MICROSCOPY

Plankton in a hamster wheel

Plankton regularly travel vast distances up and down in the ocean. A water-filled hamster wheel with glass windows now enables detailed microscopic lab observations of individual aquatic microorganisms during their vertical migrations.

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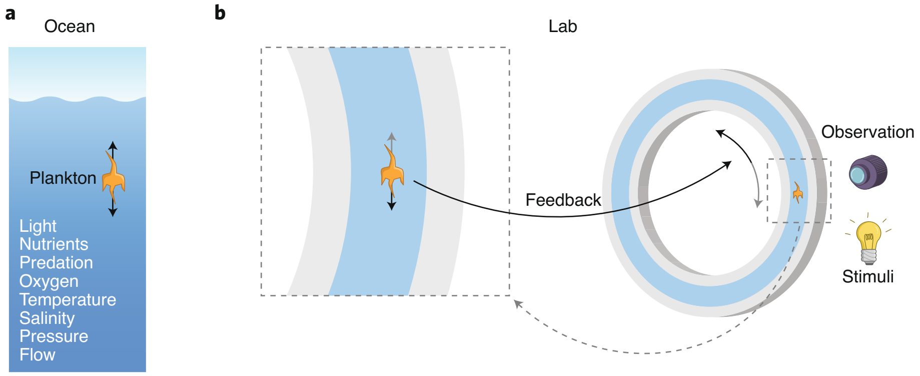

Fig. 1 | Vertical migration in the 'hydrodynamic treadmill'. **a**, Plankton migrate daily over vast vertical distances in the ocean, encountering depth-dependent changes in a wide range of environmental parameters. **b**, With the 'hydrodynamic treadmill' constructed by Krishnamurthy et al.[7], migration of individual microorganisms over large vertical distances can now be recreated in the lab. The vertical movement of the organism is automatically compensated by counter-rotation of the circular sample chamber, keeping the organism centered at a microscope's focus. Stimuli such as light patterns can be applied in a virtual depth-dependent fashion to present the organism with virtual environments.

Aquatic species ranging from microorganisms to fish migrate vertically in the water column in daily cycles[1] (Fig. 1a). Many photosynthetic plankton species move to well-lit near-surface layers during the day and deeper nutrient-rich layers during the night, while many zooplankton species follow the inverse pattern, likely to avoid predation by larger organisms that hunt by sight[2].

This so-called diel vertical migration influences global biogeochemical cycles by contributing to the 'biological pump' — a suite of biological processes that transport carbon from the upper ocean down to its eventual sequestration as sediment on the ocean floor[3]. Deciphering the behavioral mechanisms underlying the migration has been hampered by a lack of tools that allow detailed observations of individual organisms during the migration.

Recreating these migrations in the lab is challenging: researchers have observed[4] plankton populations migrating in tanks of water as tall as 10 m, but even tiny organisms such as photosynthetic dinoflagellates that measure only 200 µm across travel tens of meters vertically in the ocean[5]. Most experiments in tanks focus on population-level observations and enable inspection of individual behavior only by withdrawing liquid samples at defined positions for external inspection or by placing a camera system at a fixed position. Traveling microscopes have occasionally been used to facilitate individual observations at arbitrary positions and to even follow individuals during vertical movement[6].

Krishnamurthy et al.[7] turned this approach on its head by moving not the microscope but the sample chamber. To overcome the vertical range limitations introduced by the height of the sample chamber, they engineered a 'hydrodynamic treadmill': a circular chamber that rotates about a horizontal axis to maintain a single organism at a fixed position coinciding with the focus of a microscope (Fig. 1b). Feedback from the organism's position is used to automatically set the required rate of rotation. Keeping the optics stationary allows a wide range of microscopy techniques to be used and also allows stimuli, such as light patterns, to be presented to the organism. The researchers demonstrate that they can follow planktonic organisms with a size range of several tens to many hundreds of micrometers over virtual vertical distances up to a few meters. Trajectories end when the tracking algorithm loses the organism — for instance, as a result of floating debris obscuring the view — so improvements in the tracking routine may allow substantially extended tracking durations to cover the vertical range of natural migrations.

In a sense, Krishnamurthy et al. literally reinvented the wheel: Hardy and Bainbridge[8] presented a larger, manually rotated device based on the same principle in the early 1950s and even used light stimuli to entice responses in organisms several millimeters to several centimeters in size while following their vertical movement over virtual distances of up to 100 meters.

Modern microscopy methods and computer electronics now enable much more sophisticated behavioral assays and readouts. For example, Krishnamurthy et al. couple light stimuli to virtual depth or interrogate flow fields around the swimming organism by performing particle image velocimetry on small tracer particles in the water. In the future, the integration of fluorescence microscopy could unlock a vast cell-biological toolbox for revealing internal states of the organism during its migration.

To ensure that the water moves with the wheel during rotation, the chamber must be narrow, and thus organisms may interact hydrodynamically and sterically with the chamber wall. Hardy and Bainbridge limited water movement in their larger wheel by a sophisticated, passive system of buoyant and weighted doors that remained open around the position of the microorganism but closed elsewhere. Perhaps that idea could be revived to enable tracking in wider chambers that allow a larger range of free horizontal swimming.

Because the wheel's rotation is determined by feedback based on the organism's behavior, the assay's throughput intrinsically cannot be expanded to capture more than one individual at a time. This limitation renders it time-consuming to capture diversity in behavior between individuals, which may constitute an important component in the migration strategy of some organisms[9].

Light is likely to be a key cue in diel vertical migration, so the application of light stimuli presents a promising tool for elucidating phototactic mechanisms, as well as the function of internal circadian clocks that enable many organisms to respond to periodic light patterns in an anticipatory fashion. Virtual depth-dependent presentation of stimuli simulates migration guided by external, depth-dependent cues. Krishnamurthy et al. applied a light pattern that varies periodically with virtual depth to *Volvox*, a phototactic alga, and captured the asymmetry in its swimming response to light–dark and dark–light transitions.

Vertical migration in the ocean, however, traverses gradients in a wide range of environmental parameters beyond light, including predation, pressure, nutrients, temperature, salinity, hydrodynamic flow, and the concentrations of many chemicals, such oxygen and nitrogen. Some of these other cues likely also contribute to guiding vertical migrations. In the future, fluidic access to the sample chamber may open up the possibility of mimicking more of these natural depth-dependent variations in the lab, creating more realistic 'virtual reality' scenarios for plankton. The authors' observations, however, indicate that long-term passive observations alone, without any perturbing stimuli, may already capture a vast, previously unknown behavioral repertoire. ❐

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Competing interests

The author declares no competing interests.