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Relating cell-level swimming behaviors to vertical population distributions in *Heterosigma akashiwo* (Raphidophyceae), a harmful alga

Abstract—Cell motility may facilitate the formation of harmful algal blooms (HABs) by enabling algal cells to swim to favorable microenvironments that support explosive growth. Motility also augments the formation of algal cell aggregations that are often associated with ecological and economic consequence. In this study, we used computerized video analysis to quantify cell-level swimming characteristics by reconstructing cell trajectories in the motile raphidophyte *Heterosigma akashiwo*, a unicellular alga that forms toxic surface slicks in temperate coastal waters worldwide. *Heterosigma* cells are capable of rapid changes between at least two active swimming modes, distinguishable by the magnitude of the oscillatory component of motion. Swimming direction varied during a diurnal photoperiod, with swimming direction changing from random to upward directed shortly after the start of the light phase. Motility assays performed 6–8 h into the light phase showed that two *Heterosigma* strains from geographically distant locations differed significantly in gross swimming speeds, with mean values of 49–66 μm s^{-1} for strain CCMP452 (West Atlantic, USA), and 88–119 μm s^{-1} for strain CCAP934-1 (North Sea, Norway). A spatially explicit model of vertical distribution of *Heterosigma* cells based on strain-specific motility data suggests that cells of the two strains may diverge in water-column position within a few hours and that CCAP934-1 develops dense surface aggregations more rapidly and more robustly than CCMP452. Propensity to form toxic surface slicks, and therefore frequency and severity of HAB impacts, may vary substantially among *Heterosigma* strains, mediated by differences in cell-level motility.

Many harmful algal bloom (HAB)-forming species swim. For example, 29 of the 33 HAB-forming species identified from the west coast of North America are motile (Horner et al. 1997; Horner pers. comm.). For these species, cell-level swimming may constitute an important mechanism that regulates cell abundance and distribution, either directly by concentrating algal cells via interactions of swimming behaviors with ambient flows (Kessler 1985; Franks 1997) or indirectly by enabling cells to locate favorable microenvironments that enhance algal growth rates (Watanabe et al. 1988; Liu et al. 2001). These consequences of motility suggest that quantitative characteristics of swimming behaviors may strongly influence where and when HABs occur. Unfortunately, quantitative swimming characteristics for most HAB-forming algae remain unidentified; thus, the extent to which swimming characteristics vary across relevant ranges of physiological and environmental conditions and across distinct strains within species also remains unknown. This lack of information makes it difficult to develop and test motility-based predictive models of HAB formation or to understand fundamental biological dynamics such as coevolution of algal swimming behavior with other physiological and ecological traits.

This study quantifies the swimming characteristics of the raphidophycean biflagellate *Heterosigma akashiwo*, which forms toxic surface slicks in temperate and subtropical coastal regions worldwide (Smayda 1998). Previous studies show that *Heterosigma* cells are highly motile and suggest high variability in individual-level swimming characteristics with markedly differing implications for population distributions and HAB formation (Throndsen 1973; Bauerfeind et al. 1986). However, it remains ambiguous whether discrepancies between these studies reflect differences in culture conditions and observation methods or whether different strains of *Heterosigma* exhibit intrinsically different motility characteristics. Our study sought to quantify swimming behaviors of *Heterosigma* cells from distinct geographical origins, to establish whether these strains intrinsically differ in quantitative cell-level swimming characteristics under identical conditions, and to assess whether such differences are substantial enough to cause significant interstrain differences in the timing and location of HAB formation.

Methods—Heterosigma akashiwo cells, strains CCMP452 (Provasoli Guillard Center for Culture of Marine Phytoplankton) and CCAP934-1 (Culture Collection of Algae and Protozoa), were cultured in artificial seawater medium, O-3 (McIntosh and Cattolico 1978) at 20°C and synchronized via a 12 : 12 light : dark (LD) photoperiod. Exponentially growing cells were seen swimming freely in a 30-cm tall by 10-cm diameter Plexiglas observation chamber, which matched culture conditions (20°C, 12 : 12 LD) except that fluid motions were suppressed with a weak linear salinity gradient in the O-3 medium (salinity 20‰ at the base, 18‰ at the top). Cells were observed with dark field illumination from an infrared light source. Video was captured to a computer at 10 Hz and analyzed to produce a data file of two-dimensional cell trajectories. Each experiment began between the 6th and 8th hours of the light cycle (L6 and L8) (Cattolico et al. 1976), after which cell trajectories were observed for 1 min at 15-min intervals in the first hour, and then hourly for 24 h to assay variations over a diurnal cycle.

Like many flagellates, *Heterosigma* cells swim in a helical pattern, with an overall swimming direction that can be represented as the instantaneous axis of the helix (Crenshaw et al. 2000). Speed and direction along the axis typically vary more slowly in time than the other swimming components,

which in two-dimensional projection usually appear as rapid side-to-side oscillations superposed on low-level pixel discretization noise. The along-axis swimming component is the most relevant to long-term changes in spatial population distributions because the oscillatory components, while in some cases constituting a significant fraction of total movement, cancel out over relatively short timescales and therefore result in little or no net movement. However, the oscillatory component may contain useful diagnostic information about the type and condition of swimming cells. Along-axis components were computed from our motility data by fitting a cubic spline to each trajectory using a least-squares algorithm with 50 frames per knot. Splined trajectories were used to calculate two strain- and time-specific swimming speed statistics: gross speed, defined as the total two-dimensional projection of along-axis displacement divided by the duration of the trajectory; and vertical speed, defined as the vertical component of along-axis displacement divided by duration.

Swimming statistics were used to parameterize a spatially explicit model of vertical movements in *Heterosigma* cell populations. The model assumed that the vertical speed of each *Heterosigma* cell is a continuous time, piecewise constant Markov process. Cells were initially distributed uniformly between 10 discrete velocity classes spanning the range of observed vertical velocities and subsequently transitioned between velocity classes according to probabilistic rules defined by a reorientation vector λ and a transition matrix T: Cells in the ith velocity class changed to a new velocity at random intervals at rate λ_i. Cells moving out of the ith velocity class moved into the jth velocity class with a transition probability T_{ij} (Grünbaum 1999). λ and T were estimated directly from the observed changes in cell velocities along trajectories. By using trajectory data to estimate velocity transitions rather than mean velocities, this analysis minimized some possible observation biases (e.g., overrepresentation of rapidly upward-swimming cells in the upper part of the observation chamber shortly after cells are introduced) and better represented the effects of variability between and within cell trajectories. The model predicts effects of variations in motility on the vertical distribution of *Heterosigma* cells over a relatively short time frame of a few hours. It neglects longer term variations in other environmental factors such as wind-driven turbulence and temperature, which may directly (through mixing) or indirectly (through altered cell motility) affect the spatial distribution of *Heterosigma* cells and additional physiological dynamics such as diurnal variation in cell motility and demographics.

Variability in cell-level motility—Heterosigma is a rapid, vigorous swimmer that displays significant variability in motility characteristics on several levels. *Heterosigma* cells sampled at L6–L8 displayed multiple swimming modes, including straight trajectories in which the oscillatory components of movement were small and helical trajectories in which oscillatory components were much more pronounced (Fig. 1). Both straight and helical trajectories were observed frequently and cooccurred in many experiments. Although there was a broad range of trajectory types, possibly a continuum from helical to straight trajectories, cells are capable of clear, nearly instantaneous transitions between modes (Fig. 1).

Heterosigma cells can rapidly and dramatically alter speed and angular distribution in response to changing light conditions (Fig. 2). Cells can transition from a nearly random distribution of swimming directions in the dark (D12; mean vertical speed 11 μm s^{-1}), to an upward-biased distribution with nearly all cells oriented within a narrow range of swimming directions in the light (L2; mean vertical speed 85 μm s^{-1}). These changes coincided with (and probably caused) rapid vertical redistribution of cell densities within the observation chamber.

Heterosigma strains CCAP934-1 (North Sea, Norway) and CCMP452 (West Atlantic, New York) sampled at L6–L8 displayed significantly different motility characteristics. Distributions of gross speed and vertical speed for seven experiments on these strains are plotted in Fig. 3. Speeds varied significantly among experiments (mean values different among seven experiments, $p < 0.001$, analysis of variance [ANOVA] and nonparametric Kruskal–Wallis). All experiments with CCAP934-1 resulted in significantly faster mean gross speed than experiments with CCMP452 (comparing all pairs of experiments using Tukey–Kramer HSD (honestly significant difference), $\alpha = 0.01$). The mean vertical speed varied from 51 to 103 μm s^{-1} for CCAP934-1 and from 35 to 60 μm s^{-1} for CCMP452, indicating that cells of both strains had an upward bias in their swimming direction. However, there was considerable variability in swimming behavior among cells in each experiment. In particular, a large spread was observed in the vertical speed distribution of CCAP934-1, in which cells swam in a very directed fashion both upward and downward.

A vertical distribution predicted by the spatially explicit model is shown in Fig. 4, which represents a 4-h time series for a hypothetical mixed *Heterosigma* population with equal numbers of two-cell cohorts based on swimming behavior exhibited by CCMP452 (pooling all data from experiments 1a, 1b, 2, and 3) and CCAP934-1 (pooling data from experiments 4a, 4b, and 5). Strain CCAP934-1 has a larger upward speed component (71 μm s^{-1}, mean of all trajectories), causing the bulk of the population to move rapidly to concentrate in a surface slick. In contrast, CCMP452 has a smaller upward-swimming component (44 μm s^{-1}, mean of all trajectories), and the bulk of the population moves correspondingly less. Greater variability in upward-swimming speeds and lower transition rates between swimming speed are reflected in wider dispersion of CCAP934-1 cells across depth relative to CCMP452. These simulations suggest that from the same starting point in the water column, cells of the two strains diverge in water-column position within a few hours.

Implications for Heterosigma *biology*—Most marine environments show strong spatial and temporal heterogeneity in characteristics that are critical to phytoplankton physiology. Motility enables algal cells to more effectively exploit this heterogeneity, potentially enhancing bloom formation. We quantified the swimming behavior of *Heterosigma akashiwo*, a toxic alga whose negative effects on marine communities appear to be closely tied to its ability to form

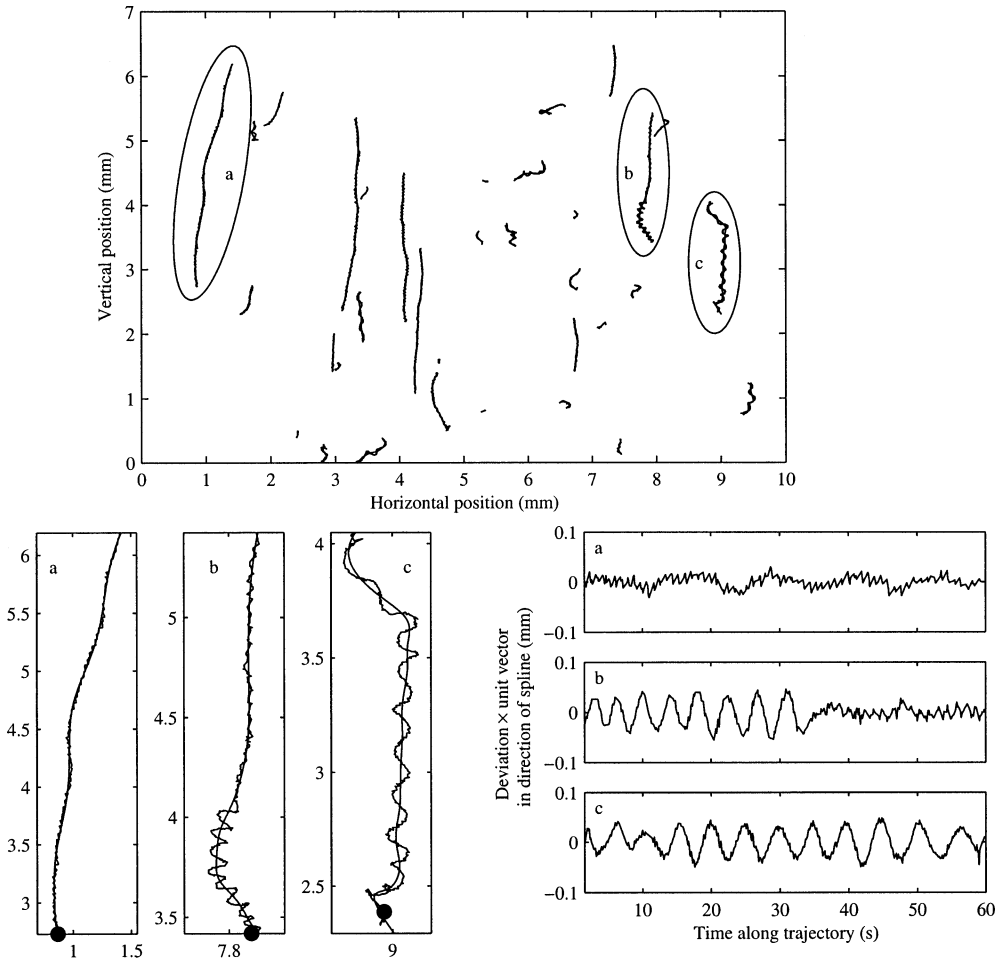

Fig. 1. *Heterosigma* cell trajectories. Trajectories that span a specified time window (frames 145–150) from 1 min of video footage in the first hour (L8) of an experiment with strain CCMP452 are plotted. Superposed on each path is the estimate of the along-axis component of swimming motion. In the lower left panels, three trajectories are shown in more detail, illustrating the diversity of swimming modes: (a) a straight trajectory; (b) a trajectory with transition from helical to straight; and (c) a helical trajectory. A large dot marks the beginning of each trajectory. The combined oscillatory/pixel noise components of swimming movements corresponding to (a–c) are plotted against time in the lower right panels. For the straight path (a) the oscillatory/noise component oscillates at higher frequency and with lower amplitude than for the helical trajectory (c). Trajectory (b) shows a rapid transition between helical and straight swimming modes.

extremely dense surface slicks. Formation of these slicks over relatively rapid timescales and *Heterosigma*'s ability to undergo substantial daily excursions through the water column (Kohata and Watanabe 1986; MacKenzie 1991) suggest that motility plays a central role in surface aggregation. Previous studies reported *Heterosigma* swimming speeds differing by an order of magnitude (20–150 μm s^{-1}; Throndsen 1973; Bauerfeind et al. 1986). Both the presence and absence of diurnal changes in water-column distributions have also been published (Hershberger et al. 1997; Smayda 1998). The implications of these observations for HAB formation by *Heterosigma* depend strongly on whether variability in motility characteristics was due to methodological, physiological, or genetic differences. Our study quantified variability in *Heterosigma*'s swimming behavior under controlled conditions, enabling us both to identify sources of variability and to assess its significance for spatial distributions.

Heterosigma cells are capable of multiple swimming modes, which are exhibited simultaneously by neighboring cells whose close proximity suggests that they are experiencing similar ambient conditions (Fig. 1). These swimming modes differ at the cell level by the relative magnitudes of the along-axis and oscillatory swimming components, which are presumably under tight flagellar control and which cells apparently can change almost instantaneously (Fig. 1b). The functional significance of these different swimming modes is unknown. However, ratios of net to gross displacement are substantially lower in helical trajectories, suggesting that cells can increase large-scale transport rates by switching to a straight swimming mode.

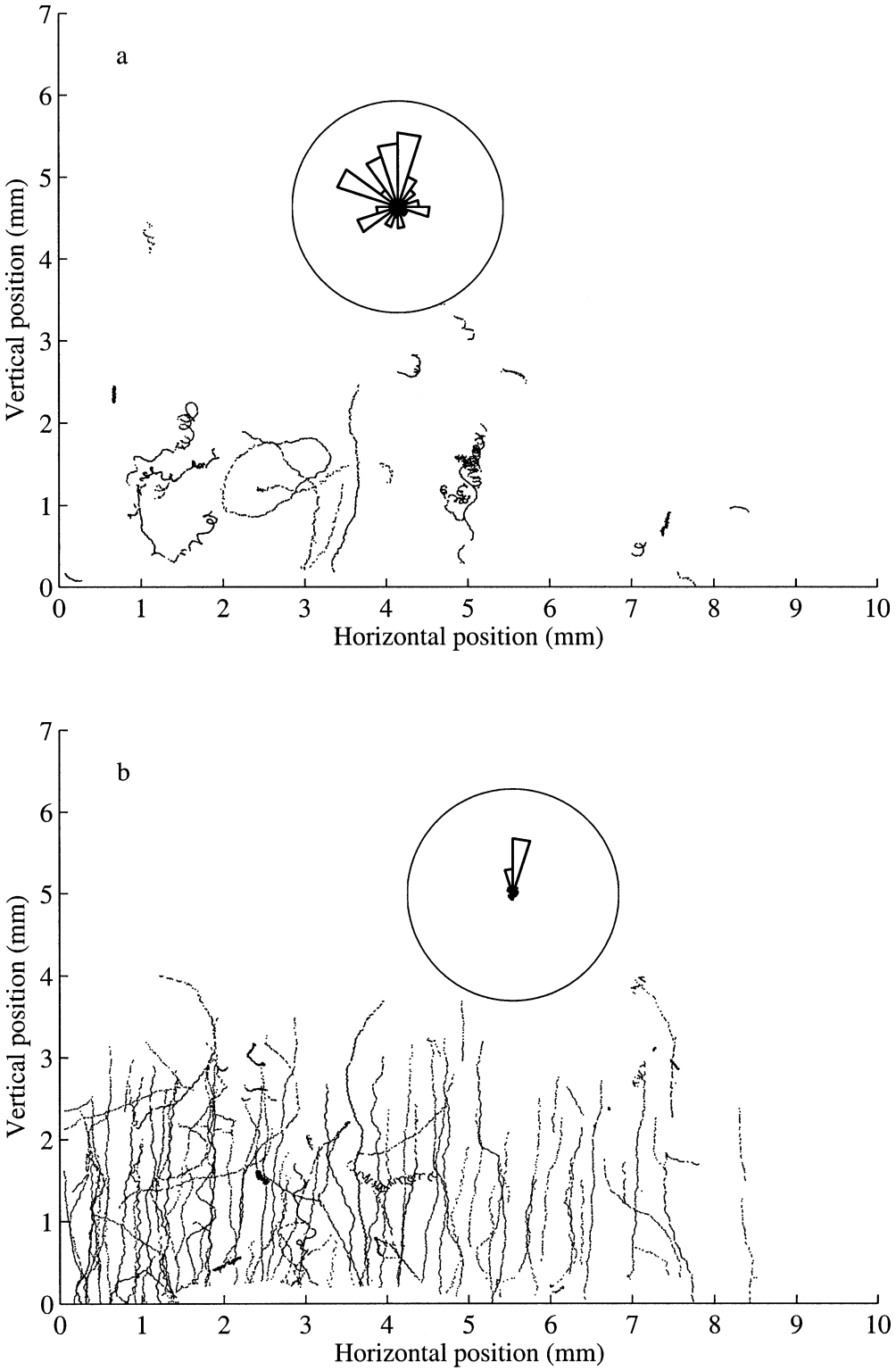

Fig. 2. Changes in cell swimming characteristics shortly after the beginning of the light phase of a 12 : 12 LD diurnal cycle. All trajectories are plotted from 1 min of video footage taken in an experiment with strain CCAP934-1. Footage was taken at (a) D12 and at (b) L2. The rose diagrams represent the direction of all trajectories plotted. Coincident with the onset of the light phase of the diurnal cycle, cells transition from random swimming (mean vertical speed 11 μm s^{-1}) to upward-directed swimming (mean vertical speed 85 μm s^{-1}). Simultaneously, the number of cells at the camera's vertical position increases dramatically. This change in cell distribution represents upward movement by a large fraction of the cell population that had been below the camera's position during the dark phase of the diurnal cycle. This behavior was typical in many experiments.

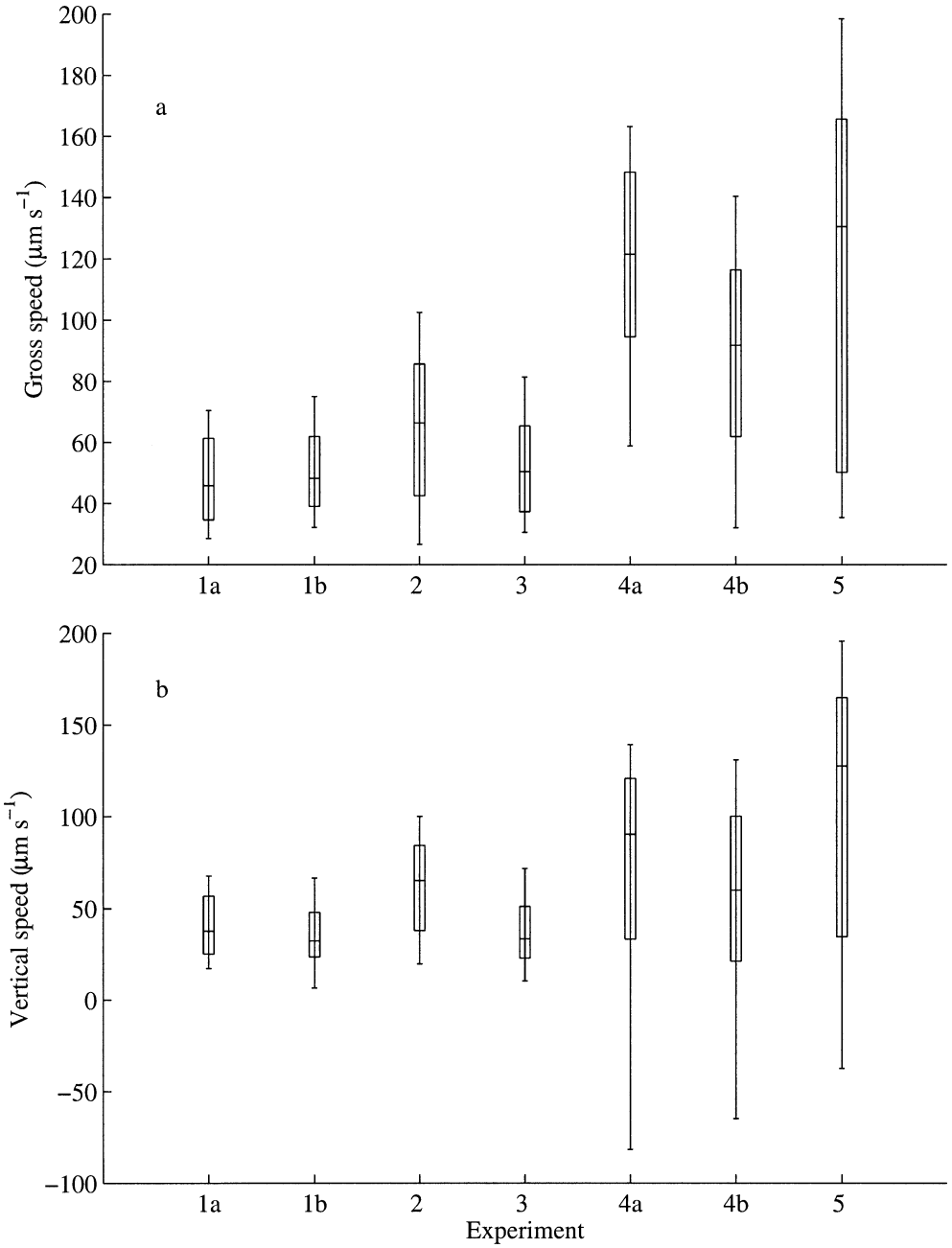

Fig. 3. Variability of swimming characteristics between two *Heterosigma* strains. Shown are the distributions of gross and vertical speeds of strain CCMP452 (Experiments 1a, 1b, 2, and 3) and strain CCAP934-1 (Experiments 4a, 4b, and 5) between L6 and L8 in the diurnal cycle. The numbers of independent trajectories analyzed in each experiment are 108, 186, 59, 127, 46, 63, and 38 respectively. Cells in experiments 1a and 1b were taken from the same culture 2 days apart; cells in experiments 4a and 4b were taken from the same culture 3 days apart. Box plots indicate the 0.1, 0.25, 0.5, 0.75, and 0.9 quantiles within each observation. These results demonstrate both substantial intrastrain variability and strong interstrain differences in swimming characteristics.

A second level of variability appeared to be associated with the diurnal light cycle. *Heterosigma* cells switched from nearly random swimming directions to strongly upwardly directed swimming in the early portion of the light phase (Fig. 2). In the field, this shift in swimming behavior would likely result in decreased mean cell depth and consequently increased light and decreased nutrient availabilities.

A third level of variability in swimming characteristics was associated with differences between *Heterosigma* strains. Previous studies suggest that *Heterosigma* cells found in geographically separate regions differ in physiological characteristics, including salinity and temperature optima (Smayda 1998), urea use (Hosaka 1992), hydrogen peroxide production (Twiner et al. 2001), ability to form resting

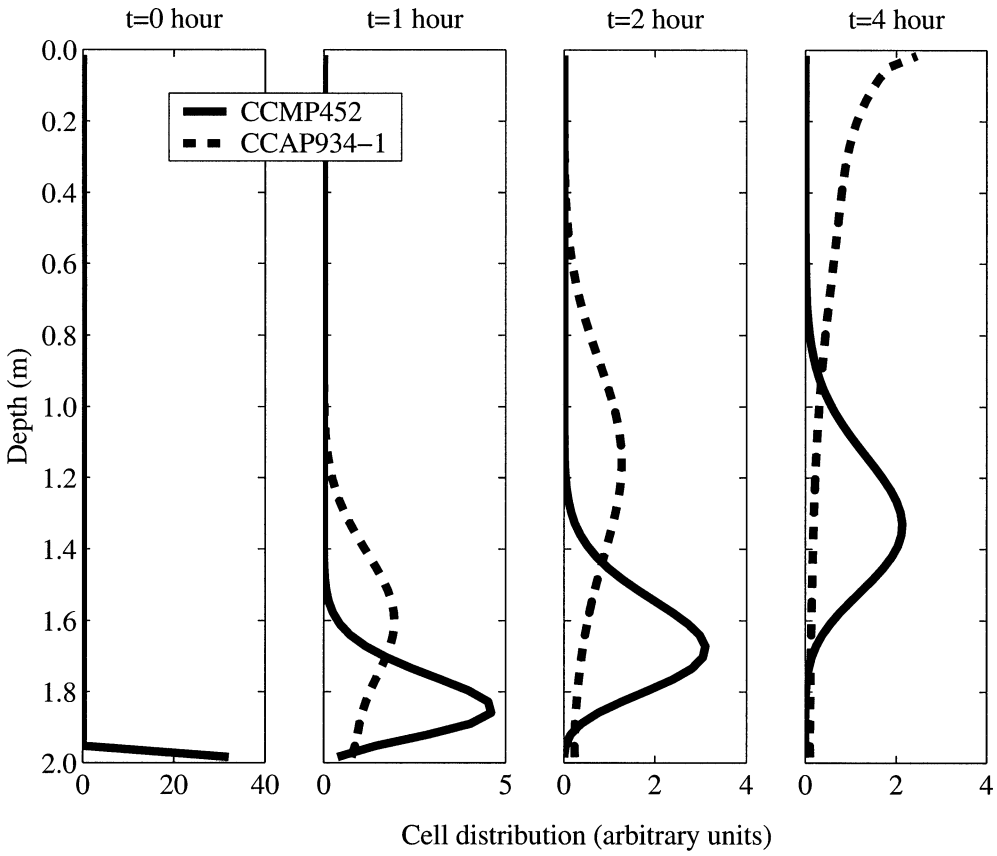

Fig. 4. Projected spatial distributions resulting from observed motility characteristics. Predictions from the spatially explicit model of *Heterosigma* water-column distribution reflect strain-dependent differences in motility between strains CCMP452 and CCAP934-1 (Fig. 3). Assuming an initial concentration of cells at a depth of 2 m, the plots represent a 4-h time series of vertical cell density profiles, with time increasing from left to right.

cells (Han et al. 2002), toxin content and icthyotoxicity (Kahn et al. 2000), and division rate (Q. Tu and R.A. Cattolico, unpubl. data). The present study shows that this pattern of interstrain differences extends to cell-level motility characteristics and, therefore, probably to propensity to form toxic slicks by redistribution of cells within the water column.

Our observations under closely matched conditions showed that strains CCAP934-1 and CCMP452 have substantial intrinsic differences in swimming speeds. Swimming speeds displayed by cells in our assays spanned nearly the entire range between mean cell speeds of 20 μm s^{-1}, found by Throndsen (1973), and 150 μm s^{-1}, found by Bauerfeind et al. (1986). Our results suggest that the relatively large speed differences may reflect distinct genetic identities of the cells used in those two studies. An implication is that strains with even wider ranges of swimming behaviors are likely to exist.

Simulation results based on observed trajectories suggested that motility differences between *Heterosigma* strains are ecologically significant. Automated analysis of cell movement provided data of sufficient quality and quantity to statistically characterize speed distributions and velocity transition rates and to subsequently determine parameters for a spatially explicit model of *Heterosigma* distribution. Our model suggested that the observed behavioral differences would lead to pronounced differences in vertical distributions of the two strains over just a few hours (Fig. 4). Furthermore, although we did not explicitly model ambient flows, the model suggests that toxic slicks formed by the more rapidly upswimming strain (CCAP934-1) may be more resistant to dispersion by wind-driven turbulence and more prone to concentration by interactions between swimming and ambient flows (Kessler 1985; Franks 1997).

In a broader context, theoretical studies of movement in heterogeneous environments indicate that no single movement behavior makes best use of all resource distributions (Grünbaum 1998). Behaviors should be tuned both to spatial patterns of environmental variation and to specific physiological requirements. Observed differences in motility among *Heterosigma* strains prompt fundamental questions about relationships between swimming behavior, physiology, and genetic identity: Do closely related strains display large differences in motility, implying rapid local adaptation of physiological characteristics in novel habitats? Do closely related strains share similar behaviors, implying slow evolution of physiological characteristics and long-distant dispersal of strains between habitats for which they are pre-

adapted? The quantitative motility assays and predictive modeling framework developed in this paper, together with new genetic fingerprinting techniques, may help resolve whether these or some alternative scenarios best explain large-scale patterns in *Heterosigma* distributions.

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