mulates at the site of the plasma membrane that senses the highest concentration of the extracellular stimulant (*8*). By directly binding to the DHR-1 domain, PIP_3 recruits DOCK2 to this site of the plasma membrane to activate Rac (*3*). Although biochemical experiments with PI 3-kinase inhibitors suggest an important contribution of PIP_3 in cell polarization, in vivo experiments with neutrophils lacking PI3Kγ (the major isoform in neutrophils) have demonstrated that these cells can nevertheless establish a polarized "leading edge" (region of the cell that extends a pseudopod) toward the chemoattractant (*3*). Thus, the signaling events leading to neutrophil polarization in the absence of PI 3-kinase activity have remained elusive.

Nishikimi *et al.* report that global membrane recruitment of DOCK2, through DHR-1–PIP_3 interaction, is not sufficient for neutrophil polarization to occur. Instead, the authors demonstrate that an additional phospholipid, phosphatidic acid, narrows and enriches the localization of DOCK2 more precisely at the membrane site that will become the growing leading edge (see the figure). Phosphatidic acid is generated from the hydrolysis of the membrane component phosphatidylcholine by phospholipase D. By using an inhibitor of phospholipase D, the authors show that a signaling pool of phosphatidic acid is responsible for targeting DOCK2 at the leading edge.

What is the mechanism by which phosphatidic acid refines the localization of DOCK2? Nishikimi *et al.* identified a polybasic region at the carboxyl terminus of DOCK2 that interacts directly with this phospholipid. Mutations in these basic residues abrogated DOCK2–phosphatidic acid binding in vitro and also prevented DOCK2 interaction with the plasma membrane at the leading edge in neutrophils. This suggests that binding to phosphatidic acid is responsible for correctly targeting DOCK2. An elegant swapping experiment of the polybasic region of DOCK2 for a polybasic region of a different signaling protein demonstrated that it is indeed phosphatidic acid binding that localizes DOCK2 to the leading edge. Abrogating DOCK2–phosphatidic acid interaction affected both the polarized accumulation of DOCK2 and the ability of neutrophils to migrate rapidly. Thus, Nishikimi *et al.* uncover a two-step mechanism for initiating polarized neutrophil movement: a more global and less specific step that depends on PIP_3 to recruit DOCK2 to the leading edge of the plasma membrane, and a second step that depends on phosphatidic acid to precisely localize DOCK2 to the exact site in the leading edge that will extend the pseudopod. Nishikimi *et al.* also found that other DOCK proteins interact with phosphatidic acid, and thus may function in response to events that alter amounts of phosphatidic acid. Interestingly, these DOCK proteins are implicated in biological processes that require cellular polarization, including myoblast fusion, phagocytosis of apoptotic cells, and neuronal development.

It is not clear how phosphatidic acid accumulates to sufficient amounts at the site of the plasma membrane that senses the highest amount of the chemoattractant. In the case of PIP_3 production, studies have demonstrated the existence of a positive feedback loop, in which PIP_3 recruits a Rac GEF and the Rac-GTP produced in situ can further enhance PIP_3 production by activating PI 3-kinases. After establishing this feedback loop, the cell generates an intracellular lipid gradient, leading to efficient signaling (*10*). Perhaps a similar feedback loop exists for phosphatidic acid production. Further studies on the regulatory mechanisms governing phospholipase D signaling, such as putative activation by Rac-GTP, are required to fully explain the signaling events that control cell motility in neutrophils and in other systems.

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OCEANS

Limits to Marine Life

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Ocean "dead zones" devoid of aerobic life are likely to grow as carbon dioxide concentrations rise.

Ocean chemistry is currently undergoing enormous change from the twinned impacts of higher carbon dioxide (CO_2) concentrations from fossil-fuel burning (*1*), inducing ocean acidification (*2*, *3*), and of rapidly declining mid-water oxygen (O_2) concentrations. The decline in O_2 results from lower sea-surface O_2 concentrations, reduced ventilation of the mid-water from ocean warming (*4*, *5*), and local eutrophication events, all of which lead to an expansion of oceanic dead zones. The reduced ventilation further elevates CO_2 concentrations at depth, because the decline in O_2 is accompanied by the equivalent respiratory CO_2 (*6*); as a result, ocean acidification penetrates more rapidly to lower depths than it would due to the fossil-fuel signal alone. Can the effects of these changes on marine life be quantified on the basis of existing data, and if so, how does one quantify them?

Initial concerns over ocean acidification focused on reduced calcification in coral reefs and other calcareous organisms (*7*, *8*), but other concerns soon arose. Elevated dissolved CO_2 concentrations may impose a physiological strain on marine animals, impairing performance and requiring energy that would otherwise be used for locomotion, predation, reproduction, or coping with other environmental stresses such as rising temperatures. However, there is as yet no formal way to estimate this impact or to relate observed oceanic chemical change to the physiological limits for marine organisms.

Ocean scientists today define the limits to aerobic life in the sea in terms of a minimum dissolved O_2 concentration (*5*), typically ~5 μM, below which it is inefficient for aerobic microbes to consume dissolved O_2; instead, the microbes turn to other electron acceptors such as IO_3, Mn(IV), and NO_3 (*9*). For higher animals, "dead zones" are defined as regions where normal respiration is greatly limited and the expenditure of effort is physiologically constrained, but there is no precise, universally accepted definition that would allow a common limit to be used when mapping changing conditions. In writing this limit in terms of dissolved O_2 [or oxygen partial pressure (pO_2)] alone, ocean scientists

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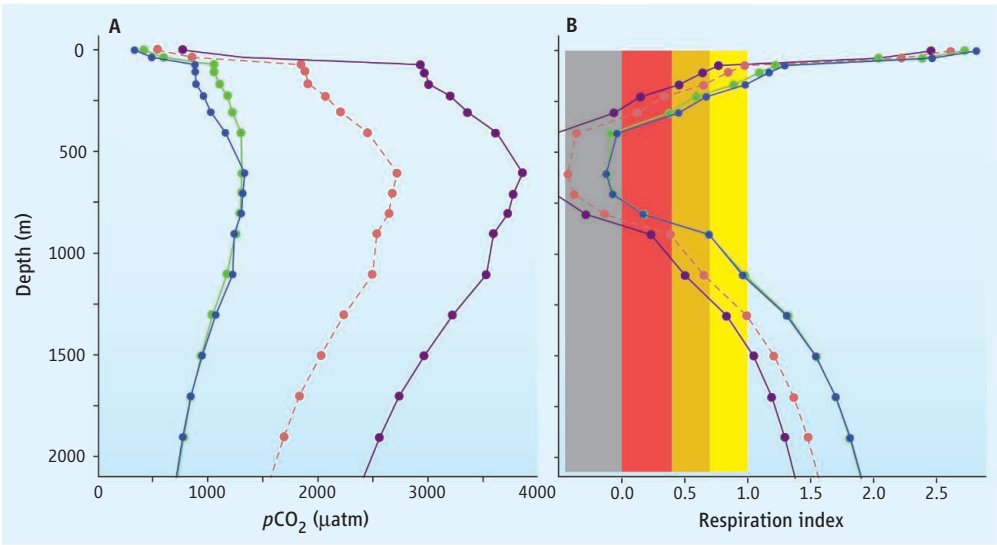

Expanding dead zones. An example of respiration stress at a station in the eastern tropical Pacific (WOCE P16C Sta 413: 13°01.75′N, 91°45.60′W). (**A**) The calculated pCO_2 rises with increasing atmospheric CO_2 concentration. The preindustrial profile was calculated from the modern data by removing the anthropogenic CO_2 at constant alkalinity. The projections (two times preindustrial and three times preindustrial) were calculated by determining the stepwise change in total CO_2 in the sea surface for each case and then propagating this change throughout the ocean. (**B**) Calculation of the respiration index with depth (RI) reveals the existence of a formal dead zone for aerobic life, where RI ≤ 0 (gray band). However, even at RI = 0.0 to 0.4 (red band), aerobic respiration is not observed. Bacteria appear to set the practical limit for all aerobic respiration at RI = 0.4 to 0.7 (orange band). Some marine animals can tolerate RI = 1 or slightly less, but others cannot (yellow band). With increasing atmospheric CO_2 concentrations, dead zones for aerobic life will grow in size. Rising ocean temperatures will further exacerbate the growth of the dead zones by decreasing oxygen saturation.

typically ignore the CO_2 side of the respiration equation, on the unspoken assumption that pCO_2 levels are low and are inversely proportional to the O_2 concentration via bacterial oxidation of marine organic matter. However, this may no longer be the case as atmospheric CO_2 concentrations rise and reset ocean chemical relations.

A simple way to approach this problem is to define the basic oxic respiration equation

$$C_{org} + O_2 \rightarrow CO_2 \quad (1)$$

and from this write out the free-energy relation

$$\Delta G = \Delta G^\circ - RT \bullet \ln\{[fCO_2]/[C_{org}][fO_2]\} \quad (2)$$

Here, ΔG° is the Gibbs free energy at standard conditions, R is the universal gas constant, T is temperature, and f is fugacity. From this equation, we can see that for all food sources there is a common term: the natural logarithm of the ratio of the gas fugacities. By substituting partial pressures for fugacities, $\log_{10}$ for the natural logarithm, and inverting the ratio to eliminate the minus sign, we obtain the expression $\log_{10}(pO_2/pCO_2)$, which provides a simple numerical constraint that is linearly related to available energy. We define this as the respiration index (RI), which may prove useful for estimating the physiological limits of deep-sea animals.

For a specific example, consider a station in the eastern tropical Pacific that is characteristic of the very large suboxic regions of the oceans. Here, the dissolved O_2 concentrations at depths between 300 and 600 m decline almost to zero, and large-scale reduction of nitrate to nitrite occurs. In this region, the calculated RI ranges from just below zero to 2 or more, depending on the ratio of pO_2 to pCO_2 (see the figure). Field data (*10*) suggest that denitrification begins to occur at RI ≈ 0.4 to 0.7, and this ratio likely sets the limit for aerobic respiration of higher animals. Actual limits will be species dependent and remain to be determined (see the figure for some hypothetical limits).

What is of concern is the impact of rising oceanic CO_2 concentrations on this ratio. Present-day pCO_2 at 500 m depth at this site is about 1000 µatm, but an increase of +280 parts per million by volume CO_2 to the atmosphere and surface ocean translates into a far greater change at depth. As surface sea water is transferred to depth, its buffer capacity is reduced by the acidic components of the normal Redfield cycle (*6*). A doubling of surface-water pCO_2 leads to a doubling or more of pCO_2 at depth (see the figure) due to the different geochemistry of the deeper water masses. From ocean equilibration with a doubled CO_2 atmosphere, the pCO_2 at the example station at 500 m depth will rise to 2500 µatm and possibly higher. Such levels have not been considered previously in many of the models designed to predict the status of the future ocean.

This simple example uses the fossil-fuel CO_2 signal alone, thereby greatly understating the case. The calculation assumes constant temperature, but oceanic warming is taking place and will drive the in situ pCO_2 higher. The calculation also assumes unchanging pO_2 levels, but deep-water O_2 concentrations are steadily declining. This affects the RI both through reducing pO_2 and through the associated increase in respiratory CO_2. Thus, we may anticipate a very large expansion of oceanic dead zones.

The expansion of dead zones also has other chemical side effects. The major redox cycles of the chemical elements are microbially driven, and thus an increase in production of N_2O—also a greenhouse gas—at depth seems likely, although any release of this to the atmosphere would be greatly limited by oceanic processes of mixing and consumption. Other redox species may serve as important tracers of the processes at work as these changes occur, with the $IO_3 \rightarrow I^-$ system being the most sensitive indicator.

For the vast areas of the ocean that are well-oxygenated, the rise in oceanic CO_2 concentrations will exert a negligible effect on the normal aerobic functioning of adult marine animals. However, based on our redefinition of dead zones, it is clear that even if oxygen levels do not decline, the oceanic dead zones will still expand as a result of rising CO_2 concentrations; with global warming reducing the oxygen levels as well, the combined effect will be severe.

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