Variation in evolved "limits to life" precludes universal tolerance indices: a critique of the "Respiration Index"

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Human emissions of carbon dioxide will affect marine ecosystems directly via ocean acidification (*2*) and indirectly via expanding oxygen minimum zones (*3*). Understanding the extent and severity of the impact requires knowledge of levels of both oxygen and carbon dioxide that are critical to organisms. Such critical levels represent species-specific adaptations in oxygen uptake and acid-base regulation that have evolved for each species within the specific habitats. One cannot set a single index for tolerance of these parameters because individual species have evolved unique tolerances to their habitats, and these limits are usually far from the ultimate limits that can be evolved in "extreme" habitats. The "Respiration Index" (*RI*, = $\mathrm{Log}_{10}\{pO_2/pCO_2\}$), proposed by Brewer and Peltzer (1) as a metric to define the operational limits of marine life, is theoretically invalid

and results in dangerous predictions regarding the tolerance of marine organisms to ocean acidification and expanding oxygen mininum zones.

A Respiration Index less than 0.4 is proposed as an approximate limit for prokaryotes while an *RI* less than 1.0 is supposedly limiting to metazoans. This fails to consider organismal physiology (*4*) assuming that the energy obtainable (free energy, ΔG) from oxidation of organic carbon (C_{org}) in cellular respiration is linearly related to the ambient partial pressures (P) of reactants and products, as if in a closed system moving toward equilibrium. Brewer and Peltzer (*1*) further assume that the gas partial pressures inside the cell, where aerobic respiration occurs, are equivalent to those in the environment. Both of these assumptions are incorrect and, as a result, the *Respiration Index* is inconsistent with available physiological data on oxygen and carbon dioxide tolerances of organisms. Intracellular concentrations of oxygen and carbon dioxide are regulated independently by kinetic and physiological mechanisms. Critical thresholds for oxygen and carbon dioxide are independent of each other and result, not from reductions in free energy of C_{org} oxidation, but from limits to oxygen supply and acid-base regulation, which vary widely among species (8).

Brewer and Peltzer (*1*) suggest that the oxidation of C_{org} to CO_2 and H_2O during cellular respiration may be expressed as a Gibbs function:

$$\Delta G = \Delta G^\circ - RT * \ln([PCO_2]/[C_{org}][PO_2])$$

where ΔG° is the free energy under standard conditions *in vitro*, R is the universal gas constant, and T is temperature. Thermodynamics is the driving force for all chemical reactions, including those mediated by organisms. However, the Gibbs function as employed by Brewer and Peltzer (*1*) is descriptive only of closed systems, in which the substrates for the reaction are consumed and the products accumulate. In such cases, the ratio of the concentrations of reactants influences the tendency of the reaction to progress in either a net forward or reverse direction until equilibrium is reached, at which point no net flux occurs and ΔG is zero. In contrast, living organisms are open systems that acquire free energy from their surroundings and operate at an approximate steady state (*5*). The concentrations of substrates and products in living systems are actively maintained at disequilibrium to ensure a constant ΔG and thus, thermodynamic drive, for forward flux through the pathways of energy metabolism (*6, 7*).

The *Respiration Index* is further flawed because it fails to distinguish intracellular from environmental gas partial pressures. This distinction is especially important within the marine oxygen minimum zones discussed by Brewer and Peltzer (*1*), where the total *quantity* of oxygen is not limiting, just its *availability* (*8*). This means that resident organisms can, via physiological adaptations, obtain sufficient oxygen despite an environmental *RI* well below 1. Oxygen is extracted by active ventilation of the seawater and rapid circulation of blood across gills of large surface area (*8*). It is concentrated and transported via high-affinity respiratory proteins in extra- and intracellular circulatory systems (*9*). The local acid-base balance can be adjusted actively to enhance oxygen off-loading at the respiring tissues. Even in organisms without

extracellular circulatory systems, the enzyme complexes in metabolic pathways contribute to oxygen flux via adapted oxygen affinities (e.g. the K_m for O_2 of cytochrome C oxidase in electron transport; *10*). Thus the effective concentration of oxygen at the site of energy metabolism may be higher than in the environment and, above a critical level, is independent of environmental oxygen concentration and rates of oxygen demand (*9*). It is this capacity for oxygen uptake, relative to energy demand, that determines hypoxia tolerance in organisms (*10*).

The effect of increasing PCO_2 (hypercapnia) on metabolism is complex but often includes pH effects on protein function. Among organisms with respiratory proteins, hypercapnia can lead to oxygen limitation because oxygen-binding affinity is adaptively pH-sensitive. Furthermore, hypercapnia can adversely affect cellular function by reducing cytoplasmic pH. However, most organisms are well adapted to control vascular and intracellular pH and PCO_2 by passive chemical buffering, catalytic conversion (to bicarbonate) and active transport (*11*). Siboglinid worms living at hydrothermal vents, for example, are capable of extruding proton equivalents at an extremely high rate to maintain an arterial pH well above the CO_2-enriched seawater in which they live (*12*). Accordingly, they are capable of carrying out aerobic respiration at an environmental *RI* well below zero (*13*). Another example of organismal adaptations is found in *Gnathophausia ingens*, a midwater crustacean living at hypoxic depths off California (*14*). The ability to extract oxygen from the ambient water is actually enhanced at low pH (7.1) in this species. In light of such adaptations to regulate internal gas partial pressures, ocean scientists "typically ignore the CO_2 side of the respiration equation", not on the

"unspoken assumption that PCO_2 levels are low" as stated by Brewer and Peltzer (*1*), but because the equilibrium state of the overall reaction is not influenced by gas partial pressures.

Dead zones do not typically result from absolute limits to biological function, but rather from evolved limits to performance among individual marine species within each habitat. The evolved limits are known to correlate with the minimum oxygen level experienced by individual species and very different limits are found among marine organisms living in different marine environments (8). As such, dead zones will likely be exacerbated by expanding hypoxia and may be influenced by ocean acidification under some circumstances. However, adaptation to specific environments cannot be accommodated by the *Respiration Index,* which consequently leads to dangerously wrong predictions. The authors state, for example, that normal aerobic function will not be affected by ocean acidification in well-oxygenated waters. However the deleterious influence of carbon dioxide on oxygen transport may be *most* pronounced in well-oxygenated surface waters where high temperature and activity levels drive oxygen demand toward the limits of transport capacity (e.g. *15*). Furthermore, the respiration index may lead readers to conclude that most marine animals are oxygen limited only at very low PO_2 ($RI < 1$). In contrast, most marine organisms are not adapted to hypoxic conditions and become limited at much higher oxygen partial pressures (e.g. at an $RI \sim 2$; *16*). Thus regions where oxygen minimum zones expand onto otherwise oxygenated shelves stand to be most dramatically affected. This may be seen off the coast of Oregon, where water from the oxygen minimum layer, which contains a rich and abundant fauna adapted to low

oxygen, creates a dead zone when it comes to shallower depths and encompasses animals that have not evolved adaptations to low PO_2 levels (e.g. *17, 18*). No general predictive index for oxygen and CO_2 tolerance is possible because tolerances to these parameters have evolved in individual species due to selection by their particular environmental conditions. Thus, tolerance of hypoxia and hypercapnia is species specific and reflects evolutionary adaptation to regional variation in environmental gas levels over long periods of time.

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