Bistability in oxidative stress response determines the migration behavior of phytoplankton in turbulence

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Turbulence is an important determinant of phytoplankton physiology, often leading to cell stress and damage. Turbulence affects phytoplankton migration both by transporting cells and by triggering switches in migratory behavior, whereby vertically migrating cells can actively invert their direction of migration upon exposure to turbulent cues. However, a mechanistic link between single-cell physiology and vertical migration of phytoplankton in turbulence is currently missing. Here, by combining physiological and behavioral experiments with a mathematical model of stress accumulation and dissipation, we show that the mechanism responsible for the switch in the direction of migration in the marine raphidophyte Heterosigma akashiwo is the integration of reactive oxygen species (ROS) signaling generated by turbulent cues. Within timescales as short as tens of seconds, the emergent downward-migrating subpopulation exhibited a twofold increase in ROS, an indicator of stress, 15% lower photosynthetic efficiency, and 35% lower growth rate over multiple generations compared to the upward-migrating subpopulation. By providing a mechanistic link between the single-cell mechanics of swimming and physiology on the one side and the emergent population-scale migratory response and impact on fitness on the other, the ROS-mediated early warning response we discovered contributes to our understanding of phytoplankton community composition in future ocean conditions.

ROS | motility | photophysiology | harmful-algal-bloom | intermittency

Turbulence is a hallmark of many phytoplankton species, giving them access to light by day and nutrients at depth by night. To migrate through the water column, motile species use gravitaxis, a form of directed motility mediated by a stabilizing torque that biases swimming in or against the direction of gravity. The physiological mechanisms mediating the nexus between turbulence and vertical migration are thus key to understanding how the hydrodynamic environment shapes phytoplankton dynamics in today's oceans as well as in future altered turbulence regimes induced by climatic changes. Vertical migration is a hallmark of many phytoplankton species, giving them access to light by day and nutrients at depth by night. To migrate through the water column, motile species use gravitaxis, a form of directed motility mediated by a stabilizing torque that biases swimming in or against the direction of gravity. The physiological mechanisms mediating the nexus between turbulence and vertical migration are thus key to understanding how the hydrodynamic environment shapes phytoplankton dynamics in today's oceans as well as in future altered turbulence regimes induced by climatic changes. Yet, a fundamental understanding of the impact of turbulence on phytoplankton migratory behavior, physiology, and fitness is lacking.

Significance

Turbulence has long been known to drive phytoplankton fitness and species succession: motile species dominate in calmer environments and non-motile species in turbulent conditions. Yet a mechanistic understanding of the effect of turbulence on phytoplankton migratory behavior and physiology is lacking. By combining a method to generate turbulent cues, quantification of stress accumulation and physiology, and a mathematical model of stress dynamics, we show that motile phytoplankton use their mechanical stability to sense the intensity of turbulent cues and integrate these cues in time via stress signaling to trigger switches in migratory behavior. The stress-mediated warning strategy we discovered provides a paradigm for how phytoplankton cope with turbulence, thereby potentially governing which species will be successful in a changing ocean.
Here, using a combination of millifluidics-based visualization, quantification of stress accumulation, photophysiology, and mathematical modeling, we report that the emergent migratory behavior of the marine raphidophyte *H. akashiwo* exposed to turbulent cues is determined by the integration of reactive oxygen species (ROS) signaling. We used time-lapse imaging to track the migration of individual cells of *H. akashiwo* in a small (12 mm × 4 mm × 1.6 mm) rotating chamber (16) that can be rotated around a horizontal axis by a computer-controlled motor with any user-defined time series of the rotation angle. *H. akashiwo* is a ubiquitous coastal species (21) known for its allelopathic effects and toxic blooms (22) and frequently used as a model system in studies of vertical migration (23, 24). We performed experiments for different rotation time series, as a model system to determine the effect of the magnitude and intermittency of small-scale turbulent eddies (25). Ocean turbulence is often intermittent or patchy and its magnitude highly variable, with turbulent kinetic energy values ranging from $ε = 10^{-10}$ to $10^{-5}$ W · kg$^{-1}$ (5, 8), which correspond to Kolmogorov timescales $τ_K = 100$ to 0.3 s. Our experimental system models intermittent turbulence as a sequence of reorientations of the chamber of magnitude $π$, each taking a time $τ_R$ separated in time by a resting time $τ_W$ during which the chamber is kept still (Fig. 1 A and B). We hypothesized that the interplay between the rotation rate, $Ω = π/τ_K$, and the time available for recovery, $τ_W$, would regulate the emergence of the downward-migrating subpopulation from an initially upward-migrating population.

### Results

**Mechanical Stability in Phytoplankton Modulates Migratory Behavior in Response to Turbulent Cues.** The change in migratory behavior for a monocolonal population of *H. akashiwo* (CCMP452) occurred within the first 10 overturning events (Fig. 1C), corresponding to only tens of seconds at the highest rotation rate used ($Ω = 1$ rad · s$^{-1}$, equivalent to a turbulence intensity $ε = 10^{-7}$ W · kg$^{-1}$; *Materials and Methods*). Exposure to additional reorientations had no further effect on migration, where the percentage of cells swimming upward changed from 77.5% before treatment (control) to 54.5% after 300 reorientations, with two almost equally abundant subpopulations: one continuously swimming upward and the other that switched to downward migration. The saturation of the behavioral response is also shown by the stable value of the upward bias index $r$ over 10 ($r = 0.17 ± 0.11$) to 300 ($r = 0.09 ± 0.06$) reorientations (ANOVA, $F_{3,11} = 0.26$, $P = 0.85$). The upward bias index, $r = (f_↓ − f_↑)/(f_↓ + f_↑)$, measures the relative proportions of upward-migrating ($f_↑$) and downward-migrating ($f_↓$) cells, and is quantified once flipping ceases and the population has had time to reach a stationary distribution inside the chamber (*Materials and Methods*). Varying the rotation rate $Ω$ (0.08 rad · s$^{-1}$ ≤ $Ω$ ≤ 1 rad · s$^{-1}$) for a fixed number of 10 reorientations with no resting time ($τ_W = 0$ s) changed the proportion of downward-migrating cells (Fig. 1D). At the fastest rotation rate tested ($Ω = 1$ rad · s$^{-1}$), the highest concentration of downward-migrating cells was observed ($r = 0.07 ± 0.10$), while at the slowest rotation rate tested ($Ω = 0.08$ rad · s$^{-1}$), the upward bias index ($r = 0.44 ± 0.05$) was not different from the nonrotating control experiment ($r = 0.49 ± 0.05$; $τ_R = 0.93$).

![Figure 1](https://static.annals.org/annals/415x415/0064/353283186739.png)

**Fig. 1.** Rotation rate and resting time between reorientations relative to gravity determine the migratory response of *H. akashiwo* to turbulent cues. (A) Time series of the orientation, $θ(t)$, of a passive sphere relative to the vertical in a three-dimensional (3D) isotropic turbulent flow, obtained from a direct numerical simulation. The signal reveals the characteristic effects of the microscale turbulent eddies, that is, periods of time where the sphere abruptly changes its orientation by up to an angle $π$ (modeled in this work by a rotation time), alternating with regions in which the orientation is more constant over time (modeled in this work by a resting time). (B) Experiments are based on a simplified characterization of intermittent turbulence in terms of two parameters: the rotation time, $τ_R$, over which the experimental chamber completes a reorientation of amplitude $π$ (one “flip”). (C) The upward bias index $r$ (*Materials and Methods*), as a function of the number of flips $N$, decreases from 0.52 to 0.17 over only 30 s of flipping ($τ_W = 0$ s, time elapsed $t = N τ_R$). (D) The upward bias as a function of the rotation time, $τ_R$, for a constant resting time, $τ_W = 0$ s (blue curve). (E) Relative reorientations smaller $τ_R$, which correspond to stronger turbulence, $ε$, cause a larger population split when evaluated over the same number (10) of flips. Our model of cell stability (dashed line, *Materials and Methods*) correctly predicts the upward bias (i.e., the fraction of downward-migrating cells that emerge for each treatment). (F) Relative distribution of the cells’ mechanical stability, expressed as the stability parameter $A$. The red curve corresponds to a population of cells before flipping. Other colors correspond to cells from the top (t) and bottom (b) subpopulations after $n = 30, 100$, and 300 flips ($τ_R = 3$ s; $τ_W = 15$ s). (F) The upward bias as a function of the resting time, $τ_W$, for a constant rotation time, $τ_R = 1$ s (blue curve). Shorter resting times (smaller $τ_W$) induced a larger population split when evaluated over the same number (100) of flips. In C, D, and F, circles and shaded regions denote mean ± SD of four replicate experiments, and corresponding controls (measured over the same time period, but without flipping) are shown in red.
$P = 0.38$ (Fig. 1D). These results show that the stronger disturbances associated with faster reorientations triggered a stronger response and more cells actively changed their direction of migration relative to gravity.

The initial stability of a cell regulates how the cell is affected by reorientations. The mechanical stability of a cell, which is typically produced by an asymmetry in the cell shape or a non-uniform distribution of cell density (16, 19), allows the cell to maintain its orientation with respect to gravity. It can be measured by the stability parameter $A = (2B)^{-1}$, where $B$ is the characteristic time for the cell to rotate back to its vertical equilibrium orientation once perturbed from it. An analysis of the stability of a cell in an eddy with rotation rate $\Omega$ predicts that if $|\Omega| < |A|$, the cell will swim with a constant angle relative to gravity (of angle $\theta_0 = \arcsin(\Omega A^{-1})$) for an upward-migrating cell or $\theta_0 = \pi - \arcsin(\Omega A^{-1})$ for a downward-migrating cell, whereas if $|\Omega| > |A|$, the cell will tumble in a periodic orbit with period $T_B = 2\pi (\Omega - A^{-2})^{-1/2}$ (Materials and Methods). From this analysis, we can predict the fraction of cells that will tumble under the effect of reorientations and therefore switch their direction of migration from upward to downward as a function of the rotation rate $\Omega$ and the initial distribution of mechanical stabilities within a population (Fig. 1D and E). We measured experimentally the initial distribution of mechanical stabilities for CCMP452 at the single-cell level (Fig. 1E and Materials and Methods), which is characteristic of stress responses in eukaryotes (27), including turbulence (3). Fast reorientations ($\Omega \approx 1$ rad · s$^{-1}$) cause an upward-migrating cell to tumble (SI Appendix, Figs. S1C and S2A) and can thus trigger the emergence of downward migration.

The Migratory Switch Is Mediated by a Bistability in the Stress Response. The migratory behavior was further affected by the resting time $\tau_W$, a measure of the signal’s intermittency (Fig. 1B). This was revealed by experiments with fast reorientations ($\Omega = 3.14$ rad · s$^{-1}$), which induce a population split in CCMP452 in the absence of resting time (Fig. 1D and Materials and Methods). When the resting time was varied in the range $\tau_W = 0$ s to 100 s, we found the population split to occur for values of $\tau_W$ below a threshold of 40 s (Fig. 1F), a value in line with the typical interval between reorientations experienced by CCMP452 cells in strong turbulence (SI Appendix, Fig. S2 B and C). A threshold response is characteristic of stress responses in eukaryotes (27), including dinoflagellates (28) and diatoms (29), and led us to hypothesize that a progressive intracellular accumulation of oxidative compounds resulting from the reorientations is the physiological mechanism underlying the change in migration direction.

To test this hypothesis, we performed experiments with cells stained using a marker (CM-H$_2$DCFDA) that forms a fluorescent compound in the presence of ROS, which are signaling molecules that mediate the perception of diverse environmental stress conditions (30). Intracellular ROS accumulation was quantified by flow cytometry (SI Appendix, Fig. S3 and Materials and Methods). For these experiments, we used continuous rotation on a roller device ($\tau_W = 0$ s, Materials and Methods) with a sample volume (2 mL) larger than the millifluidic chamber (75 $\mu$L) and thus more suitable for analysis by flow cytometry. The migratory response was found to be independent of whether rotation was continuously in one direction (i.e., rolling) or alternating between clockwise and counterclockwise (i.e., flipping) (SI Appendix, Fig. S4). After just 1 min of rolling ($\Omega = 1$ rad · s$^{-1}$), downward-migrating cells were found to have accumulated twofold more ROS compared to upward-migrating cells, and the difference in the accumulated stress between the two subpopulations was consistently detected also after 5 min and 20 min of rolling (two-sample t test, $P = 0.04$; $t_6 = 2.5$) (Fig. 2A). A similar stress response was detected in the other strain of *H. akashiwo*, CCMP3374, when exposed to the same treatment (SI Appendix, Fig. S5).

Additionally, we characterized the differences in the ROS accumulation between upward- and downward-migrating subpopulations as a function of the rotation rate of the rolling device. We found that for rotation rates of $\Omega < 1$ rad · s$^{-1}$, faster reorientations caused a progressively higher ROS accumulation in the cells harvested at the top (Fig. 2B). For the range of rotation rates $\Omega > 1$ rad · s$^{-1}$, the subpopulation of upward-swimming cells contains both cells with high mechanical stability ($A > \Omega$) and part of the cells with low mechanical stability ($A < \Omega$) that accumulated less ROS beyond the threshold and therefore switched in migratory direction. For rotation rates such that $\Omega \geq 1$ rad · s$^{-1}$, the cells with low mechanical stability fully undertake the behavioral switch and accumulate progressively higher stress compared to upward-migrating cells (Fig. 2B). Taken together, these observations indicate that a bistability in oxidative stress response is associated with the split in migratory behavior of phytoplankton cells experiencing turbulent cues.

To further support the finding that ROS affects migration behavior, we observed the migration of cells exposed to different exogenous stressors known to cause ROS accumulation. In a first set of experiments, we added hydrogen peroxide (H$_2$O$_2$) to the medium. H$_2$O$_2$ diffuses across the cell membrane, mimicking the physiological intracellular accumulation of ROS caused by the reorientations. We determined the ROS levels induced by H$_2$O$_2$ for both *H. akashiwo* strains, and we compared those levels with the ROS levels generated by exposure to 20 min of rolling. The results show that the stress response driven by ROS generation during a downward-migrating subpopulation after exposure to H$_2$O$_2$ (at a concentration $C = 33$ $\mu$M) quantitatively match the ROS accumulation observed upon rolling, where we observed a bistable stress response (Fig. 2C). Exposure to exogenous H$_2$O$_2$ induced the population split in migratory behavior above a threshold concentration of 15 $\mu$L H$_2$O$_2$ (Fig. 2D), with a threshold-like behavioral response akin to that caused by fast reorientations (Fig. 1F). Most notably, the ROS levels of CCMP452 cells increased sharply upon increasing the concentration of exogenous H$_2$O$_2$ from 10 to 33 $\mu$M (SI Appendix, Fig. S6), a concentration range that coincides with the H$_2$O$_2$ concentration causing the population split (Fig. 2C and D). In a second set of experiments, we exposed CCMP452 cells to light for 30 min at intensities known to lead to ROS accumulation (31) and characteristic of ocean surface waters (32). We observed the emergence of a downward-migrating subpopulation for cells exposed to near-UV-A light (380 to 400 nm) at intensities greater than 80 $\mu$mol photons m$^{-2}$ · s$^{-1}$ or to full-spectrum light (320 to 800 nm) at intensities greater than 650 $\mu$mol photons m$^{-2}$ · s$^{-1}$ (Fig. 2E and SI Appendix, Fig. S7A). Finally, we repeated the overturning experiments for cells pretreated with the ROS scavenger potassium iodide (KI; Materials and Methods) at an exogenous concentration of 100 $\mu$L (Fig. 2F and SI Appendix, Table S1). No emergence of a downward-migrating subpopulation was observed in this case. To summarize, this suite of experiments, in which we exposed cells to ROS scavengers and...
inducers, showed that the behavioral response could be blocked by adding a ROS scavenger (KI) to the medium and that the behavioral response could be triggered by applying external ROS (in the form of H₂O₂ and high irradiance) that activated the response downstream in the signaling cascade. Taken together, these experiments demonstrate the causal link between intracellular stress accumulation mediated by ROS and the behavioral switch in migration direction.

To determine the dependence of the stress threshold above which downward-swimming emerges on cell physiology, we conducted rolling experiments with cell cultures under different conditions. Because ROS production patterns in raphidophytes vary across different growth phases (33–35), we investigated the dependence of the stress threshold by performing rolling experiments using a population at 72 h after inoculation in the fresh medium (early exponential phase) to compare to the standard treatment between 96 h and 120 h after inoculation (midexponential phase) (16). While the stress accumulation in *H. akashiwo* after exposure to rolling is regulated by the growth phase, we found that the value of the stress threshold relative to the baseline stress level is conserved across different growth phases (SI Appendix, Fig. S8). Before rolling, cells in early exponential phase presented a higher baseline ROS production rate compared to cells in mid-exponential phase. After rolling, the accumulated stress and the value of the threshold were higher in early exponential phase (SI Appendix, Fig. S8A). These results are the consequence of a less-efficient scavenging machinery and an increased endogenous ROS production, in line with the literature on ROS production patterns in raphidophytes (33–35). However, the increase in the ROS after rolling, s, relative to the baseline stress level, s₀, is comparable between the two growth phases for both upward- and downward-migrating subpopulations (SI Appendix, Fig. S8 A, Inset). This analysis allowed us to identify the threshold h = s₀/h₀ = 2.3 ± 0.6 for the emergence of the migratory switch, because below this value of relative stress, cells still perform upward swimming after exposure to rolling.

**Phytoplankton Navigation under Turbulence Is Regulated by Stress Accumulation–Dissipation Dynamics.** To predict the emergence of the behavioral switch in the migratory response, we devised a mathematical model of stress dynamics in cells exposed to turbulence. In the model, the cell’s mechanical stability prevents overturning by weaker eddies (Fig. 1D and SI Appendix, Figs. S1C and S2A), thus creating resting times between periods during which the cell is overturned (Fig. 3A and SI Appendix, Fig. S2 B and C). Accordingly, a cell accumulates ROS whenever it is tumbled by an eddy and dissipates stress by means of its intracellular antioxidant capacity (34, 36), with a characteristic dissipation timescale τₘ (Materials and Methods and Eqs. 3 and 4 and SI Appendix). During a tumbling event, a cell is rapidly reoriented relatively to gravity, and it experiences an impulsive force of typical magnitude Fₘ ~ 1 pN (SI Appendix). We assumed that, when tumbling, intracellular stress is generated in the cell in the form of a nearly instantaneous release (i.e., a spike) of ROS of amplitude Δs (a free parameter of our model, Materials and
Methods. We quantified stress dissipation dynamics and the timescale $\tau_{S}$ experimentally by observing the reduction of ROS over time for cells after exposure to continuous rolling for 5 min ($\Omega = 1 \text{ rad} \cdot \text{s}^{-1}$). These experiments showed that stress decays exponentially over time with a timescale $\tau_{S} = 87 \pm 32$ s (Fig. 3B).

Using this model of stress accumulation–dissipation dynamics, we can predict the time series of stress accumulation for individual cells exposed to flipping ($\Omega = 3.14 \text{ rad} \cdot \text{s}^{-1}$) for the same range of rolling times $\tau_{W}$ (Fig. 1F) or to rolling for the same range of rotation rates $\Omega$ (Fig. 2B) studied experimentally. This allowed us to compute the stress accumulated by cells during reorientations as a function of the resting time (Fig. 3C) or the tumbling period $T_{B}$ (Fig. 3D) and to compare the model predictions with the experimentally measured ROS concentrations at which downward migration emerged (Fig. 3A and B). We used the ROS accumulation as a function of the rotation rate to constrain the value of the parameter $\Delta s/\sigma_{0} = 0.40 \pm 0.07$ (Fig. 3D), where the baseline level of stress $s_{0}$ is quantified by observing the ROS production in the cells before rolling. By using the stress threshold $h = 2.3$ (obtained through rolling experiments; SI Appendix, Fig. S8A, Inset) above which a cell would switch its migratory strategy, we find that the theoretical prediction for the maximum resting time in the flipping experiments (or equivalently for the maximum tumbling period in the rolling experiments; see SI Appendix) associated with the emergence of downward-migrating cells ($\tau_{W} = 32 \pm 13$ s, Fig. 3D) quantitatively matches the value of $\tau_{W}$ observed experimentally ($\tau_{W} = 40$ s, Fig. 1F).

In order to model stress accumulation while capturing the effect of intrinsic variability in the mechanical stability and in the ROS scavenging efficiency, we developed an analytical model of stress accumulation during navigation under fluid rotations that accounts for heterogeneity in these two phenotypic traits (SI Appendix, Fig. S9 and SI Appendix). For the subpopulation performing upward swimming even after exposure to strong turbulence or high concentrations of $\text{H}_{2}\text{O}_{2}$, we implemented a smaller dissipation timescale. This follows from the observation of a bistable stress response upon induction with $\text{H}_{2}\text{O}_{2}$ without turbulence, in which the stability parameter does not play any role (Fig. 2C) (SI Appendix). Using the same fitting parameter $\Delta s/\sigma_{0} = 0.40$ estimated through the accumulated stress at the population scale (Fig. 3D) and the experimental distribution of the stability parameter (Fig. 1E), we found good agreement between the stress distributions for the two subpopulations in the experiments and in the stochastic model capturing single-cell variability (SI Appendix, Fig. S10).

Our model further predicts the temporal dynamics for the saturation of the stress response in which 98% of the total stress is accumulated within 5 min of rolling (at a rotation rate $\Omega = 1 \text{ rad} \cdot \text{s}^{-1}$), similarly to the stress saturation dynamics observed in the experiments (Fig. 2A). The timescale for saturation of the stress response is insensitive to changes in $\Delta s$, which is a multiplicative factor in the model. Conversely, changing the ratio...
τw/τs would change the number of rotations N, and therefore the time, needed to reach saturation of the stress response (SI Appendix, Eq. S6). The model reveals a criterion for the emergence of downward migration: when τw/τs < 1, the stress increases hyperbolically as a function of the ratio τw/τs (SI Appendix, Eq. S8), because the ROS scavenging machinery of a cell is too slow in dissipating stress relative to the rate at which stress accumulates owing to the short interval between reorientations by turbulence, and the accumulated stress induces the switch in migratory behavior.

Brief Exposure to Turbulence Has Long-Lasting Effects on Phytoplankton Fitness. The accumulation of ROS in response to turbulent cues directly affected cell physiology for multiple cell divisions after cessation of the cue. Single-cell photo-physiological measurements using pulse-amplitude–modulated chlorophyll fluorometry (PAM; see Materials and Methods) showed that the downward-migrating cells emerging after 5 min of continuous rolling (Ω = 1 rad · s⁻¹) had 15% lower photosynthetic quantum yields (Fv/Fm) compared to upward-migrating cells (Fig. 4A and SI Appendix, Table S2). This reduction may stem directly from endogenous ROS, which can reduce photosynthetic quantum yields (37) via the general suppression of PSII D1 protein synthesis and repair (38, 39), activation of nonphotochemical pathways, or photoactivation of PSII reaction centers (40). This reduction in photosynthetic performance in H. akashiwo is acute when compared to the reductions of typically less than 10% caused by high light exposure in diatoms (31, 37). We further found evidence for longer-term physiological damage induced by 5 min of continuous rolling corresponding to strong turbulence (Ω = 1 rad · s⁻¹), with the downward-migrating subpopulation exhibiting a 35% lower growth rate over 4 d (g1 = 0.47 ± 0.03 d⁻¹) than the upward-migrating subpopulation (g2 = 0.74 ± 0.02 d⁻¹) (Fig. 4B), with the growth of the latter comparable to the growth rate obtained for control cells (g = 0.69 ± 0.06 d⁻¹) (SI Appendix, Fig. S11). We also estimated the instantaneous growth rate for the two subpopulations between 72 h and 96 h using log(n96/n72), where n72 and n96 are the cell concentrations at 72 h and 96 h after exposure to rolling. Within the time period 72 h to 96 h after exposure to turbulent cues, we observed a comparable growth rate in the two subpopulations (g1 = 0.83 ± 0.39 d⁻¹ for the upward-migrating subpopulation and g2 = 1.01 ± 0.21 d⁻¹ for the downward-migrating subpopulation). The growth reduction over multiple generations (approximately three) indicates that the reorientation-induced ROS accumulation has systemic consequences for H. akashiwo and suggests the potential presence of a transgenerational stress memory, akin to epigenetic effects observed in plants (41). This result is in contrast with stress propagation in Escherichia coli and yeast (27), where mother cells retain the oxidized aggregated protein, leaving daughter cells cleared of damaged proteins (36).

Discussion

The evidence we have presented for the role of stress in vertical migration provides a view of the ecological implications of the active response of phytoplankton to turbulence. Our results demonstrate that the overturning of cells, a fundamental yet to-date-unappreciated mechanical cue due to turbulence in the ocean, can trigger behavioral and physiological responses over timescales spanning tens of seconds to multiple generations. The good agreement between our model and observations suggests that motile phytoplankton use mechanical stability to sense the intensity of turbulent cues (Fig. 5A) and integrate these cues in time via ROS signaling (Fig. 5B): when ROS accumulates beyond a threshold, it triggers the switch in migratory behavior (Fig. 5 C and D) underpinned by a rapid modulation of the cellular morphology, which, together with the internal distribution of organelles, determines the sign of the stability parameter and thus the direction of migration (16). This ROS-mediated early-warning strategy may be advantageous owing to the heterogeneity in mechanical stability within monoclonal populations (Fig. 1E). The reorientations used in our experiments, corresponding to moderate to strong levels of turbulence (ε = 10⁻⁶–10⁻⁶ W · kg⁻¹, Materials and Methods), did not inhibit motility (SI Appendix, Fig. S124). By responding to the ROS-mediated early warning upon first encountering a region of turbulence, cells with weaker mechanical stability will avoid swimming into the “eye of the storm” where they could get trapped (42), damaged, or lose motility (8–10) (Fig. 5D). Heterogeneity is also seen in the antioxidant capacity, potentially the product of a tradeoff in which cells with low antioxidant capacity are more sensitive to turbulent cues via ROS signaling but at the cost of weaker protection against other environmental cues eliciting oxidative stress. The cells belonging to the upward-migrating subpopulation might have a potentiated scavenging machinery (represented in our model by a shorter timescale τs) and/or a decreased baseline stress rate. The increased scavenging machinery in part of the population could originate as natural variability (43) or could be linked to a different phase of the cell cycle, which in a population in exponential phase is dictated by the time from the last division.

The emergence of downward migration upon exposure to well-known ROS inducers (H₂O₂, UV-A radiation, and high irradiance), in the absence of turbulent cues, shows that ROS accumulation is the cause of the migratory response but at the same time begs the question of how specificity of ROS signaling (30, 36) toward turbulence might be achieved in H. akashiwo. In fact, specificity of response may not be necessary if avoidance is a universally appropriate response to accumulation of ROS. Elevated levels of exogenous H₂O₂ (100 μM), UV-A radiation (300 μmol photons m⁻² · s⁻¹), and full-spectrum light (650 μmol photons m⁻² · s⁻¹) negatively impacted motility (SI Appendix, Figs. S7B and S12 B and C). Exposure to excessively high levels of irradiance can cause photoinhibition (31, 37), and downward migration would be a relevant response in the upper layers of the ocean, can trigger behavioral and physiological responses over timescales spanning tens of seconds to multiple generations. The good agreement between our model and observations suggests that motile phytoplankton use mechanical stability to sense the intensity of turbulent cues (Fig. 5A) and integrate these cues in time via ROS signaling (Fig. 5B): when ROS accumulates beyond a threshold, it triggers the switch in migratory behavior (Fig. 5 C and D) underpinned by a rapid modulation of the cellular morphology, which, together with the internal distribution of organelles, determines the sign of the stability parameter and thus the direction of migration (16). This ROS-mediated early-warning strategy may be advantageous owing to the heterogeneity in mechanical stability within monoclonal populations (Fig. 1E). The reorientations used in our experiments, corresponding to moderate to strong levels of turbulence (ε = 10⁻⁶–10⁻⁶ W · kg⁻¹, Materials and Methods), did not inhibit motility (SI Appendix, Fig. S124). By responding to the ROS-mediated early warning upon first encountering a region of turbulence, cells with weaker mechanical stability will avoid swimming into the “eye of the storm” where they could get trapped (42), damaged, or lose motility (8–10) (Fig. 5D). Heterogeneity is also seen in the antioxidant capacity, potentially the product of a tradeoff in which cells with low antioxidant capacity are more sensitive to turbulent cues via ROS signaling but at the cost of weaker protection against other environmental cues eliciting oxidative stress. The cells belonging to the upward-migrating subpopulation might have a potentiated scavenging machinery (represented in our model by a shorter timescale τs) and/or a decreased baseline stress rate. The increased scavenging machinery in part of the population could originate as natural variability (43) or could be linked to a different phase of the cell cycle, which in a population in exponential phase is dictated by the time from the last division.

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would result in a considerable difference in cell number after strategies, exemplified in our work through the mechanism of a mechanics and by ROS bistability. Deepening our understanding species, mediated by high phenotypic variability in swimming the stochasticity of turbulence (46). One example of this is the intensities and more energetic local storm events (20, 45). We directly through their response to decreased mean turbulence phytoplankton physiology and metabolism directly but also in-

To study the long-term impact of turbulent cues on growth rates, both the top and bottom subpopulations of CCMP452 were harvested (300 μL each) from a 2-mL cell culture vial that had been exposed to turbulent cues in the form of rolling for 5 min (SI Appendix, Generation of turbulent cues, in turbulence

(ii) Rolling). Prior to harvesting the subpopulations, the cell culture was allowed to attain the postturbulence stationary vertical distribution driven by their migration behavior (SI Appendix, Upward bias index). The harvested cells were introduced into the supernatant of the initial cell culture (from which the 2-mL suspension had been taken) in a 1:12 ratio and allowed to grow over 96 h (Fig. 4B). Cells were counted every 24 h by flow cytometry (CytoFLEX S, Beckman Coulter), and in parallel, their motility was checked using phase-contrast microscopy (Nikon Ti-E, Nikon, Japan). The cell concentration of each of the subpopulations was fitted over the 96-h period using the least squares method to obtain exponential growth curves (Wolfram Mathematica version 11.3, Champaign, IL).

Quantification of Endogenous Stress Production. To quantify the accumulation of ROS, cells exposed to turbulence or static conditions were incubated under dark conditions for 30 min in 10 μM CM-H2DCFDA (Ex/Em: 492 to 495/517 to 527 nm, Thermo Fisher Scientific, diluted in f/2). CM-H2DCFDA is a chloromethyl derivative of H2DCFDA that enables the detection of low concentrations of ROS. The marker is a suitable indicator for long-term quantification of ROS as it passively diffuses into live cells and forms a highly stable fluorescent adduct when oxidized. After the 30-min incubation period, fluorescence intensities of single cells were quantified using a flow cytometer (CytoFLEX S, Beckman Coulter), in the FITC-A channel (Ex/Em: 488/520 nm). Single-cell oxidative stress levels are represented as (relative) fluorescence units. To obtain the stress levels, we subtracted the FITC-A values for the control in the absence of CM-H2DCFDA staining from the FITC-A values for the stained cells. This additional step ensured the subtraction from the stress measurement of the autofluorescence of H. akashiwo over the green portion of the spectrum, characteristic of raphidophytes (51). Fluorescence levels were obtained for the turbulence-exposed population for the top and bottom subpopulations and for the control population (no turbulence) with and without the addition of CM-H2DCFDA.

PAM Chlorophyll Fluorometry Experiments. PAM was used to quantify the photosynthetic performance of cells after exposure to turbulent cues. Microscopic multicolor-variable chlorophyll fluorescence imaging (IMAG-RGB; Walz, Effeltrich, Germany) was employed experimentally: 1) a continuous solid body rotation (i.e., rolling) of the organism, which here exemplifies the characteristic reorientation rate of the cell, and 2) multiple, fast (≤ 1 Hz) changes in cell orientation by flipping the organism. By taking the partial sum in the summation in Eq. 5, we can solve the system with the Laplace transform, which gives the stress level as a function of time

\[ S(t) = \sum \delta(t - t_i) \left( e^{-\delta t_i} \sigma_i + \eta_i \right), \]

where the Dirac delta function \( \delta(t - t_i) \) records the stress spikes \( \sigma_i \) (assumed to have the same value \( \delta \sigma_i \)) occurring at times \( t_i \) for a given swimming trajectory and \( \eta_i \) is the baseline stress rate. Eq. 3 can be solved by performing the Laplace transform, which gives the stress level as a function of time

\[ S(t) = \sum \delta(t - t_i) \left( e^{-\delta t_i} \sigma_i + \eta_i \right), \]

where the sequence of times \( t_i \) at which stress is generated is \( S = (T_{0}, T_{1}, \ldots, T_{N_{T}}) \). \( T_0 \) is the period of the orbit for the tumbling cells given in Eq. 2, which depends on the stability parameter \( A \) and on the rotation rate \( \Omega \). In SI Appendix, Supplementary Text, we further model the stress dynamics for cells exposed to turbulent cues for the two paradigmatic cases that we employed experimentally: 1) a continuous solid body rotation (i.e., rolling) with rotation rate \( \Omega = \omega_{A} \) (no resting phases, \( \tau = \omega = 0 \), and 2) multiple, fast (≤ 1 Hz) changes in cell orientation by flipping the organism. To take into account population heterogeneity in the mechanical stability and stress dissipation parameters observed experimentally, we also derived a stochastic model of stress dynamics under rolling and flipping (SI Appendix).

Data Availability. All study data are included in the article and/or SI Appendix.

Acknowledgments. We thank G. Boffetta and M. Cencini for sharing the direct numerical simulations data and Russell Naisbit for help with the editing of this manuscript. This work was supported by Gordon and Betty Moore Foundation Marine Microbial Initiative Investigator Award GBMF3783 (to R.S.), Gordon and Betty Moore Symbiosis in Aquatic Systems

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Investigator Award GBMF9197 (to R.S.; https://doi.org/10.37807/GBMF9197), and Simons Foundation 542935 (to R.S.) as part of the Principles of Microbial Ecosystems Collaborative (PriME), Swiss National Science Foundation Grant 315230_176189 (to R.S.), the Israeli Science Foundation grant 71223 (to A.V.), funding from the Science for Life Laboratory (to L.B.), the Independent Research Fund Denmark (DFF-1323-00747/DFF-1325-00069) (to L.B.), the Swedish Research Council (2019-04401) (to L.B.), the Human Frontier Science Program Cross Disciplinary Fellowship LTF000993/2014-C (to A.S.), and the ATTRACT Investigator Grant A17/M5/1175282/MBRACE of the Luxembourg National Research Fund (to A.S.).

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https://doi.org/10.1073/pnas.2005944118

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