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The real limits to marine life: a further critique of the Respiration Index

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Abstract

The recently proposed "Respiration Index" (RI = log[PO_2]/[PCO_2]) suggests that aerobic metabolism is limited by the ratio of reactants (R, oxygen) and products (P, carbon dioxide) according to the thermodynamics of cellular respiration. Here we demonstrate that, because of the large standard free energy change for organic carbon oxidation ($\Delta G° = -686\,\mathrm{kcal\,mol^{-1}}$), carbon dioxide can never reach concentrations that would limit the thermodynamics of this reaction. A PCO_2 to PO_2 ratio of 10^{503} would be required to reach equilibrium (equilibrium constant, $K_{eq} = 10^{503}$), where $\Delta G = 0$. Thus a respiration index of −503 would be the real thermodynamic limit to aerobic life. Such a Respiration Index is never reached either in the cell or in the environment. Moreover cellular respiration and oxygen provision are kinetically controlled such that, within limits, environmental oxygen and CO_2 concentrations have little to do with intracellular concentrations. The RI is fundamentally different from the aragonite saturation state, a thermodynamic index used to quantify the potential effect of CO_2 on calcification rates, because of its failure to incorporate the equilibrium constant of the reaction. Not only is the RI invalid, its use leads to incorrect and dangerous predictions of the threat of changing oxygen and carbon dioxide to marine life. We provide a physiological model that identifies oxygen thresholds, and allows for synergistic effects of ocean acidification and global warming.

1 Introduction

Growing evidence suggests that oxygen minimum zones are expanding, while ocean acidification is well-documented (Feely et al., 2008; Stramma et al., 2008; Keeling et al., 2010; Bograd et al., 2010). The biological effects of these climate-induced changes are well characterized (Seibel and Fabry, 2003; Melzner et al., 2009; Pörtner and Knust, 2007; Seibel and Childress, 1998; Seibel 2011; Hochachka and Somero, 2002), but no single metric has been identified that facilitates straightforward ecological predictions relating to climate change in the marine environment. A recent paper by Brewer and Peltzer (2009) proposes a "Respiration Index" that purports to quantify the combined effects of carbon dioxide and oxygen partial pressures on the aerobic metabolism of marine organisms. The RI is based on the idea that the energy available to perform work (ΔG), obtained from cellular respiration, is directly related to the partial pressures of oxygen and carbon dioxide in seawater, as if the reaction were taking place in a closed system and moving toward equilibrium.

$$C_{org} + 6O_2 \longleftrightarrow 6CO_2 + 6H_2O \tag{1}$$

They express this as a Gibbs free energy equation

$$\Delta G = \Delta G^\circ + RT \cdot \ln K_{eq} \tag{2}$$

where K_{eq} is the equilibrium constant equivalent to the ratio of products (e.g. PCO_2) to reactants (e.g. PO_2), ΔG is the Gibbs free energy defined as the energy available to do work, and ΔG° is the standard free energy defined as the free energy at standard temperature and where the concentrations of products equal reactants. Brewer and Peltzer (2008) defined a Respiration Index, RI, as the ratio of the concentrations of reactants to products, a metric of the presumed direct effect of these concentrations on ΔG.

$$RI = \log PO_2/PCO_2. \tag{3}$$

Note that Brewer and Peltzer inverted the ratio of products to reactants from Eq. (2) to eliminate the negative sign that was erroneously inserted in their formulation of the Gibbs equation. Equation (1) is correct as written above but we retain the inversion for consistency with their paper.

The RI is alluring because of its simplicity and apparent mechanistic underpinnings. However, Brewer and Peltzer's suggestion that the R/P ratio can present a thermodynamic limit to aerobic life is incorrect because it ignores kinetic and physiological control and does not include the standard free energy for the reaction, ΔG°. We argue here, based on well-studied bioenergetic considerations, that the Respiration Index is irrelevant and that cellular metabolism is irreversible and tightly regulated in the cell. As a result, the energy obtained from the oxidation of organic matter is effectively independent of environmental gas concentrations.

2 ΔG of C_{org} oxidation and the K_{eq}

Brewer and Peltzer suggest that a RI of 0 (i.e. PO_2:pCO_2 =1) represents a thermodynamic limit to aerobic metabolism as if the reaction were at equilibrium when PO_2 is equal to PCO_2. However, ΔG does not equal zero, and the reaction is not near equilibrium, when the RI is zero. Rather, an RI of zero at 25° C is the definition of standard conditions, where $\Delta G = \Delta G^\circ$ (Eq. 2, Fig. 1). The ΔG° for glucose oxidation is equal to $-686\,\mathrm{kcal\,mol^{-1}}$ (Nelson and Cox, 2008). This means that glucose oxidation is an extremely exergonic reaction that is very far from equilibrium under standard conditions, when $PCO_2 = PO_2$ (RI = 0). Under these standard conditions, oxidation of C_{org} is still a highly favorable reaction with tremendous potential for energy extraction (Fig. 1). Brewer and Peltzer (2008) appear to confuse equilibrium ($\Delta G = 0$ and RI $\sim K_{eq}$) with standard conditions ($\Delta G = \Delta G^\circ$ and RI = 0).

The oxidation of organic carbon remains energetically favorable across all realistic CO_2 partial pressures, whether intracellular or environmental. Inserting the value for ΔG° for Glucose into the Gibbs equation, we calculate the ratio of products to substrates at equilibrium (the equilibrium constant, K_{eq}), which is the actual absolute thermodynamic limit to aerobic life as envisioned by Brewer and Peltzer (2009), ignoring kinetic control of metabolic processes. At equilibrium, $\Delta G = 0$ and no free energy is available from the reaction to perform work. In that case, the Gibbs equation simplifies.

$$-\Delta G/RT = \ln K_{eq} \tag{4}$$

where R =1.987 cal mol^{-1} × K (note that ΔG° is in kcal mol^{-1}), and T = 298°K. We calculate a K_{eq} of 10^{503}. That is, at equilibrium when ΔG = 0, the ratio of CO_2:O_2 would be 10^{503}, equivalent to a RI of −503. Even when coupled to ATP synthesis, a highly endergonic reaction, the effective K_{eq} is 10^{170}. Obviously this value is never obtained in the cell or in the environment and the RI is thus completely invalid and provides no useful information. This is the reason that biologists have ignored the CO_2 side of the respiration equation, not "*on the unspoken assumption that* PCO_2 *levels are low and are inversely proportional to the* O_2 *concentration*" as asserted by Brewer and Peltzer.

Why is the RI any different from the aragonite saturation state (Ω, Eq. 5), a thermodynamic index of calcification in marine organisms? To the extent that calcification and cellular metabolism are both kinetically controlled processes carried out as open systems, the aragonite saturation state does suffer some of the same problems faced by the RI and is, at best, a rough predictor of the response of calcifying organisms to ocean acidification (Ries et al., 2009). However, unlike the respiration index, the aragonite saturation state incorporates the equilibrium characteristics of the reaction (i.e. the solubility product, K_{sp}) and the concentration of carbonate and calcium ions in seawater is near the equilibrium value such that ocean acidification can have an impact on the reaction. Moreover, the reaction is reversible and the back reaction, dissolution, often occurs in direct contact with seawater and is not kinetically controlled. Thus the aragonite saturation state is a valid, albeit imprecise, thermodynamic index.

$$\Omega = [\mathrm{Ca}^{2+}][\mathrm{CO}_3^{2-}]/K_{\mathrm{sp}} \tag{5}$$

The formulation that Brewer and Peltzer appeared to strive for would be RI = $(PCO_2/PO_2)/K_{\mathrm{eq}})$. When such a metric approaches 1, ΔG is zero and no net energy is obtainable from cellular respiration. However, such a condition is only met in nature when oxygen is well below the limits of detection and beyond the extraction capabilities of any organism. Even so, the effect of ocean acidification on the thermodynamics of cellular respiration would be negligible. Carbon dioxide does influence organismal metabolism, but only indirectly (see below).

3 Kinetic and physiological control of organismal metabolism

Even if one ignores the thermodynamic considerations above, the RI is completely irrelevant. The RI assumes that the gas partial pressures inside the cell are equivalent to those in the environment when, in fact, they are quite different. The differences exist, not only because of differing solubilities between CO_2 and O_2, but because organisms maintain, within physiological limits, gas partial pressures at levels consistent with basic cellular function regardless of the partial pressures in the environment. For example, by comparing aquatic and terrestrial organisms it becomes clear that intracellular gas levels are actively maintained, rather than passively reflective of environmental conditions. Because oxygen is not limiting in air, the rate of ventilation can be much lower in air-breathers and carbon dioxide can build up to very high partial pressures (5 kPa). Intracellular concentrations of gases are regulated by independent physiological mechanisms and limits at the whole-organism level result from limits to oxygen supply and acid-base regulation. These vary widely between species. These kinetic objections were raised in an earlier critique (Seibel et al., 2009).

The RI does not distinguish between intracellular and environmental gas partial pressures. This distinction is important because marine organisms adapted to hypoxic environments can obtain sufficient oxygen regardless of the RI, via enhanced oxygen extraction capabilities. Active ventilation across the high-surface area gills and rapid circulation of blood with high-affinity respiratory proteins ensures a sufficient gradient of oxygen from the seawater to the sites of cellular respiration (Childress and Seibel, 1998; Sanders and Childress, 1990; Seibel, 2012). The acid-base balance can be actively adjusted to enhance oxygen release at the respiring tissues. The oxygen affinities of enzyme complexes in metabolic pathways also contribute to oxygen flux (Connett et al., 1990). Via these mechanisms, the concentration of oxygen may be higher in the respiring tissues than in the environment. The cellular PO_2 is, above a critical limit, independent of environmental partial pressures (Seibel, 2011).

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4 Physiological effects of hypercapnia

Elevated carbon dioxide (hypercapnia) may decrease (Seibel et al., 2012) or increase (Stumpp et al., 2011) organismal metabolism. These effects are often mediated by available energy, but not via the thermodynamic mechanism proposed by Brewer and Peltzer. Depending on the buffering capacity of intra- and extracellular fluids and on the capacity of organisms to compensate via transport of acid-base equivalents, elevated CO_2 will acidify tissues just as it does seawater (Seibel and Fabry, 2003; Melzner et al., 2009). This may have important implications for protein function among other things (Hochachka and Somero, 2002). One well-studied effect of CO_2 on organismal function is on the oxygen-transport capacity via pH-sensitive oxygen binding proteins (e.g. hemoglobin and hemocyanin). For example, the classic paper by Redfield and Goodkind (1929) shows that CO_2 has no toxic effect on squids except "*through its influence on the oxygenation of the blood*". They demonstrate that asphyxiation in squids (*Loligo pealei*) occurs at a constant blood-oxygen concentration regardless of CO_2 but that CO_2 can influence the blood oxygen level. Thus the blood oxygen level that causes asphyxiation is constant but the environmental PO_2:PCO_2 (i.e. the RI) that results in that particular blood-oxygen level can be quite different. Extreme pH sensitivity of oxygen binding has been shown for a number of other squids (Bridges, 1994). The effect of CO_2 on blood-oxygen binding is believed to be responsible for reduced levels of metabolism under ocean acidification scenarios in the squid, *Dosidicus gigas* (Rosa and Seibel, 2008; Seibel, 2012).

5 Dangerous predictions

The respiration index results in inaccurate and potentially dangerous predictions. For example, Brewer and Peltzer (2009) state that "*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, carbon dioxide may be most problematic in well-oxygenated surface waters because high temperature elevates oxygen demand above the limits of transport capacity (Rosa and Seibel, 2008). Conversely, the RI suggests that only very low PO_2 (RI <1) will limit marine animals. However, most marine organisms are poorly equipped for hypoxic conditions and become stressed at much higher oxygen levels (for instance, at an RI ~ 2; Vaquer-Sunyer and Duarte, 2008). Regions where oxygen-minimum zones expand onto shelves may be particularly affected. For example, upwelled hypoxic water recently created a dead zone along the Oregon coast because the animals there are not equipped for low PO_2 levels (Feely et al., 2008; Chan et al., 2008).

6 What are the oxygen limits to marine life?

Useful criteria for establishing limits for O_2 tolerance must rely on measures of physiological or ecosystem performance and recognize the capacity of organisms to adjust O_2 provision and demand, via acclimation and adaptation, to match oxygen availability. A measure of particular importance is the critical oxygen partial pressure (P_{crit}; Childress and Seibel, 1998), which integrates the systems involved in oxygen uptake and transport with those that determine energy usage in whole animals (Connett et al., 1990; Pörtner and Knust, 2007). The P_{crit} is defined as the oxygen partial pressure below which metabolism cannot be regulated independently of PO_2. Broad comparative studies have revealed that, below a "saturation threshold" (~ 5 kPa; Seibel, 2011), the P_{crit} closely matches the oxygen level to which a species is exposed in its natural range (Childress and Seibel, 1998; Seibel, 2011; Richards 2011). This means that existing oxygen levels are the effective limit for marine life. Any reduction in PO_2 from the current level requires acclimation, adaptation or migration, the capacity for which is largely unknown. A lower "adaptation" threshold exists near 1 kPa below which further reductions in oxygen do not elicit further reductions in P_{crit}. In other words, an apparent limit to adaptation is reached beyond which further increase in gill surface area, blood oxygen affinity or ventilation capacity is not possible (Seibel 2011). Ecosystems poised near this lower threshold, such as the California Current oxygen minimum zone, may undergo dramatic community restructuring with small reductions in oxygen content.

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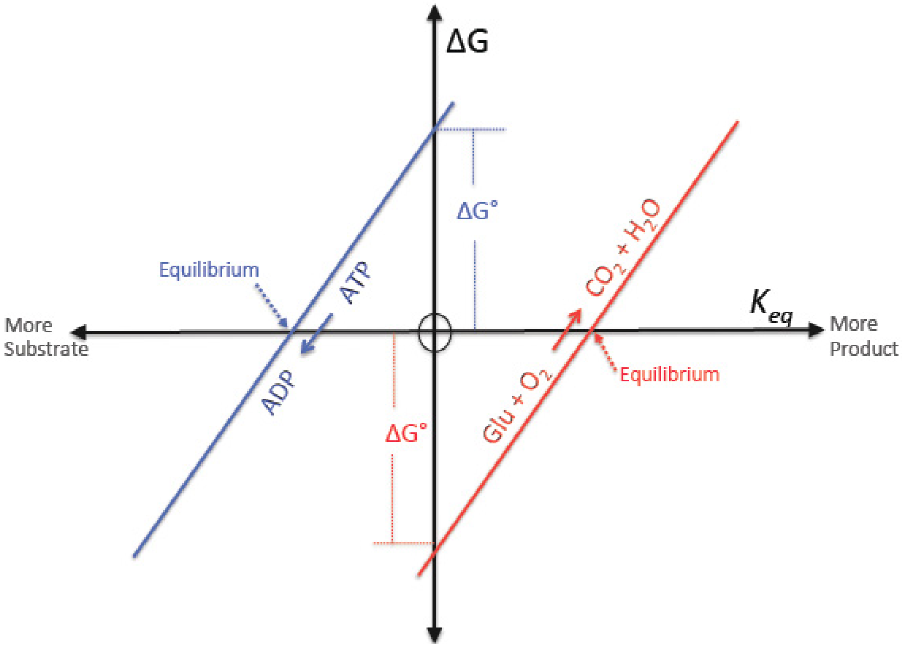

Fig. 1. A schematic illustrating the relationship between the free energy change (ΔG), the free energy change under standard conditions (ΔG°) and the equilibrium constant (K_{eq}). At equilibrium ($\Delta G = 0$), the glucose (Glu) oxidation reaction (red line) is shifted far to the right resulting in a high K_{eq} (10^{503}, ratio of product, CO_2, to reactant, O_2). ΔG°for glucose oxidation is very large and negative ($-689\,\mathrm{kcal\,mol^{-1}}$) meaning that, under standard conditions ($K_{eq} = 1$), the reaction is highly favored in the forward reaction. The energy derived from this highly exergonic reaction is used to drive the synthesis of ATP (blue line), a highly endergonic and unfavorable reaction.