is significant (Kiørboe, 2008). In addition to a diffusion dominated environment, there is substantial evidence for pervasive heterogeneity on all scales, including the microscale at which plankton operate, which has substantial implications for the rates of encounter between predator and prey (Haury, et al., 1978; Fenchel, 2002; Menden-Deuer, 2008; Durham & Stocker, 2012). Furthermore, most marine environments are extremely dilute and plankton will typically account for a very small percentage of suspended constituents, usually less than 10 ppm by volume (Wolfe, 2000). Therefore, a major challenge for heterotrophic protists, as well as for phytoplankton, is to efficiently locate resources at sufficient concentrations to survive (Caron, et al., 2012). Motile heterotrophic protists have adapted a wide range of behavioral responses which are utilized under different environmental circumstances to maximize foraging efficiency, such as in the absence or presence of prey patches (Montagnes, et al., 2008). One such strategy involves the interpretation of the sharp physicochemical gradients associated with prey patches to direct and modulate predatory swimming behaviors which can increase rates of encounter between predator and prey. The enhanced capacity for heterotrophic grazers to actively search out and exploit these plankton rich patches, can result in a

heterogeneous distribution of predator biomass on the order of minutes (Fenchel & Blackburn, 1999), subsequently challenging models that assume constant predatory consumption rates on small (minutes) temporal scales. Therefore, in order to gain a more complete and mechanistic understanding of the planktonic predator-prey interaction and improve modeling efforts, quantitative investigations into environmental signals, such as the role of intraspecific cell concentration, are needed to adequately conceptualize this major trophic pathway in the marine food web (Sherr & Sherr, 2007).

While the quantitative study of signaling is still in its infancy, it has been established that all organisms, whether dead or living, release chemicals into their surrounding environment which are potentially available to be interpreted by any organism with the correct machinery to receive and process such information (Vos, et al., 2006). There have been many laboratory studies that have observed the quantitative changes in both predator and prey swimming behaviors in response to infochemicals. In Menden-Deuer and Grünbaum 2006, *Oxyrrhis marina* responded to the exuded chemical cues from thin layers of *Isochrysis galbana* by modulating their pair of constantly beating flagella that decreased vertical velocities and increased turning rates in order to remain in position to exploit this prey-rich area. Behavioral responses to chemical cues have also observed in some motile prey species. When subjected to predator-derived cues, *Heterosigma akashiwo* increased fleeing behaviors, which resulted in reduced encounter rates and a net positive population growth, as opposed to a net negative population growth when fleeing was not an option (Harvey &

Menden-Deuer, 2012). The results of these studies suggest that both prey and predator-derived cues are significant in mediating swimming behavior in autotrophs and heterotrophs.

Oxyrrhis marina was an ideal candidate for our study for a number of reasons. First, it is a highly studied species (reviewed in Lowe, et al., 2011) and its feeding and foraging behaviors have been well-characterized in the literature, which provided context in which to interpret hypothesized modifications of swimming behaviors in response to environmental cues. Second, *O. marina* is maintainable at high cell densities in culture due to its ability to tolerate a range of conditions and prey sources (Boakes, et al., 2010; Lowe, et al., 2011). Third, the helical swimming trajectories exhibited by *O. marina* are mainly linear and continuous (Cosson, et al., 1988) which makes it a suitable candidate for establishing a standard 3D framework via video microscopy to quantify swimming behaviors at the individual level. A causal and mechanistic understanding of individual interactions at this level are necessary to establish the basis for population level models that aim to study more representative, and often more complex, scenarios (Kiørboe, 2008). Lastly, *O. marina* has the potential to serve as a model species to be incorporated into future multi-tropic level behavioral models (Mariani, et al., 2008; Davidson, et al., 2011).

While there have been numerous studies that have observed modulated swimming behaviors in predators in response to prey-derived signals, the role of intraspecific signals in mediating swimming behaviors has yet to be investigated. It is not yet known whether *O. marina* has the right biological machinery to

receive and process their own exuded infochemicals and how it might respond, but the possible trade-offs associated with increased predator accumulation and motile behaviors can carry large scale implications. For example, if there were a thin layer of prey in the water column which led to an accumulation of predator biomass within this layer, would this result in a temporal shift in swimming behavior as the food signal weakens and predator-derived signal strengthens? Would a competitive signal arise between individuals and how would swimming behaviors change as a result? How does the signal between so-called competitors compare to the prey-derived signal? Fundamental differences in swimming behaviors are associated with different motivations (i.e. increasing encounter rates with prey to enhance foraging efficiency as compared to decreasing encounter rates with other predators to avoid competition), one might expect a selective pressure on swimming behaviors to optimize fitness in terms of this trade-off (Visser & Kiørboe, 2006). This raises the question, do individuals behave differently in the presence of intraspecific competitors than in the presence of prey? Here, we investigate the role of intraspecific signaling by (1) quantifying the individual swim behaviors of *O. marina* at three different intraspecific cell concentrations in the absence and presence of a competing prey signal, and (2) associating these individual-level changes with the resulting population distributions and estimates of dispersal and encounter rates.

METHODS

Culture conditions of predator and prey – The heterotrophic protist, *Oxyrrhis marina* (CCMP3375), was cultured in triplicate in 29.6 psu, 0.2 µm sterile-filtered autoclaved seawater (SFSW) collected from Narragansett Bay and incubated at 15°C under low light conditions (~10 µmol photons m^{-2} s^{-1}) on a 12 hour light: 12 hour dark cycle. Cultures were not axenic and fed every 4-5 days with 80 mL of the haptophyte prey alga *Isochrysis galbana* (CCMP1323) which was grown in SFSW, enriched with f/2 nutrients minus silica (Guillard, 1975). *O. marina* cultures were transferred every two weeks or on filming days and the *I. galbana* cultures were transferred every 4-5 days to maintain exponential growth. Prior to filming, the *O. marina* cultures were starved for approximately 24 hours in order to minimize cell size variations between treatments, prevent significant *I. galbana* addition to the filming tank and maximize the predator's motivation to forage. Under these conditions, *O. marina* was maintained at cell concentrations between 3,000 to 5,500 cells mL^{-1} and averaged 10-22 µm in body size. Both predator and prey cell concentrations were monitored daily with a Beckman Multisizer III. To ensure a more precise number of both predator and prey cells were added to reach target tank concentrations, *O. marina* and *I. galbana* samples were fixed with 1% Lugol's solution and counted on a Nikon E800 microscope with a 1 mL Sedgewick rafter or hemocytometer, respectively.

Experimental design – The experiments involved the testing of two different treatments: predator concentration and the addition of prey cells. A total of nine different treatments were filmed at least in triplicate over a three

week period, which included six different target *O. marina* concentrations (ranging from 200-2,000 cells mL^{-1}) while in the presence and absence of *I. galbana* cells (10,000 cells mL^{-1}) to the tanks. For prey added treatments, *I. galbana* cells were thoroughly mixed into SFSW before being added to the tanks by peristaltic pump. For all treatment designs, *O. marina* cultures were gently condensed through a 10 μm mesh to an approximate volume of 20-25 mL in order to minimize the introduction of turbulence around the injection site, while still adding the appropriate number of cells to achieve target tank concentrations. The condensate was then added to a 30 mL syringe and slowly added to the bottom of the tanks through silicone tubing with a 1 mm internal diameter. Due to a high loss of *O. marina* cells in the condensing process (~30-60% of target tank concentration), the entire filming volumes were collected and counted, in triplicate, immediately after the conclusion of each film. These averaged counts were used to determine the tank concentrations of replicate treatments which were 171 ± 52, 384 ± 61, and 704 ± 100 *O. marina* cells mL^{-1} for low, medium, and high *O. marina* cell concentrations, respectively. Following a 15 minute adjustment period for the organisms after injection, each tank was then filmed as outlined below.

Tank setup and filming procedures – A 2 psu vertical salinity gradient (27.6 to 29.6 psu) was established using a peristaltic pump in each of the three, 30 cm x 5.5 cm, 1-L octagonal filming tanks to create a stable filming environment by suppressing otherwise dominant water movements associated with convection.. The same source SFSW used to maintain cultures was used to fill the tanks. The

filming tanks were covered and held in a temperature controlled room to prevent temperature and air pressure changes from destabilizing the density gradient. These were essential steps for optimal viewing conditions and the digital reconstruction of the microscale planktonic swimming tracks used to calculate swimming statistics and compare the treatment effects. The methods for video capture were followed and adapted from Menden-Deuer & Grünbaum (2006) and Harvey & Menden-Deuer (2011). Two infrared-sensitive Pixelink cameras with Nikon 60 mm Micro Nikkor lenses were mounted opposite two infrared (960 nm) light-emitting diodes and at a 45° angle to achieve maximal viewing window overlap between both cameras within the center of the tank, avoiding known wall effects on swimming behaviors. Tanks were filmed in the dark (to eliminate known light mediated behaviors) and within one hour of the light to dark transition to limit disruptions in each culture's preconditioned, 24 hour circadian rhythm (Jakobsen & Strom, 2004). Five evenly spaced horizons were monitored throughout each of the three filming tanks. The filming order of horizons was initially randomized and the resulting order was followed across all treatments and replicates. Each replicate resulted in 180 two minute video segments: 5 horizons filmed 6 times (later defined as intervals) in each of the 3 columns for two cameras each at 15 frames per second at 1024 x 768 resolution over the course of 3 hours.

Video Analysis – The methods for video analysis were also detailed in Menden-Deuer & Grünbaum (2006) and the same protocol was followed across all treatments. The x, y pixel position of every organism in each frame was

determined using ImageJ image-processing software and extracted from background particles by optimization of user defined pixel size and threshold parameters. The 3D paths of individual organisms in each film were digitally constructed by compiling the pixel positions over time using Tracker 3D, a Matlab-based motion analysis script, also detailed in Menden-Deuer & Grünbaum (2006). A physical 3D calibration grid was used to fit pixel positions and distances to the actual dimensions of the tank, thereby creating an approximate 0.8 cm x 0.4 cm x 0.3 cm viewing window. Images from both cameras, along with the associated calibration file, were zipped together to form the raw 3D tracks. The raw tracks were smoothed by taking 0.1 second subsamples and these smoothed 3D tracks were used to calculate the four aspects of swimming behavior outlined in the following section. Only tracks with a minimum length of 3 seconds were used in the calculation of swimming behaviors.

Statistical analysis – The non-parametric Kruskal-Wallis test was used to determine significant differences ($p < 0.05$) in swimming behaviors by comparing the mean group ranks of turn rate (degrees $second^{-1}$), vertical velocity (μm $second^{-1}$), swimming speed (μm $second^{-1}$) and vertical deviation angle (degrees) between treatments. Post hoc, one way ANOVA tests were conducted to identify the specific treatments that had significantly differences in individual swimming behaviors (Tukey-Kramer, $p < 0.05$). All analyses were performed in Matlab, using various scripts outlined in Harvey & Menden-Deuer (2011). Two approaches were then taken to quantify differences in swimming behavior in response to the two treatment stimuli of these experiments: predator

concentrations and presence of prey cells. First, for each of the four swimming statistics (turn rate, vertical velocity, swimming speed, vertical deviation angle), an average, spanning over all intermediate time points and horizons, was calculated as a measurement of the response to varying *O. marina* cell concentrations and are reported as medians and interquartile ranges (IQR). The resulting distributions were represented as box plots with respect to *O. marina* cell concentration and each of the individual swimming behaviors were ranked in both the presence and absence of prey. The second approach aimed to illustrate the temporal changes in intraspecific mediated *O. marina* swimming behavior in the absence and presence of prey, where each point represents the compilation of all available tracks per tank per film interval. The group ranks of each swimming behavior were analyzed over time within treatment (low, medium, high *O. marina* cell concentrations) as well as across treatments at each specific time interval.

Dispersal rates – In addition to the analysis of the specific individual swimming behaviors, the resulting rate of dispersal ($\mu m^2\ s^{-1}$) of advancing particles with given movement behaviors was calculated in order to analyze the potential impacts that intraspecific signaling plays in mediating the foraging behavior of *O. marina* as a whole. The following dispersal rate equation was outlined in Visser & Kiørboe (2006) which was modified from Taylor's equation (1921),

$$\text{Dispersal Rate } (\mu m^2\ s^{-1}) = \frac{\nu^2 \tau}{3}$$

where ν is the effective movement speed ($\mu m\ s^{-1}$) and τ is correlation time scale (s). These two parameters were estimated from a least squares regression curve

fit of the average root mean square distance versus time. Due to the lack of a significant number of sufficiently long trajectories, our correlative timescale did not extend beyond 30 seconds.

Encounter rates between O. marina and prey cells – To understand the potential implications of the observed shifts in aggregative swim behaviors in response to an enhanced intraspecific signal, we calculated encounter rates as a function of the total volume swept clear by *O. marina* using the following model from Gerritsen & Strickler (1977).

$$\text{Encounter rate (Z)} = \frac{\pi R^2}{3}\left(\frac{u^2 + 3v^2}{v}\right) \times [I.\ galbana]$$

We used a predator detection radius (R) of 10 μm, which was the sum of the radii for both *O. marina* cells (~8 μm) and *I. galbana* (~2 μm), and assumed that predator swimming speeds (v) were much greater than the swimming speed of I. galbana (u), which is known to be a weak swimmer. Therefore, we used a prey swim speed (u) of 5 μm s^{-1} and our observed predator (v) swimming speeds (μm s^{-1}), and a prey concentration of 10,000 cells mL^{-1} for treatments with added *I. galbana*. In our estimates of encounter rate, we did not attempt to model the decrease in prey concentrations as a result of grazing over 3 hours and assumed a constant *O. marina* concentration which allowed for comparison of encounter rate variation based on modifications of individual movement behavior instead of prey concentration.

It was determined through one-way ANOVA testing that the horizon depth within each filming tank and differences between replicate tanks were not

significant in mediating any of the four analyzed aspects of swimming behavior. Also, there were no significant differences in swimming behaviors between the low target concentrations of 200 and 400 *O. marina* cells mL^{-1} (minimum $p > 0.45$ for all four aspects of swimming behaviors) as well as between the high 1,500 and 2,000 *O. marina* cells mL^{-1} treatments (minimum $p > 0.31$ for all four behaviors). Therefore, data from the same treatment, but different replicates, horizons and tanks were combined in subsequent analyses. The total number of horizons for respective low, medium, and high *O. marina* treatments in the absence of prey cells were 178, 210, and 150 horizons which were compiled from 5, 7, and 6 replicates. For experiments with prey added, 89, 90, and 90 horizons were compiled from respective triplicate treatments of low, medium, and high concentrations of *O. marina.*

RESULTS

Individual O. marina swimming behaviors – Turning rate (degrees s^{-1}) is a measure of the directional change along a swimming path over time, where higher turning rates are associated with more frequent changes in direction and backtracking through previously encountered volumes of water. The mean group ranks of *O. marina* turning rates averaged across all time points and horizons were significantly different across all treatments ($p = 0.0002$, Figure 1A). Slower turn rates were more frequently observed at the low *O. marina* concentration in the absence of *I. galbana* prey cells (median = 64 degrees s^{-1}, IQR = 56 – 74), which was significantly slower than the individuals observed in the corresponding no-prey medium and high concentration treatments by 8.4% and 6.8%, respectively (Table 2). In the presence of prey, the turning rates among different *O. marina* concentrations treatments with prey cells did not significantly differ as median turn rates ranged from 66 to 71 degrees s^{-1}. For all treatments, the fastest turning rates were most frequently observed within the first 30 minute, which was followed by a sharp decrease in the next 30 minute interval (Figure 2A, 2B, Table 3). This large temporal variation within treatments diminished within the first hour of observation and the inclusion of these time points did not result in significantly different mean group ranks.

The mean group ranks of swimming speed (μm s^{-1}) were significantly different across all treatments ($p << 0.0001$) and increased significantly by 8.7% from low (median = 271 μm s^{-1}, IQR = 236 – 292) to high (294 μm s^{-1}, IQR = 265 – 310) *O. marina* concentration in the prey devoid treatments (Figure 1B). Linear

regression analysis reveals a close relationship ($p < 0.0001$) between swimming speed and the *O. marina* cell concentration in the absence of prey cells. In the presence of prey, there were no differences in swimming speeds among treatments of different *O. marina* concentrations (median swimming speeds ranged from 279 to 284 μm s^{-1}). For all treatments, faster swimming speeds were observed more frequently within treatments after the first hour (Figure 2C, 2D). In the absence of prey, *O. marina* swam consistently faster at the high concentration treatment over time as compared to the two less concentrated treatments. For all treatments with prey cells, the increases in swim speed were less pronounced and occurred over a longer time period, but faster swimming speeds were eventually observed at the higher concentrations of *O. marina* after 3 hours. Averaged over the entire 3 hour observational period, encounter rates increased by 22 ± 5% from low to high *O. marina* concentrations in the absence of prey cells and by 16 ± 10% in the presence of prey cells, as a result of increased swimming speed.

Vertical velocity (μm s^{-1}) is the z-component of swimming speed, where positive and negative values indicate the respective upward and downward direction of swimming trajectories. Despite the high variation in all treatments (interquartile ratios (median/IQR) ranged from 68% to 141%), the mean group ranks of vertical velocity were still significantly different ($p << 0.0001$) and vertical velocities at low concentrations of *O. marina* (median = 80 μm s^{-1}, IQR = 10 – 124) increased by 41% at high concentrations (median = 113 μm s^{-1}, IQR = 62 – 148) in the absence of prey cells (Figure 1C). In the presence of prey, the

intraspecific signal did not induce a significant change in the mean group ranks of vertical velocity treatments with medians ranging from 108 to 117 μm s^{-1}. Upward trajectories were more frequently observed than downward trajectories in all treatments. In the absence of prey cells, the magnitude of variation in vertical velocities increased significantly after 1 hour of filming as a greater proportions of tracks were directed downward in the low and medium *O. marina* treatments (Figure 3A, 3B). At high concentrations of *O. marina* or in the presence of prey, *O. marina* swam with consistently positive vertical velocities at each time point throughout the 3 hour film interval.

The vertical deviation angle (θ_z) is the angle between the overall direction of an individual trajectory and the vertical axis, and ranges from 0 to 180 degrees identifying the overall vertical displacement as upward ($0 < \theta_z < 90$ degrees) or downward ($90 < \theta_z < 180$ degrees). For all treatments, θ_z most frequently ranged from 0 to 90 degrees, meaning that *O. marina* primarily swam with some degree of upward trajectory (Figure 1D). The mean group ranks across all treatments were significantly different ($p << 0.0001$) despite the observed variability in θ_z (interquartile ratios ranged from 36% to 63%). There were no significant differences between the distributions of low, medium and high concentration treatments in either the absence or presence of prey; however, consistently lower θ_z for all treatments were observed in the presence of prey (median vertical deviation angles ranged from 56 to 63 degrees) than in its absence (median vertical deviation angles ranged from 48 to 54 degrees). Similar to the temporal trends observed in vertical velocity, the presence of prey or a high *O.*

marina cell concentration led to persistent overall upward swimming trajectories (Figure 3C, 3D).

Oxyrrhis marina dispersal rates – The cumulative modifications in individual swimming behavior between treatments led to different rates of dispersal in *O. marina*, particularly in the presence of *I. galbana* cells (Figure 4). On a 15 second time scale, the root-mean-square distance (RMSD) deviated significantly above the 1:1 correlative distance to time ratio for all treatments signifying highly correlated and ballistic movements. At a longer 30 second time scale, the RMSD dipped below the 1:1 line indicative of non-ballistic trajectories for nearly all treatments. On this time scale, swimming trajectories in both treatments of low *O. marina* treatments were more ballistic than respective medium and high treatments. In the absence of prey, the dispersal rates for low, medium and high *O. marina* concentrations were $4.8 \times 10^4 \pm 610$ $\mu m^2 s^{-1}$, $4.0 \times 10^4 \pm 300$ $\mu m^2 s^{-1}$, and $4.9 \times 10^4 \pm 280$ $\mu m^2 s^{-1}$ suggesting that the *O. marina* cell concentration is a possible contributing factor in mediating motile swimming behaviors (Figure 5). In the presence of prey, dispersal rates decreased from the $1.4 \pm 0.03 \times 10^5$ μm^2 at low concentrations of *O. marina* by factors of approximately 2-3 times the dispersal rates of medium and high treatments, respectively.

Population Distribution of O. marina – The magnitude of variation in swimming behaviors and dispersal rates in response to the intraspecific signal in the absence (Figure 6) and presence (Figure 7) of prey cells was reflected in each treatment's vertical population distributions of *O. marina*. For all treatments,

significant variations were observed between the first 30 minute interval and the remaining five 30 minute intervals. Following the first 30 minute interval, which was characterized by a relative maximum abundance (~40%) within the bottom 10 cm of the tank, we did not observe significant differences in the distributions of *O. marina* over the remaining five 30 minute intervals (represented as a single averaged point). Over the course of the 3 hour film, the proportion of *O. marina* cells observed at the top horizon decreased by 22 ± 5% in the absence of prey cells and 16 ± 10% in the presence of prey cells.

DISCUSSION

Research over the past century has found numerous examples demonstrating the ability of motile heterotrophic protists to respond to biotic and abiotic stimuli (reviewed in Boakes, et al., 2010). Modifications that enhance *O. marina*'s ability to navigate a heterogeneously distributed environment can provide a distinct competitive advantage which may lead to altered growth and grazing rates (Montagnes, et al., 2011). *O. marina* is known to respond to signals derived from prey and higher order predators by modulating swimming behaviors that mediate rates of encounter; however, it is not yet known if *O. marina*'s behavioral response is dependent on the intraspecific cell concentration. Our investigation into this unexplored, yet potentially ever-present signal, suggests that the variation in individual swim behaviors (e.g. increases to swim speed, vertical velocity) was dependent on both 1) the strength of the intraspecific signal and 2) the presence of a competing prey signal which resulted in increased encounter rates with prey, and variations in population distributions and dispersal rates. The analyzed individual swim behaviors were all significantly different across low, medium, and high *O. marina* concentrations and resulted in varying dispersal rates and vertical population distributions when in the absence of prey cells. The presence of prey cells largely overshadowed the role of the intraspecific signal in mediating individual swimming behaviors, but still resulted in observable differences at the population-level. These observations served as the contextual basis in which we interpreted modifications in swim behavior as behavioral responses that would

enhance prey encounters in different environmental circumstances (Grimm & Railsback, 2005; Visser, 2007). Simultaneous analysis of *O. marina* swimming behaviors with dispersal rates, encounter rates, and population distributions established the theoretical framework to translate the intraspecific signal beyond the level of the individual.

Recognition of self: consequence of intraspecific signals in mediating O. marina swim behaviors – An increase in dispersive individual swim behaviors (e.g. significant increases in swim speeds and vertical velocities, moderate decrease in turn rates) from low to high *O. marina* concentrations resulted in a 22% reduction in the proportion of the population at the top horizon suggesting that *O. marina* is capable of modifying individual swim behaviors in response to the intraspecific cell concentration. There was virtually no difference in dispersal rates across low to high treatments devoid of prey cells suggesting that the intraspecific signals between *O. marina* cells did not affect their dispersal. The population distributions observed under low and medium *O. marina* cell concentrations largely align with known motility behaviors of starved *O. marina* that increase dispersal and encounters with prey, as larger proportions of the population were observed at the top horizon of the tank (Visser & Kiørboe, 2006; Kiørboe, 2008). One possible interpretation for the observed behaviors could be that starved *O. marina*, lacking other environmental cues in our prey devoid experiments, responded to the increased intraspecific signals associated with high *O. marina* cell concentrations as a false indication of a prey-rich

environment (Durham & Stocker, 2012), thereby increasing localized searching over time and reducing overall dispersal rates.

For all treatments in the absence of prey cells, *O. marina* was observed to immediately swim upwards within the first 60 minutes as vertical velocity and vertical deviation angle ranged from 121 – 156 μm s^{-1} and 23– 40 degrees, respectively, indicating upward trajectory. This directional bias persisted over the entire observational period for treatments with high *O. marina* concentrations, whereas downward swimmers became more frequent after 90 minutes in low and medium *O. marina* treatments. The presence of a strong intraspecific signal may serve to mediate the direction of swimming trajectories over time as *O. marina* is not known to exhibit any significant tendencies towards upward or downward trajectories. In the absence of other stimuli and vertical velocity distributions have been observed to be bimodal (Schuech & Menden-Deuer, 2014). It is noteworthy that predators were injected at the bottom of the tanks which would eliminate the contribution of all swimmers that immediately swam below the lowest filming horizon, resulting trajectories with an upwards bias; however, over time, *O. marina* were still most frequently observed to swim upwards. The upward trajectory bias agrees well with the foraging strategy of *O. marina*, which is suggested to specialize in encountering horizontally extensive thin layers of prey at the expense of exiting an encountered patch and benefitting from prey patches with other geometries (Menden-Deuer & Grünbaum, 2006). Therefore, we hypothesize that a strong intraspecific signal could serve as a

useful stimulus in the absence of other environmental cues to direct predators towards the surface in an attempt to encounter areas of elevated prey density.

Recognition of prey in the presence of other competitors – In the presence of prey, all analyzed swimming statistics did not significantly differ between treatments. However, we still observed a reduction in dispersal rates by 54% from low to medium *O. marina* cell concentrations and 68% from low to high treatments, and a decreased proportion of overall trajectories observed at the top horizon with increasing *O. marina* concentrations. This suggests that in the presence of prey, higher concentrations of *O. marina* were subjected to stronger aggregative conditions for longer periods of time as compared to lower concentrations similar to the trend observed prey devoid treatments. Remaining highly aggregated while in the presence of other individuals at low prey to predator ratios may seem counterintuitive from a competitive standpoint; however, this behavior may not be completely unexpected. For example, processes such as cell growth, cell proliferation and cell death can be dependent on the local cell concentration and has been demonstrated in a number of multicellular organisms (Søren, et al., 1997). However, aggregative conditions also carry detrimental effects such as increased risk of predation from higher trophic level predators, increased competition for food and increased risk of population wide subjugation to harmful conditions (Schuech & Menden-Deuer, 2014). In terms of this trade-off, our results suggest that *O. marina* favors the short-term benefit of increased prey encounters within a prey patch over the long-term risks associated with remaining aggregated.

Additionally, *O. marina* swimming behavior has been observed to vary with prey concentration. At high prey concentrations (~10^4–10^5 cells mL^{-1}), longitudinal flagellum (associated with higher swim speeds) have been observed to beat more frequently as compared to low prey concentrations (~10^1–10^3 cells mL^{-1}) where the beating of the transverse flagellum was more frequent which is related to higher turning rates (Roberts, et al., 2011). While the exact predator-to-prey ratio was not calculated throughout the 3 hour observational period, *O. marina* consumed *I. galbana,* and reduced the prey concentration below the 10,000 cells mL^{-1} threshold suggested by Roberts, et al. (2011), which would predict a simultaneous decrease in swim speeds and increase in turn rates over time. However, our results do not agree with these observations as average swim speeds increased and turn rates decreased. We hypothesize that the transition in individual swim behaviors on the 3 hour time scale were dependent on the absence or presence of prey signals, rather than the actual concentrations of prey, and were facilitated by a shift behaviors as starved *O. marina* consumed prey.

Aggregative behaviors in the presence of intraspecific signal and prey cells – We observed an overall decrease in RMSD in nearly all treatments over time, which is characteristic motile behavior for biological organisms which balances increased encounters with prey while mitigating predation risk from higher order predators (Visser, 2007; Kiørboe, 2008). These retentive swimming behaviors are further enhanced by the presence of prey exudates, or the excreted chemical cellular material, as the distance that potential consumers can perceive

prey is increased (Larsson & Dodson, 1993). There is increasing evidence to support that *O. marina* has surface receptors that bind to these prey-derived chemical cues, comparable to the signal transduction pathway observed in the model freshwater protist, *Paramecium tetraurelia* (Hartz, et al., 2008); however, it is not known if other signals are similarly interpreted (Søren, et al., 1997; Breckels, et al., 2010). Due to time constraints, we did not characterize the intraspecific signal as mechanical, chemical or a combination of both, but *O. marina* exudates could serve as an effective stimulus to decrease encounter with intraspecific competitors. Theory predicts that in environments with high intraspecific signals that dispersive, ballistic motile behaviors would increase the distance between predators, benefitting the individual by simultaneously decreasing encounters with competitors while increasingly encounters with prey patches. Our results suggest that *O. marina* did not increase dispersive behaviors in the absence of prey cells at high concentrations of intraspecific cells and were observed to increase retentive behaviors. This is a puzzling and largely counterintuitive response as starved cells in this environment would have been subjected to the greatest competition and presumably would have modified behaviors to increase dispersal rates between competitors. One possible interpretation for this observation is that since it is likely that the intraspecific signal has a chemical component (Vos, et al., 2006) and *O. marina* is known to have a strong chemotactic response to prey patches (Durham & Stocker, 2012), it is possible that the surface receptors or signal transduction pathways of *O. marina* are more generalized which would allow the interpretation of a greater

variety of signals at the expense of forming specialized behavioral responses in the presence of multiple signals. This would still permit for well-known prey selectivity through physical encounters between predator and prey (Montagnes, et al., 2008), but does reaffirm doubts concerning *O. marina*'s ability to differentiate chemical signals emanating from mixed assemblages (Martel, 2006). Further testing is needed to determine the validity of these theories which could be achieved by studying the chemotactic response of *O. marina* to intraspecific exudates and a deeper investigation into the internal mechanisms used to interpret external chemical cues.

Evidence of unicellular group behavior in protists? – The ability to interpret intraspecific cues is significant and can serve as the hypothetical basis for coordinated group behaviors, a strategy typically associated with larger multicellular organisms that function to benefit the overall population through the enhancement of specific individual level behaviors. Coordinated behaviors within intraspecific populations has yet to be effectively demonstrated in protists, but has been observed in other microorganisms, most notably in bacteria with regards to quorum sensing (Crespi, 2001). This form of cell-to-cell communication allows bacteria to interpret local conditions (e.g. community composition, strength of chemical cues) and modify individual cell behaviors which has implications at the population-level (Waters & Bassler, 2005). A communicative mechanism that signals the use of a specific set of swim behaviors in *O. marina* that increase the encounter rate with prey cells would be particularly advantageous during foraging. The topic of protistan group behavior

has yet to be thoroughly investigated and *O. marina* is not known to designate specialized roles within populations, even though each cell presumably have particular swim behaviors that are employed under certain favorable environmental conditions. The few existing studies that have investigated this topic tend to sit at the precipice of what defines group behavior. For instance, *Pfiesteria*, a single-celled dinoflagellate species was observed to simultaneously release toxins to ambush their prey, which resulted in a large scale fish kill and allowed the dinoflagellates to feed on the carcasses (Burkholder, 1999). The synchronized release of certain chemicals in response to an increased presence of prey can serve as an important trigger of individual foraging behaviors that would increase the overall fitness of the population. In light of very few observations, the result that *O. marina* significantly altered its swimming behavior as a function of *O. marina* cell concentration is an intriguing observation with implications for how we study and understand the marine food web structure and function in the ocean.

Consequence of aggregative behaviors for encounter rates – The model of Gerritsen and Strickler (1977) provided a useful mechanism to compare encounter rates based on intraspecific variations in swimming behaviors. Over the 3 hour observational period, the approximate 20 μm s^{-1} increase in median swimming speed across low to high *O. marina* concentrations in the absence of prey resulted in a 9% increase in the volume swept clear. This simplified model does not account for increased encounter rate due to turning rate (Visser & Kiørboe, 2006) or an enhanced detection radius of predators through

interpretation of chemical cues (Kiørboe, 2008). However, the de-correlation length scale (mm) was far greater than the detection radius (μm) between predator and prey, so it is likely that modulations in swimming speeds alone could account for a significant increase in encountered water volumes and prey cells that ultimately influence predation pressure (Harvey, et al., 2013). *Oxyrrhis marina* have been observed to have maximum ingestion rates of 250 *I. galbana* cells flagellate^{-1} day^{-1} (Goldman, et al., 1989), which would require a prey concentration of approximately 31,000 cells mL^{-1} at high *O. marina* concentrations, assuming each encountered prey cell was successfully captured and ingested. In contrast, *O. marina* at low concentrations would require a prey concentration of approximately 34,000 cells mL^{-1} to achieve the same encounter rate facilitated by a 20 μm s^{-1} increase in swim speed. The prey concentration of this experiment (10,000 cells mL^{-1}) likely limited ingestion rates below *O. marina*'s maximum ingestion rate; however, an approximate ambient prey concentrations of 11,000 cells mL^{-1} would be required to match the increased encounter rates resulting from the faster swimming speeds observed at high *O. marina* concentrations. This suggests that the behavioral response to intraspecific signaling is significant in altering encounter rates and in the context of more dilute marine environments, would enable *O. marina* to meet its daily consumption requirement at lower prey concentrations.

Limitations of methods – Considering the significant modifications in *O. marina* swimming behaviors, dispersal rates and population distributions to an inherent signal, this study supports the continued study of intraspecific signaling

in other heterotrophic protists and environments. Our laboratory study was sufficient to demonstrate the ability of *O. marina* to interpret its own intraspecific signal; however, a number of methodological restraints including tank size, tank environment and technological limitations may have limited the scope of our results. First, the 0.3 m tanks used in this project likely fell on the shorter end of relevant spatial scales in which to observe the response to the intraspecific signal (Menden-Deuer & Grünbaum, 2006). Relative maximum abundances were observed at or near the top horizon within the first hour of filming which sat only a few mms from the surface. It is possible that over the course of 3 hours, *O. marina* further modified swim behaviors as a result of its interaction with this physical boundary. However, even in this relatively small environment, predators were still distributed throughout the tank as only 3 out of the total 810 two minute films did not contain *O. marina* cells within the viewing window. Future studies of *O. marina* swimming behavior should account for *O. marina*'s ability to swim great distances and the incorporation of larger tanks would facilitate an extended viewing window in which to observe swimming behaviors not mediated by tank limitations.

Second, the highly controlled and artificially enhanced concentrations of *O. marina* likely contain our observations to a very narrow range of environmental conditions. However, because quantitative databases of swimming behaviors for *O. marina*, and other motile protists, are limited, we cannot extrapolate our results to more realistic environments in the presence of multiple signals. The role of chemical cues in mediating individual swimming

behaviors is a relatively new study that has only recently been explored due to advancements in observational capabilities. In this study, the use of 3D video microscopy required a highly controlled environment to prevent larger scale water movements from overshadowing fine scale swimming behaviors and a previously unknown response to intraspecific signaling led us to design experiments that favored the enhancement of treatment response over *O. marina* concentrations that can be found in more realistic environments. While these simplified and highly controlled laboratory conditions likely constrained our observations to a very narrow range of environmental conditions, they were optimal for establishing a contextual framework in which future studies may explain observed modifications in swim behavior in more realistic environments, further advancing our mechanistic understanding of predator-prey interactions.

Lastly, significant advancements in 3D video microscopy have furthered our ability to quantify swimming behaviors, but could still be improved. One limitation of this technique is the inability to track individuals on greater time scales which would provide longer trajectories and limit the contribution of resampled individuals, though given the large number of observations, we would not expect resampling to significantly alter the results. Proper characterization of swimming tracks is scale dependent and the randomly diffusive motility patterns typically attributed to biological organisms are often only observed on large scales (Kiørboe, 2008). Individuals that swim in and out of the viewing window result in shorter tracks that could inherently bias observations towards more ballistic motile behaviors; however, even with a static camera system, swimming

tracks became characteristically diffusive over time for most treatments suggesting that our correlative time scale was sufficient to describe swimming behaviors over time.

CONCLUSIONS

The findings of this study suggest that *O. marina* has the capability to interpret its own intraspecific signal in the absence of competing signals which resulted in quantifiable modifications to individual swim behaviors. In the presence of a prey signal, intraspecific signals were not significant in inducing a behavioral shift on the individual level, but still resulted in significantly different rates of dispersal and *O. marina* population distributions. The particular aggregative or dispersive swimming behaviors were hypothesized to be adapted to fit specific biological needs in the organism's current environment. In terms of more realistic environments, enhanced rates of biological processes and community distributions are likely to be affected on relevant spatiotemporal scales (Woodson & McManus, 2007). Predatory foraging behavior may be enhanced by any number of environmental signals, particularly those emanating from prey; however, if swimming behaviors are influenced by the local environmental signals and the behavioral response occurs rapidly, then swim behaviors could be predicted based on specific environments (Visser, 2007). In order to fully describe the range of possible behavioral capacity of *O. marina*, and possibly many other species of heterotrophic protists, the intraspecific signals between predators should be taken into consideration as an inherent stimulus of swimming behaviors.

TABLES

Table 1. Summary of p-values from the non-parametric Kruskal-Wallis test between the mean group ranks of swimming behaviors at low (L), medium (M), and high (H) *O. marina* concentrations in the absence (-) and presence of prey (+). For tests with $p < 0.05$, post hoc one-way ANOVA tests were performed to identify which treatments were significantly different in the individual swimming behaviors.

	Turning Rate	Swimming Speed	Vertical Velocity	Vertical Deviation Angle
p-value	0.0002	2.22e-07	4.79e-08	2.22e-07
L-/M-	yes	yes	no	no
M-/H-	no	yes	no	no
L-/H-	no	no	yes	no
L+/M+	no	no	no	no
M+/H+	no	no	no	no
L+/H+	no	no	no	no

Table 2. Summary of medians, percentiles, interquartile ranges and whisker values for box plots reported in Figure 1, for low, medium and high *O. marina* concentrations in the presence (+) and absence of prey cells (-). The interquartile ranges (IQR) are the difference between the 25th and 75th percentiles. Low whiskers and high whiskers were calculated as Q1 – (1.5 x IQR) and Q3 + (1.5 x IQR), respectively. Data points outside of this range were identified as outliers.

Turning Rate	Low -	Med -	High -	Low +	Med +	High +
Low Whisker	30	48	48	51	41	43
25th percentile (Q1)	56	63	63	66	60	60
Median	64	69	68	71	70	66
75th percentile (Q3)	74	75	74	77	76	75
High Whisker	96	90	88	88	93	85

Swimming Speed	Low -	Med -	High -	Low +	Med +	High +
Low Whisker	160	188	202	226	211	177
25th percentile	236	255	265	261	259	250
Median	271	280	294	279	286	284
75th percentile	292	299	310	294	303	306
High Whisker	334	333	328	333	341	324

Vertical Velocity	Low -	Med -	High -	Low +	Med +	High +
Low Whisker	-156	-184	-46	-19	-37	-33
25th percentile	10	2	62	70	77	69
Median	80	88	113	108	119	117
75th percentile	124	129	148	149	158	151
High Whisker	233	256	220	219	243	219

Vertical Deviation Angle	Low -	Med -	High -	Low +	Med +	High +
Low Whisker	14	15	20	23	18	22
25th percentile	47	50	46	46	38	41
Median	63	60	56	54	48	52
75th percentile	86	87	69	66	65	68
High Whisker	140	143	98	93	104	97

Table 3. Summary of the number of analyzed trajectories, time points, median, and standard error of turn rate (degrees s^{-1}), vertical velocity (μm s^{-1}), swimming speed (μm s^{-1}), and vertical deviation angle (degrees) plotted in Figures 2-3.

Treatment	Trajectories (x10^2)	Time (minutes)	Turn Rate	Std. Error	Swim Speed	Std. Error
Low - prey	451	30	55	0.4	171	0.4
	66	60	42	0.9	259	1.4
	31	90	43	1.4	276	2.0
	31	120	46	1.4	289	1.8
	37	150	46	1.3	284	1.6
	29	180	46	1.4	302	1.8
Low + prey	98	30	54	0.8	256	1.0
	23	60	46	1.7	276	2.2
	13	90	45	2.2	280	3.3
	13	120	45	2.1	283	3.0
	14	150	45	2.0	282	2.9
	16	180	47	1.4	289	2.0
Med - prey	1132	30	51	0.2	206	0.2
	171	60	43	0.6	273	0.8
	88	90	45	0.8	283	1.1
	96	120	46	0.8	273	1.0
	113	150	47	0.7	264	0.9
	105	180	47	0.7	281	0.9
Med + prey	441	30	56	0.4	221	0.4
	86	60	50	0.9	254	1.1
	34	90	45	1.3	289	1.8
	29	120	41	1.4	300	1.9
	30	150	42	1.3	300	1.9
	31	180	43	0.7	302	1.1
High - prey	2052	30	56	0.2	217	0.2
	354	60	49	0.4	271	0.5
	238	90	44	0.5	299	0.6
	180	120	44	0.5	297	0.7
	160	150	44	0.6	299	0.7
	133	180	44	0.6	305	0.8
High + prey	508	30	52	0.4	212	0.4
	101	60	48	0.8	241	1.0
	56	90	43	1.0	278	1.3
	62	120	41	0.8	292	1.2
	65	150	41	0.8	306	1.1
	68	180	43	0.6	306	0.8

Treatment	Trajectories ($x10^2$)	Time (minutes)	Vertical Velocity	Std. Error	Vertical Deviation Angle	Std. Error
Low - prey	451	30	121	0.3	23	0.1
	66	60	137	1.9	39	0.6
	31	90	56	3.7	59	1.1
	31	120	3	3.8	98	1.1
	37	150	71	3.4	63	1.0
	29	180	125	4.1	49	1.2
Low + prey	98	30	156	1.1	28	0.3
	23	60	136	3.5	40	1.0
	13	90	104	5.0	50	1.4
	13	120	122	5.1	46	1.4
	14	150	151	4.8	40	1.3
	16	180	138	3.5	45	1.0
Med - prey	1132	30	146	0.3	23	0.1
	171	60	148	1.3	37	0.4
	88	90	111	2.2	47	0.6
	96	120	4	2.1	93	0.6
	113	150	-117	1.9	138	0.6
	105	180	-83	2.1	129	0.6
Med + prey	441	30	148	0.4	25	0.1
	86	60	126	1.6	36	0.5
	34	90	154	3.1	35	0.9
	29	120	162	3.7	37	1.0
	30	150	169	3.6	37	1.0
	31	180	144	2.0	44	0.6
High - prey	2052	30	153	0.2	24	0.1
	354	60	125	1.0	36	0.3
	238	90	150	1.5	38	0.4
	180	120	151	1.6	38	0.5
	160	150	136	1.8	45	0.5
	133	180	195	1.8	32	0.5
High + prey	508	30	136	0.4	26	0.1
	101	60	123	1.4	33	0.5
	56	90	92	2.8	51	0.9
	62	120	85	2.9	64	0.9
	65	150	185	2.8	35	0.8
	68	180	173	2.0	39	0.6

Table 4. Summary of dispersal rates ($\mu m^2\ s^{-1}$), the parameters motility parameters of effective movement speed, ν (μm) and correlative timescale, τ (seconds) derived from the least squares regression curve and the associated goodness of fit (r^2) with the average root mean square distance (RMSD).

	Dispersal Rate ($\mu m^2\ s^{-1}$)	Std. Error ($\mu m^2\ s^{-1}$)	CV (%)	ν ($\mu m\ s^{-1}$)	τ (second)	Correlation Coefficient (r^2)
Low-	48153	610	1.27	337	1.3	0.9846
Low+	140577	3016	2.15	185	12.3	0.9853
Med-	40069	296	0.74	215	2.6	0.9969
Med+	64196	444	0.69	123	12.7	0.9756
High-	49381	283	0.57	205	3.5	0.9937
High+	44650	269	0.60	155	5.6	0.9928

FIGURES

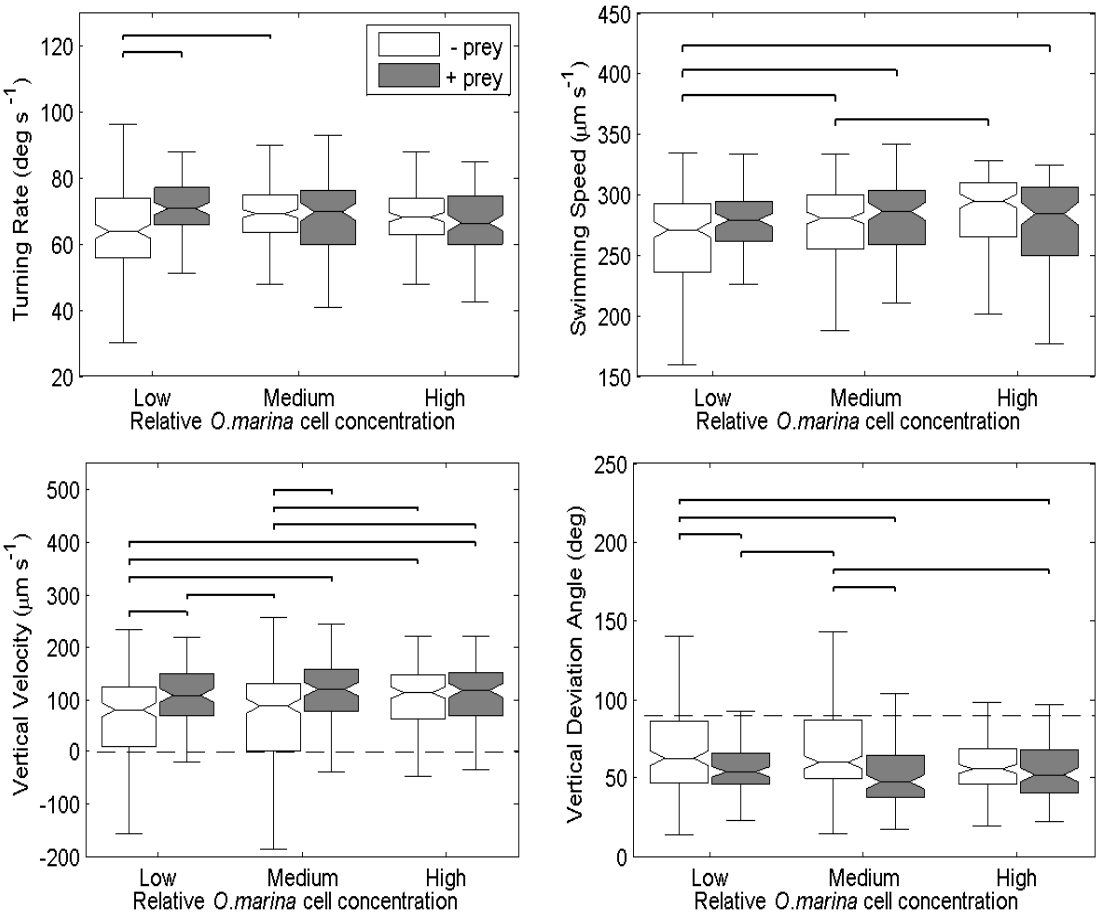

Figure 1. The mean group ranks of *O. marina* swimming behavior in the absence (white) and presence (grey) of *I. galbana* were significantly different (all p-values < 0.0002) in (A) turning rate (degrees s^{-1}), (B) swimming speed (μm s^{-1}), (C) vertical velocity (μm s^{-1}) and (D) vertical deviation angle (degrees). Box plots with non-overlapping notches have significantly different group means ($\alpha = 0.05$) and are indicated by brackets. Mid-box line = median; box = 25th and 75th percentiles; bars = box ± 1.5 x interquartile range (IQR); horizontal dashed lines separate downward and upward swimming trajectories.

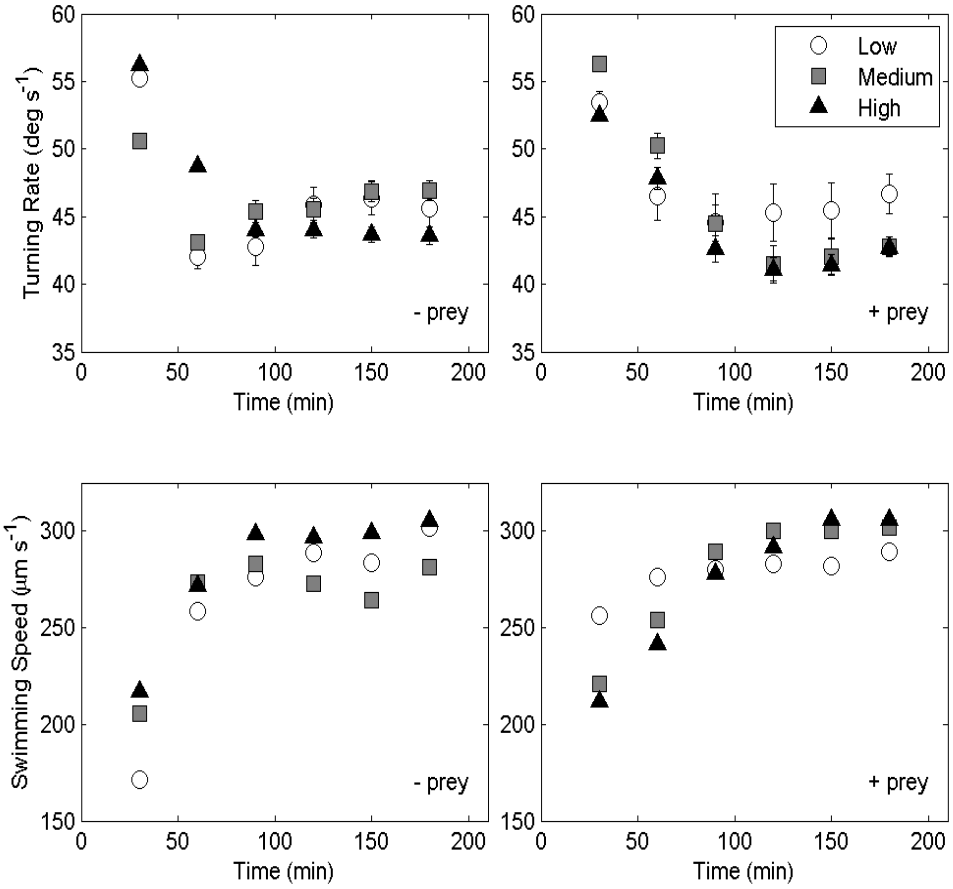

Figure 2. Median turning rate (degrees s^{-1}) and swimming speed (µm s^{-1}) of all analyzed tracks across replicate treatments and compiled by low (white circles), medium (grey squares), and high (black triangles) *O. marina* cell concentrations over time (hours) in the absence and presence of prey. Relative maximum turning rates and minimum swimming speeds were observed in the first 30 minute film interval. Error bars represent one standard error about the median and are largely contained within the symbols.

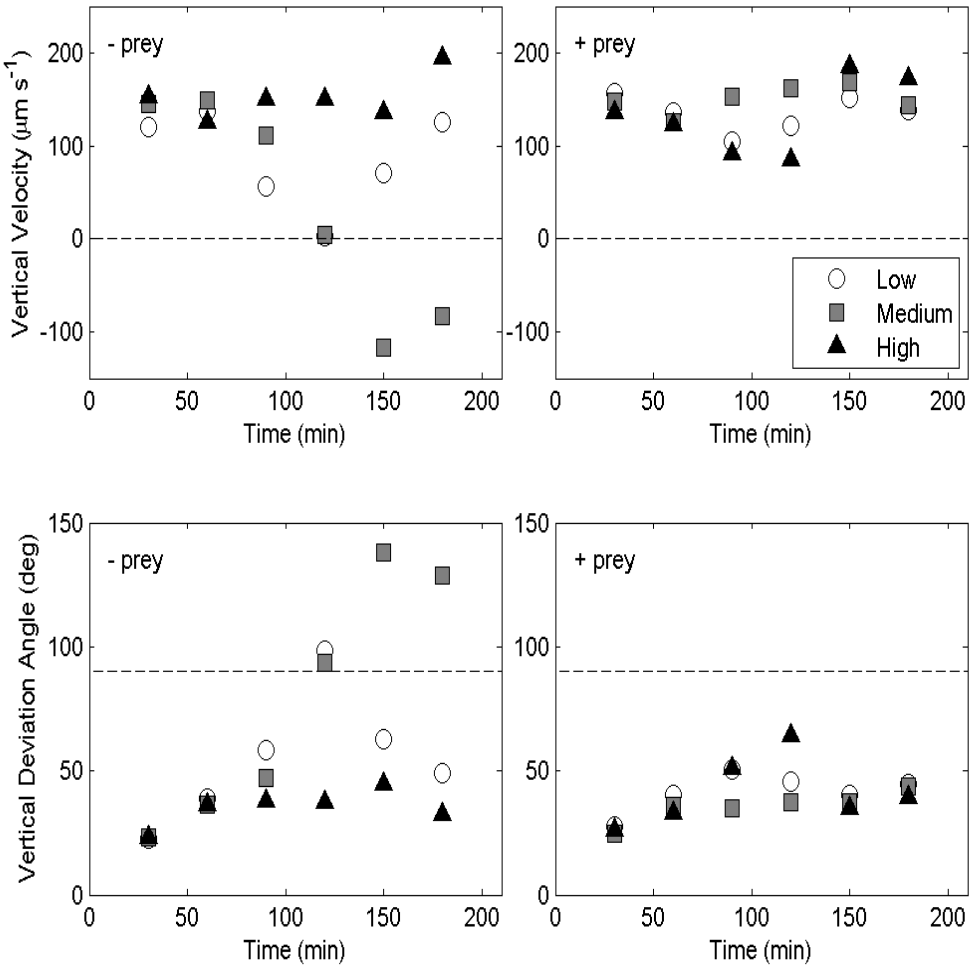

Figure 3. Median vertical velocity (μm s^{-1}) and vertical deviation angle (degrees) over time (minutes) of the analyzed tracks compiled by replicate treatments. The horizontal, dashed lines indicate the direction of swimming trajectory, as downwards (vertical velocity < 0 μm s^{-1}, θ_z > 90 degrees) or upwards (> 0 μm s^{-1}, θ_z < 90 degrees). See Figure 2 for definition of error bars.

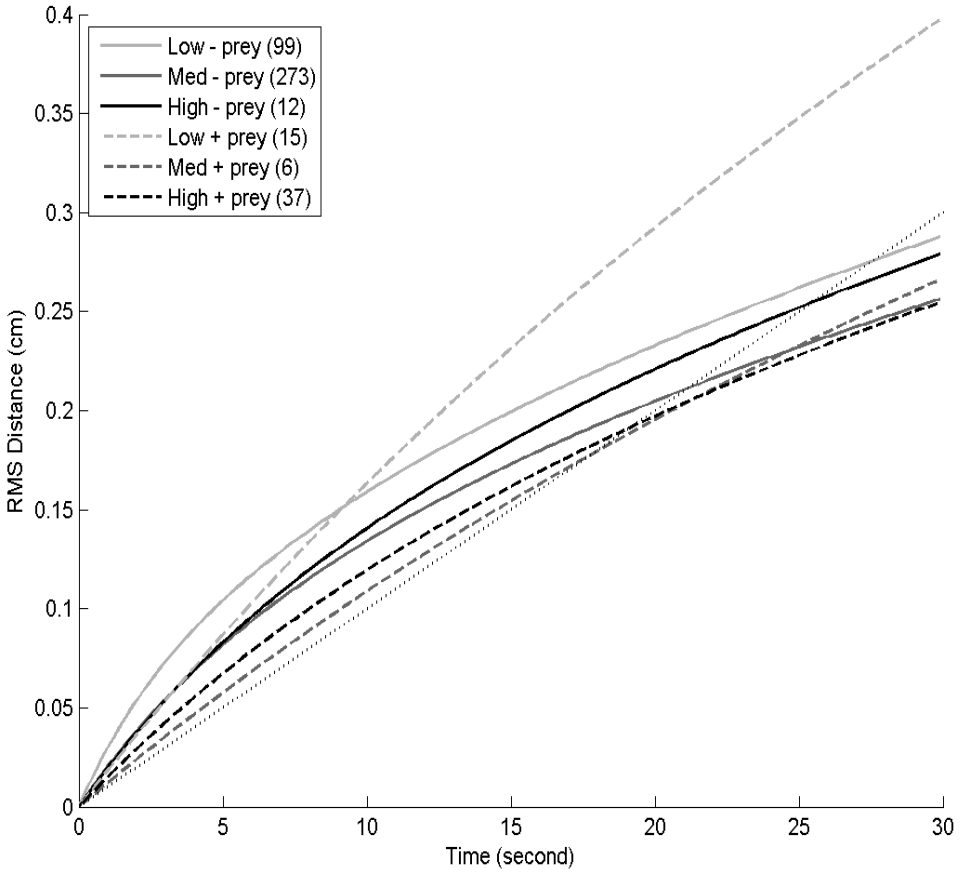

Figure 4. The root mean square (RMS) distance (cm) over time for each treatment of low, medium and high *O. marina* concentration in the absence (solid lines) and presence (dashed lines) of prey cells. The number of tracks used to compose each curve is indicated in parentheses. The dashed line indicates the 1:1 correlative distance to time relationship distinguishing super ballistic (> 1:1) or diffusive (< 1:1) motile behavior. All treatments, except for the low *O. marina* cell concentration in the presence of food, exhibited the characteristic diffusive behaviors of biological organisms over the 30 second time scale.

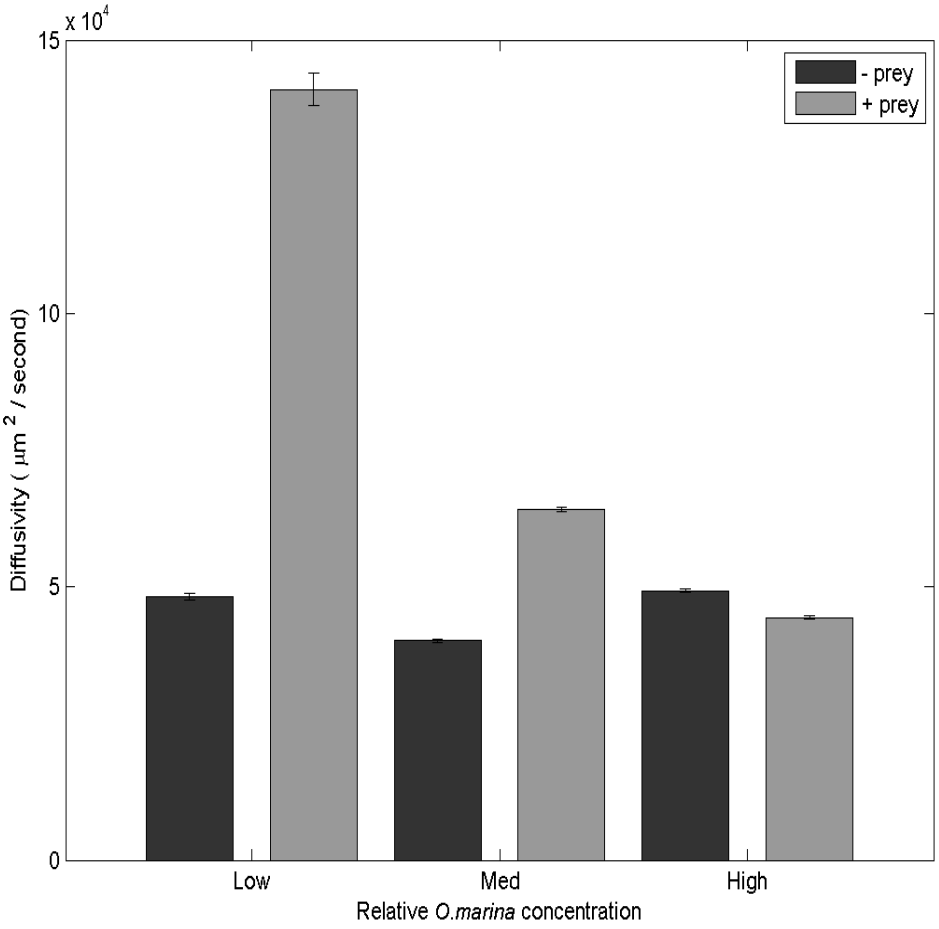

Figure 5. Rates of dispersal ($\mu m^2\ s^{-1}$) as calculated from effective movement speed (v, $\mu m\ s^{-1}$) at a 30 second correlation times (τ). In the absence of prey cells, diffusivity of *O. marina* cells varied significantly less than when in the presence of prey cells, which significantly decreased with *O. marina* cell concentration. Error bars represent one standard error about the mean and the number of tracks composing each bar can be found in Figure 4.

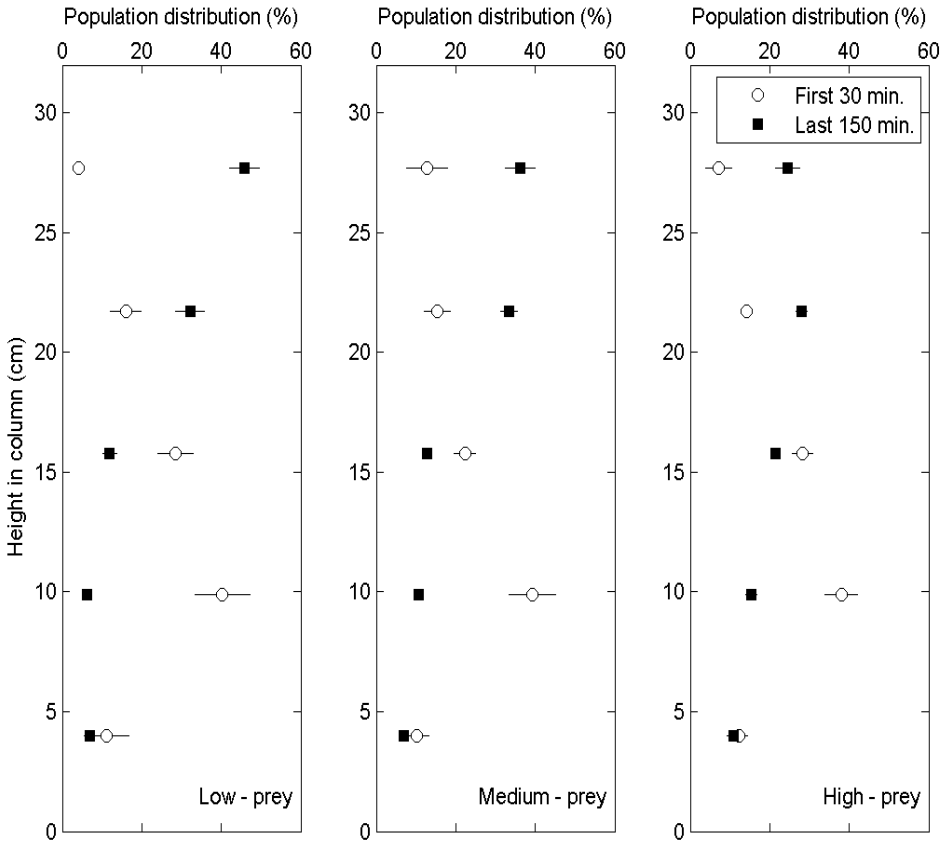

Figure 6. Population distributions for replicate treatments of low, medium and high cell concentrations of *O. marina* in the absence of prey cells. For all treatments, the distribution for the first 30 minute interval (white circles) differed significantly from all remaining time which were compiled as a single averaged point per horizon (black squares). As *O. marina* cell concentration increased from low to high, a lower proportion of the population were found at the top horizon decreased by 22 ± 5%. Error bars represent one standard error of the mean.

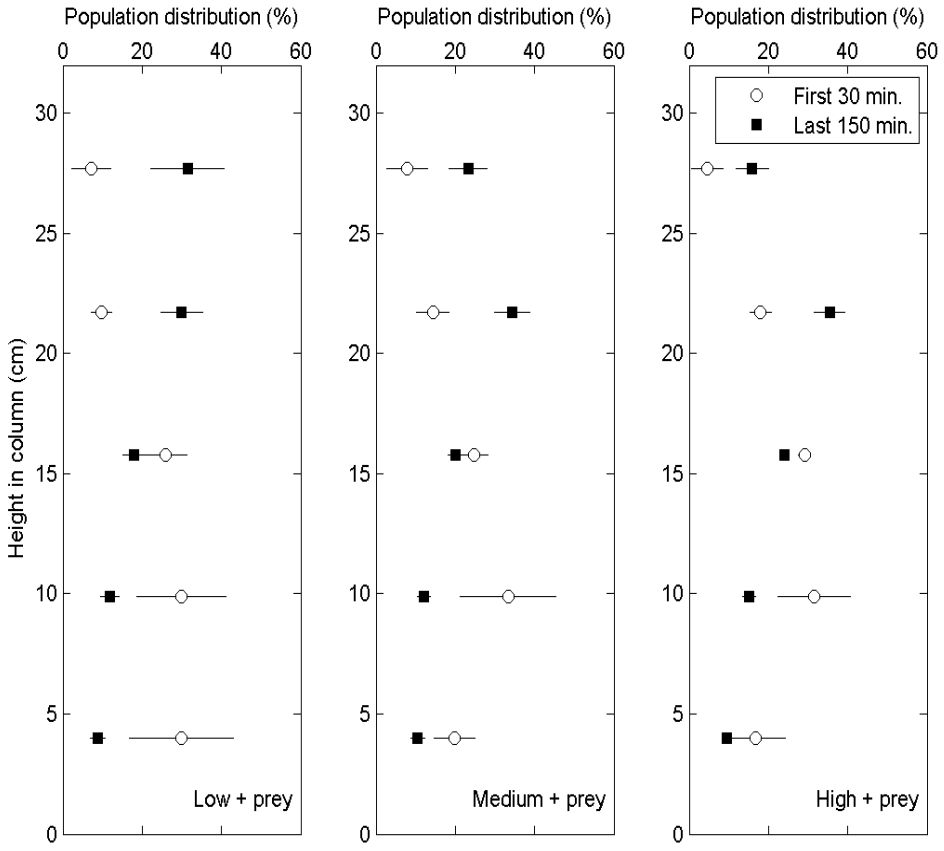

Figure 7. Distributions of *O. marina* in the low, medium and high cell concentrations in the presence of *I. galbana* prey cells for the first film interval (white circles) and the average of the remaining 150 minutes (black squares). The distributions of the remaining 150 minutes in the presence of prey varied less significantly than the distributions in the absence of prey, but we still observed a 16 ± 10% lower proportion of the population from low to high *O. marina* concentrations. Error bars represent one standard error of the mean for triplicate films.

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