Exploring a first-principles-based model for zooplankton respiration

Ted T. Packard and May Gómez

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Oxygen consumption (R) is caused by the respiratory electron transfer system (ETS), not biomass. ETS is ubiquitous in zooplankton, determines the level of potential respiration (Φ), and is the enzyme system that ultimately oxidizes the products of food digestion, makes ATP, and consumes O_2. Current respiration hypotheses are based on allometric relationships between R and biomass. The most accepted version at constant temperature (T) is $R = i_0 M^{0.75}$, where i_0 is a constant. We argue that, for zooplankton, a Φ-based, O_2-consuming algorithm is more consistent with the cause of respiration. Our point: although biomass is related to respiration, the first-principles cause of respiration is ETS, because it controls O_2 consumption. Biomass itself is indirectly related to respiration, because it packages the ETS. Consequently, we propose bypassing the packaging and modelling respiration from ETS and hence Φ. This Φ is regulated by T, according to Arrhenius theory, and by specific reactants (S) that sustain the redox reactions of O_2 consumption, according to Michaelis – Menten kinetics. Our model not only describes respiration over a large range of body sizes but also explains and accurately predicts respiration on short time-scales. At constant temperature, our model takes the form: $R = \Phi S/(K_m + S)$, where E_a is the Arrhenius activation energy, R_g, the gas constant, and K_m, the Michaelis – Menten constant.

Keywords: biomass, electron transfer system, Kleiber's law, metabolic theory of ecology, metabolism, respiration.

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T. T. Packard: Institut Ciències del Mar (CSIC), Passeig Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain, and Bigelow Laboratory, West Boothbay Harbor, ME 04575, USA. M. Gómez: Biological Oceanography Group, Marine Science Faculty, Universidad de Las Palmas de Gran Canaria, Campus Universitario de Tafira, 35017 Las Palmas de Gran Canaria, Spain. Correspondence to T. T. Packard: Institut Ciències del Mar (CSIC), Passeig Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain. tel: +34 928 452905; fax: +34 928 452922; e-mail: ted@icm.csic.es.

Introduction

A first-principles model of respiration should be based mechanistically on the biochemical basis of respiration. Currently, no such model for zooplankton exists, although the metabolic theory of ecology (MTE) by Brown *et al.* (2004) and the enzyme-kinetic-based models of Packard *et al.* (1996a,b, 2004) and Roy and Packard (2001) purport to be. Here, we examine the suitability of these two types of metabolic model for predicting zooplankton respiration in different physiological states and on a short time-scale. We find the MTE applicable on large scales and the enzyme kinetic approach on small scales.

During the past 10 years, a series of studies has put forth a MTE based on the allometric equation of Kleiber (1932; West *et al.*, 1997, 2000; Enquist *et al.*, 1998; Brown *et al.*, 2004). These studies argue that metabolism is determined by biomass (M), temperature (T), and the flux of elemental materials (i_0) through an organism (Brown *et al.*, 2004). They invoke a similarity of the fractal scaling nature of plant and animal circulatory and distribution networks, along with metabolic thermodynamics and kinetics, to explain the widespread applicability of the allometric equation. The mathematical model of the MTE takes the form

$$R = i_0 M^{3/4} e^{-E_a/kT}. \quad (1)$$

The biomass part is from Kleiber's law, $R = aM^{3/4}$ (Kleiber, 1932; Whitfield, 2006), where $a = R$ when $M = 1$. With an uncertainty of two orders of magnitude, Kleiber's law holds true over 20 orders of magnitude in biomass (Hochachka and Somero, 2002; Whitfield, 2006). The relative ease with which biomass can be measured, as well as the fact that biomass is a basic property of living systems, explain why Kleiber's law is so well entrenched in biological thinking and why the MTE is so appealing to ecologists.

Temperature dependence in Equation (1) is based on the atomic-scale Boltzmann factor, $e^{-E_a/kT}$, where E_a is the Arrhenius energy of activation in electron-volts, k the atomic-scale Boltzmann constant (1.38×10^{-23} J atom^{-1} K^{-1} or 0.33×10^{-23} cal atom^{-1} K^{-1}), and T is calculated in degrees Kelvin (K). For the stoichiometric factor or resource availability, the MTE uses a constant, i_0 (Brown *et al.*, 2004). This MTE equation has been applied to secondary production, respiration (Gillooly *et al.*, 2001), growth, developmental time (Gillooly *et al.*, 2002), etc., in both plants and animals. Recently, it has been applied to the calculation of both phytoplankton productivity and total plankton respiration in the world ocean to demonstrate that respiration will exceed photosynthesis in both the oligotrophic as well as the warming ocean waters (López-Urrutia *et al.*, 2006). On large scales, the MTE provides a basis for improving ocean models.

The models based on enzyme-kinetics of metabolism argue that, at the cellular level, each metabolic process is controlled by the maximum velocity (V_{max}) of the enzymatic reaction that controls the process (Packard *et al.*, 2004), the temperature, and substrate availability (S). In the case of respiratory oxygen

consumption (R_{O_2}) in zooplankton, this approach asks questions about the biochemistry of respiration and builds the logic of the model on the answers. At constant temperature, what is the direct cause of R_{O_2} and what controls it? Cytochrome oxidase at the end of the electron transport chain is the enzyme that reduces O_2 to H_2O. However, this O_2 reduction rate is controlled at the other end of the electron transport chain by the dehydrogenases that oxidize NADH, succinate, and NADPH and that, in turn, are produced by the Krebs cycle and other metabolic pathways. Packard *et al.* (1996a, b) argue that it is the V_{max} of these enzymes and the availability of their substrates (S) that, according to the theory of Michaelis–Menten, control the *in vivo* dehydrogenase activity, the electron transfer system (ETS) activity, and potential respiration. The V_{max} of the dehydrogenases and the ETS is largely controlled by M, but the substrates are controlled by the organism's nutritional and physiological state.

The equation, for the enzyme-kinetic-based model for any oxygen consumption rate (R_1) at temperature T_1 as a function of V_{max_0} measured at another temperature (T_0), takes the form

$$R_1 = \frac{S(V_{max_0}\, e^{-\lambda})}{K_m + S}. \quad (2)$$

V_{max_0} is the potential rate of the process (in the same units as the physiological rate, R_1) and $\lambda = E_a(1/T_1 - 1/T_0)/R_g$, an exponent derived from the Arrhenius equation ($R_0 = Ae^{-E_a/R_gT_0}$ and $R_1 = Ae^{-E_a/R_gT_1}$), where A is the Arrhenius frequency factor (a reaction constant), E_a the molar-scale Arrhenius energy of activation ($\approx$62.7 kJ mol^{-1} K^{-1} or 15 kcal mol^{-1} K^{-1}; Packard *et al.*, 1971, 1975), R_g the molar-scale gas constant (8.31 J mol^{-1} K^{-1} or 1.987 cal mol^{-1} K^{-1}), and T_0 and T_1 the temperatures (°K) of the measured potential rate and the predicted rate, respectively, between 0°C and the temperature optimum of R_{O_2}. The term $e^{-\lambda}$ represents the fraction of the measured Φ_0 that is available to function at T_1. If $T_1 > T_0$, then $\Phi_1 > \Phi_0$ and $R_1 > R_0$; if $T_1 < T_0$, then $\Phi_1 < \Phi_0$ and $R_1 < R_0$, as observed in nature, up to the optimum temperature for the organism's R_{O_2}. Because A is a constant for a particular reaction, it is eliminated when solving $R_1 = f(R_0)$, i.e. $R_1 = R_0\, e^{-E_a(1/T_1 - 1/T_0)/R_g}$. The concentration of the substrate, S, represents the reactant of the enzyme reaction controlling R_{O_2}. The constant, K_m, for a single-substrate reaction ($S \rightarrow P$), where P is the product of the reaction, is the Michaelis–Menten constant. In the case of a bisubstrate reaction as in the ETS (Savenkoff *et al.*, 1995; Gómez *et al.*, 1996), ($S_1 + S_2 \rightarrow P_1 + P_2$), S becomes [S_1S_2] and K_m becomes $K_\beta = (K_{S1})(K_{ia}) + (K_{S2})[S_1] + (K_{S1})[S_2]$, where K_{S1} and K_{S2} are the Michaelis–Menten constants for S_1 and S_2, and K_{ia} the dissociation constant for the enzyme-S_2 complex (Packard *et al.*, 1996a, b, 2004). Note that, if T_0 and T_1 are equal, λ becomes 0, and $e^{-E_a(1/T_1 - 1/T_0)/R_g}$ becomes 1, $R_1 = R_0$, and Equation (2) simplifies to

$$R_1 = R_0 = \frac{S\, V_{max_0}}{K_m + S}. \quad (3)$$

Note also the difference between $e^{-E_a(1/T_1 - 1/T_0)/R_g}$ in Equation (2) and $e^{-E_a/kT}$ in Equation (1). Both exponential distribution functions trace back to Maxwell in 1859, but Maxwell, Boltzmann, van't Hoff, and Arrhenius used them for different purposes. It was Arrhenius who used this function to predict the temperature dependence of biological processes, as well as chemical and biochemical reactions (Arrhenius, 1915). At a numerical level, R_g in Equation (2) is greater than k in Equation (1) by 23 orders of magnitude. This is because $k = R_g/N$, where N = Avogadro's number, 6.022×10^{23} atoms mol^{-1}. This would not be a problem if E_a values were reported in molecular units, but historically biologists, biochemists, and chemists, following Arrhenius (1889, 1915), have reported their measurements of E_a in molar units (Martínez and Estrada, 1992; Treacy *et al.*, 1997; Oxtoby *et al.*, 1999; Ramirez *et al.*, 2006; Angelova *et al.*, 2007).

Previously, this enzyme-kinetic-based model predicted respiratory CO_2 production rates in bacteria from V_{max} measurements of isocitrate dehydrogenase activity, a proxy for potential respiratory CO_2 production (Figure 1a, and Roy and Packard, 2001). About the same time, it was applied to respiratory oxygen consumption in bacteria, as we plan to do here for zooplankton. The model predicted rates in bacteria from V_{max} measurements of respiratory ETS activity, kinetic constants from the literature, and modelled time courses of the two main ETS electron donors (reactants), NADH and NADPH (Figure 1a, and Packard *et al.*, 1996b). Here, we argue that this ETS-based model can be used to predict zooplankton respiratory oxygen consumption.

Material and methods

The data used in this paper have been drawn from the zooplankton studies of King and Packard (1975) and the bacterial studies of Berdalet *et al.* (1995) and Packard *et al.* (1996a, b). The older ETS data have been converted to values consistent with the newer methodology, using the correction factor of 3.3 according to Owens and King (1975). The use of the bacterial studies is based on the understanding that, because of the universal nature of the relationship between respiration and the respiratory electron transport system in bacteria and zooplankton, lessons learned from a study of respiration in bacteria can guide our thinking about respiration in zooplankton.

The zooplankton respiration and ETS activity measurements are described in Packard *et al.* (1974) and King and Packard (1975). Bacterial respiration in *Pseudomonas nautica* (strain 617) was measured according to Berdalet *et al.* (1995), and the bacterial ETS was measured kinetically using the method of Packard and Williams (1981).

Results

The two metabolic models, as defined by Equations (1) and (2), have three components. Both models claim to be based on first principles, and both claim to be applicable to multiple physiological processes. Here, we compare them for the specific case of respiratory oxygen consumption in both zooplankton and bacteria.

Other than the conceptual physics–chemistry and mathematical differences described in the introduction, a major difference between the two models is the physiological scale of focus. The MTE focuses on explaining the difference in respiration between organisms of different size, for example a euphausid and a tintinnid. It finds that biomass, as in Kleiber's (1932) law, serves as a good proxy for respiration over this scale of difference. If we examine this claim for zooplankton over a biomass range of 0.001–432 mg dry weight per animal and a respiration range of 0.004–183 μl O_2 h^{-1} (animal)$^{-1}$, we find that Kleiber's law and the MTE describe the data well over these five orders of magnitude (Figure 2). The equation is $R = 1.5\, M^{0.76}$ with M the dry weight per animal.

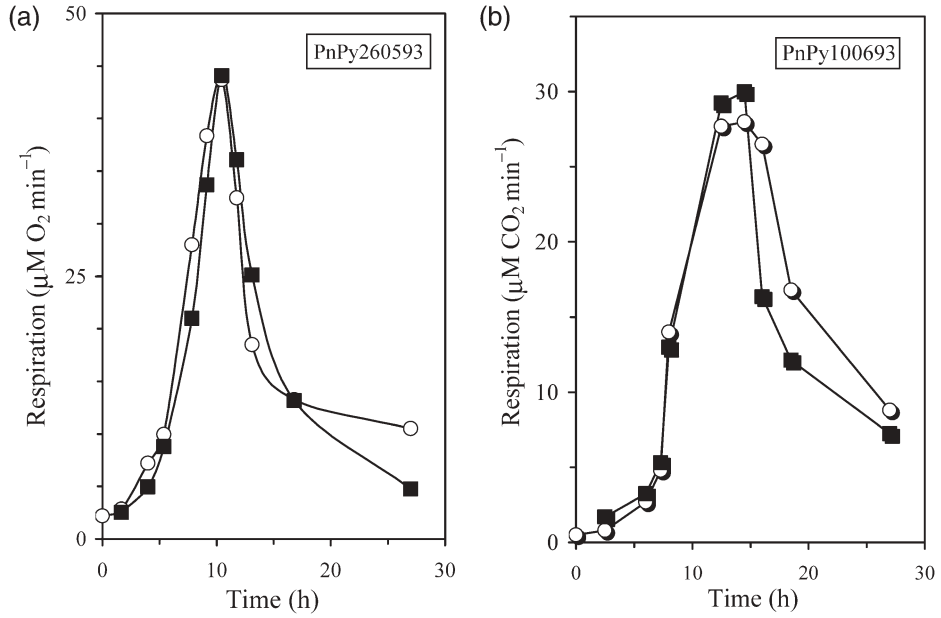

Figure 1. Modelled (open circles) and measured (solid squares) respiratory O_2 consumption rate (R_{O_2}) and CO_2 production rate (R_{CO_2}) in the marine bacterium *Pseudomonas nautica* grown at constant temperature on pyruvate in batch culture (Berdalet *et al.*, 1995). (a) O_2 consumption model, based on respiratory potential respiration (Φ), from Packard *et al.* (1996b). The algorithm of the model is a bisubstrate case of Equation (2), where S_1 is reduced nicotinamide adenine dinucleotide (NADH), S_2 is reduced nicotinamide adenine dinucleotide phosphate (NADPH). The model is: $R_{O_2} = \Phi^*[NADPH]^*[NADH]/((K_{NADH}^*[NADPH]) + (K_{ia}^*K_{NADH}) + (K_{NADPH}^*[NADH] + [NADPH]^*[NADH]))$, where K_{ia}, K_{NADPH}, and K_{NADH} were 6.7, 9.0, and 26.0 μM, respectively, and the [NADPH] and [NADH] time courses were modelled from the pyruvate and cell protein in the culture. The kinetic constants were determined by optimization from ranges of values in the literature. (b) CO_2 production model, from Roy and Packard (2001), based on isocitrate dehydrogenase activity (IDH). It is similar to the simple model used in Packard *et al.* (1996a; their Figure 1a). As in (a), the algorithm of the model is the two substrates case of Equation (2), where S_1 is oxidized nicotinamide adenine dinucleotide phosphate ($NADP^+$) and S_2 is isocitrate (ISO). The model is: $R_{CO_2} = 3^*IDH^*[NADP^+]^*[ISO]/((K_{iso}^*[NADP^+]) + (K_{ia}^*K_{NADP}) + (K_{NADP}^*[ISO] + [NADP^+]^*[ISO]))$, where the measured values of K_{ia}, K_{NADP}, and K_{iso} were 23.1, 33.4, and 5.37 μM, respectively (Roy and Packard, 1998), and the [$NADP^+$] and [ISO] time courses were modelled from the pyruvate and cell protein in the culture. The measurements of R_{O_2} and R_{CO_2} were made according to Berdalet *et al.* (1995). Experiment identification numbers are given in the upper corners of the plots.

In contrast to this large-scale focus, the enzyme kinetic model (EKM) aims to predict changes in the respiration rate during changes in physiological state in the same species, over relatively short periods (i.e. 24 h). In these situations, the biomass will change several fold, but not by orders of magnitude. Here, we examine the case of a marine bacterium growing on pyruvate. Figure 3 contrasts the measured respiration rate of a growing, well-nourished *P. nautica* culture (batch mode), with the respiration rate as the culture falls into a nutrient-deficient physiological state. The respiration increases with biomass (cell-protein) while pyruvate is present in the culture, but when the culture becomes pyruvate-deficient, the respiration drops while the biomass remains high. An attempt to model respiration in this culture with the MTE finds that biomass alone cannot serve as a proxy for respiration after nutrient limitation (after $h = 12$), because the biomass forces the MTE-predicted respiration to remain high while the measured respiration falls (Figure 3). Here, both Kleiber's law and the MTE are not able to predict respiration during nutrient limitation.

Another difference between the two models is that the EKM seeks a more fundamental understanding of respiration than biomass and resource availability [Equation (1)]. It seeks to build on causality. It argues that respiration and biomass track each other well in eukaryots (Figure 2a), because biomass contains mitochondria, and mitochondria contain the respiratory electron transport system. Because the respiratory ETS is a constitutive-enzyme system, it correlates with cell biomass (Figure 2b) even better than does respiration (Figure 2a), the difference likely caused by differences in physiological or nutritional states. For resource availability, the EKM proposes that enzyme kinetics, via substrate-dependent regulation of the maximum rate capacity (V_{max}), can explain mechanistically the variations in respiration (Packard *et al.*, 1996b, 2004). EKM argues that respiration is controlled by the levels of pyridine nucleotides available to the ETS and that the ETS controls the delivery rate of electrons to cytochrome oxidase and consequently the reduction of O_2 to H_2O. Accordingly, this implies that, for respiratory oxygen consumption, potential respiration, the V_{max} of the ETS is an appropriate predictor. For zooplankton, if in Figure 2a we replace dry weight with potential respiration (Φ), we find the correlation improved (Figure 4a) and, because the slope is so close to 1, we can eliminate the use of logarithms (Figure 4b). This figure shows that over more than five orders of magnitude, respiration and Φ are linearly related. Furthermore, the R–Φ ratio of 0.47 (Figure 4b) argues that the *in vivo* substrate level of these organisms (representing five phyla) was close to the Michaelis–Menten constant (K_m), i.e. the point at which the respiration rate = one half of the V_{max} or the potential rate (Figure 5).

In addition to using V_{max}, the EKM also uses the Michaelis–Menten rectangular hyperbolic expression, $S/(K_m+S)$, as in

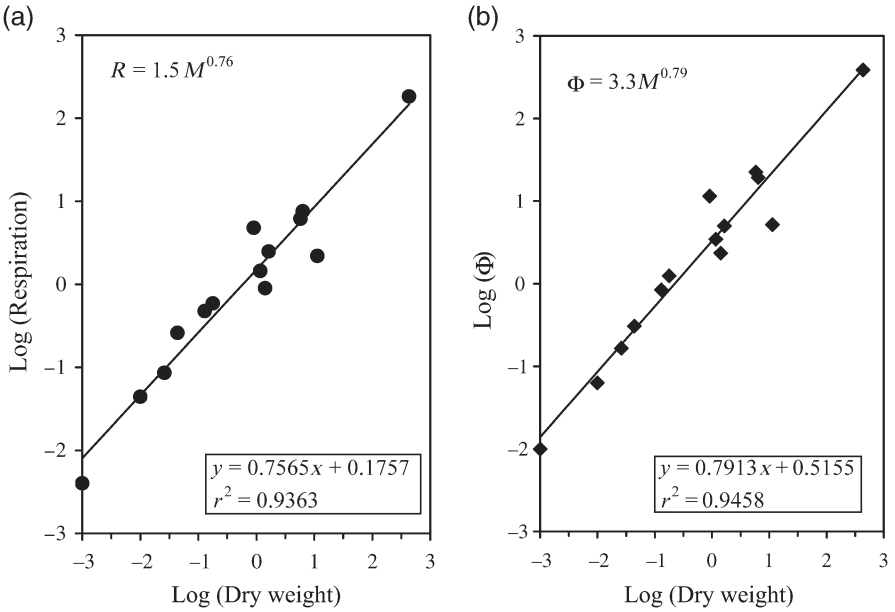

Figure 2. (a) Logarithmic plot of respiratory O_2 consumption [μl O_2 h^{-1} (animal)$^{-1}$] as a function of dry weight (mg animal^{-1}) in five phyla of zooplankton. *Tomopteris septentrionalis* represented Annelida; *Sagitta elegans*, Chaetognatha; and *Pleurobrachia bacheii*, Ctenophora. Arthropoda was represented by eight species of crustaceans that included Amphopoda, Copepoda, Decapoda, and Euphausiacea. Coelenterata was represented by *Leuckartiara octona*, *Phialidium gregarium*, and *Stomotoca artra*. The power function $R = 1.5\,M^{0.76}$ is in close agreement with Kleiber's law (Hochachka and Somero, 2002; Whitfield, 2006). (b) Logarithmic plot of potential respiratory O_2 consumption [μl O_2 h^{-1} (animal)$^{-1}$] as a function of dry weight (mg animal^{-1}) in the same zooplankton. The data are recalculated and replotted from King and Packard (1975).

Equation (3) (Packard *et al.*, 1996b, 2004). The parallel-decline in the pyruvate and the respiration rate between hours 10 and 13 in Figure 3 and the decline in the enzyme activity as the substrate for the enzyme falls in a Michaelis–Menten plot (Figure 5a) suggest that, as the pyruvate in the culture medium falls, so do the ETS reactants (Figure 5a and b) and the respiration rate (Figure 3). When the pyridine nucleotide substrates of the ETS are modelled as a time function (Figure 5b) and inserted in the bisubstrate version of Equation (2) along with the Φ time course in Figure 6, the EKM can accurately calculate respiratory O_2 consumption during periods of both nutrient limitation and nutrient sufficiency (Figure 6). To our knowledge, no other model of

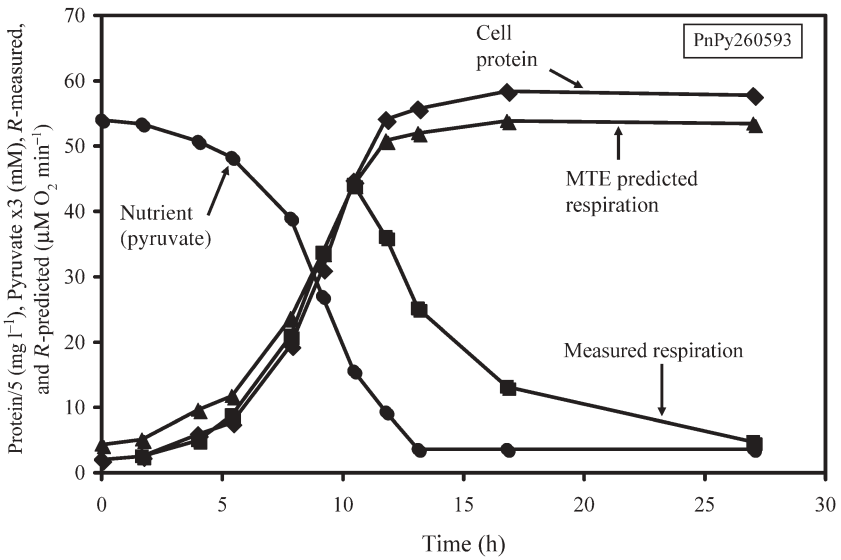

Figure 3. Respiration at different physiological states in the marine bacterium *Pseudomonas nautica* as in Figure 2(PnPy260593). Note the drop in the respiration (solid squares) after the pyruvate (solid circles) became limited. The MTE prediction of respiration from biomass according to Kleiber's law using the equation $R = 0.76\,M^{0.75}$ from Figure 2a, where M is the cell protein in the bacteria culture. Note that, as the nutrients become limiting after hour 10, the predicted R and the measured R diverge. Kleiber's law does not apply here.

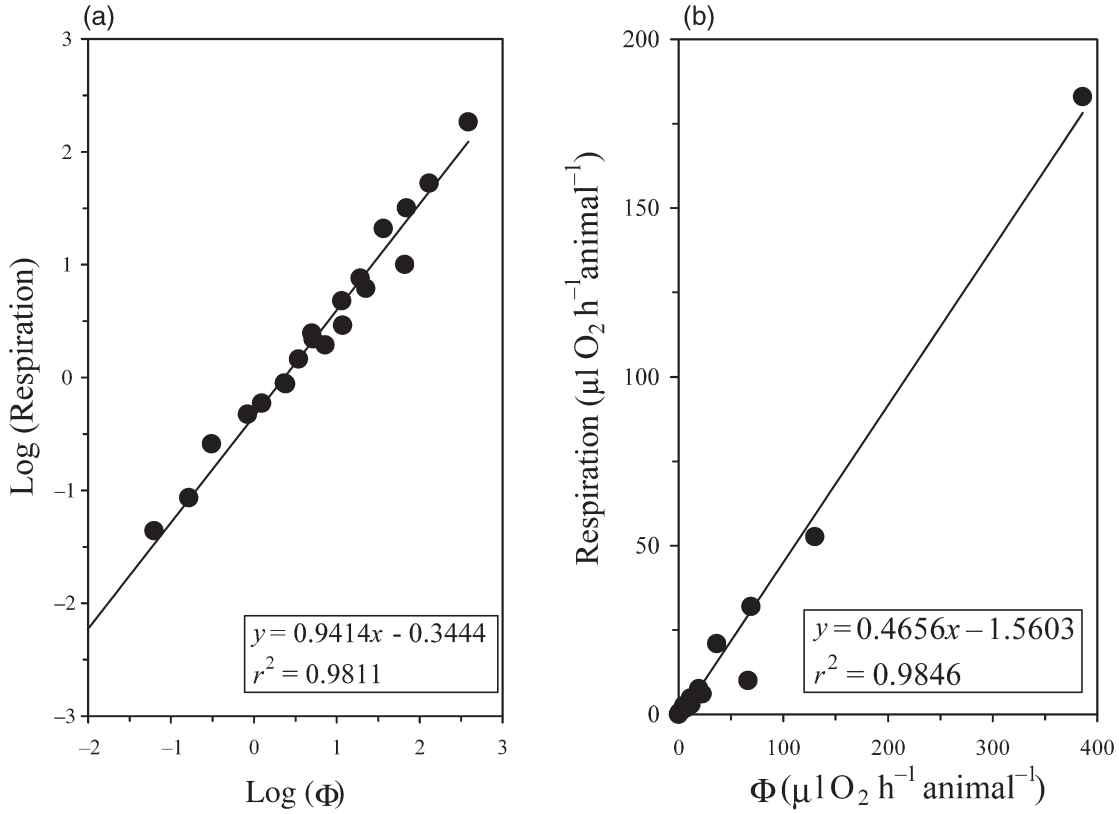

Figure 4. (a) Respiratory oxygen consumption as a logarithmic function of potential respiration [µl O_2 h^{-1} $(animal)^{-1}$] in the same zooplankton given in Figure 2 and King and Packard (1975). (b) Respiratory oxygen consumption as a linear function of Φ [µl O_2 h^{-1} $(animal)^{-1}$] in the same zooplankton given in Figure 2a and King and Packard (1975). Accordingly, $R = f(\Phi)$ is a linear function in the EKM [Equation (2)] rather than a power function.

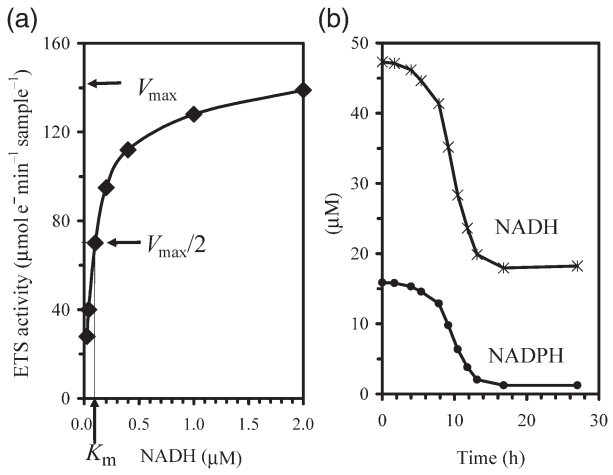

Figure 5. (a) Michaelis–Menten plot explaining the idealized relationship between the activity (velocity) of an enzyme reaction and the concentration of its substrate. Shown are the locations of V_{max} and the Michaelis–Menten constant, K_m. The relationship between ETS activity and one of its substrates (NADH) is used as an example. (b) NADH and NADPH concentrations as a function of time, modelled according to the algorithms in Packard *et al.* (1996b). Both substrates were modelled as functions of the carbon source (pyruvate) and the biomass (cell-protein) according to the equations: NADH (µM) = 2.6*(Pyr) + 0.052*(Pro) and NADPH (µM) = 0.882*(Pyr) + (5.9 10^{-4})*(Pro).

respiration can predict the oxygen consumption rate at the level of accuracy shown in Figure 6.

Discussion

The final difference in the two models is the temperature term. The MTE uses the Boltzmann factor ($e^{-E_a/kT}$) from statistical mechanics with the atomic-scale Boltzmann constant, the absolute temperature, and atomic-scale Arrhenius energy of activation in electron volts (eV), according to Gillooly *et al.* (2001). The atomic scale is used by atomic, nuclear, and particle physicists, because it is convenient (Feynman, 1998), but normally it is not used by other scientific communities. On the other hand, the EKM works at the molar level using Arrhenius' theory and equation, $R = Ae^{-E_a/R_gT}$, relating absolute temperature to biological, biochemical, and chemical rates according to more than a century of research following Arrhenius's seminal paper in 1889 (Arrhenius, 1889, 1915; Seiwell, 1937; Sizer, 1942; Moore, 1955; Segel, 1976; Ikeda *et al.*, 2000; Hochachka and Somero, 2002; Angelova *et al.*, 2007). It calculates the Φ_1 at T_1 from the Φ_0 measured at T_0 by exploiting the fact that A is constant for the reaction. Thus, the temperature expression in the EKM [Equation (2)] is $e^{-E_a(1/T_1-1/T_0)/R_g}$ rather than $e^{-E_a/kT}$ as in the MTE algorithm [Equation (1)]. The scale difference in the two models is large, i.e. Avogadro's number (6.022×10^{23}atoms mol^{-1}). However, if one does not mix scales and calculates e^{-E_a/R_gT} using molar-scale units of J mol^{-1}, J mol^{-1} K^{-1}, and $K^{-1}$and calculates

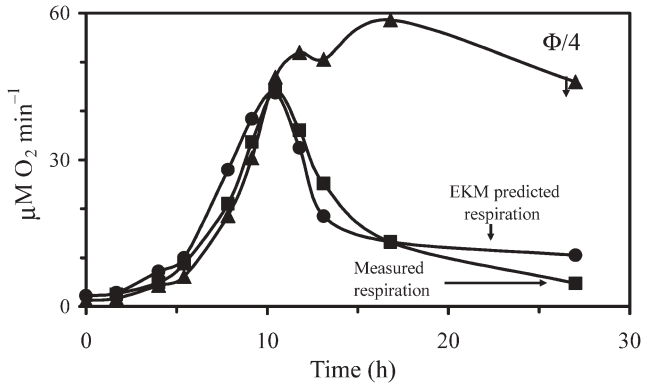

Figure 6. Potential respiration (as $\Phi/4$), measured respiration (solid squares), and the EKM predicted respiration (solid circles) in the marine bacterium, *Pseudomonas nautica* as in Figure 1a (PnPy260593). Note that Φ does not drop with the respiration after pyruvate was exhausted, but from Φ, with the reduced substrates (Figure 5b), and using Equation (2), the EKM predicts low respiration after pyruvate was exhausted. Note also the parallelism between the measured respiration and the EKM-predicted respiration through the entire 27 h time course of the experiment.

$e^{-E_a/kT}$ using the atomic-scale units of eV, eV K^{-1}, and K^{-1}, then e^{-E_a/R_gT} will equal $e^{-E_a/kT}$. However, the great body of biological, biochemical, and chemical literature over the last century dealing with energy of activation and temperature-dependence of biological rate processes is in molar units. Switching to eV units, which are not mainstream SI units, will confuse and fractionate the biological community. Electron volt is a unit that even Richard Feynman, the Nobel laureate physicist, argued against (Feynman, 1997). For these reasons, the EKM uses the Arrhenius equation, embedded in Equation (2), to calculate respiration.

The MTE, based on Kleiber's law, uses mass as its primary predictor of respiration and, although respiration can be predicted over as much as 20 orders of magnitude in body mass, the uncertainty in the prediction may be as much as several orders of magnitude (Hochachka and Somero, 2002; Whitfield, 2006). Such a large margin of error will handicap the MTE in resolving fine-scale problems. We argue that, by thinking about causality, one can construct a model based on dynamic biochemistry that can yield more understanding of respiration than can a model based on biomass. In the 20th century, Warburg, Keilin, Szent-Györgyi, Krebs, Lehninger, Chance, Green, Slater, Boyer, and Mitchell explained the role in respiration of the cell-free extracts, oxidative enzymes, the cytochromes, the mitochondria, ATP, the electron transfer chain, oxidative phosphorylation, and proton motive force (Fruton and Simmonds, 1958; Keilin, 1966; Mahler and Cordes, 1971; Lehninger *et al.*, 1993; Nelson and Cox, 2005). They do not invoke biomass in any of their explanations. Knowing this, respiratory biochemistry yields so much more understanding of respiration than can be attributed to biomass alone that it is hard to understand why we do not focus our attention down through the biomass of an organism, the organs and tissues, down past the non-respiring ribosomes, endoplasmic reticulum, and vacuoles to the mitochondria. From there, we can continue to focus down into the mitochondrial inner membrane where the five complexes of the ETS control the oxidation of the reduced pyridine nucleotides (produced in carbon oxidation), the production of ATP, and the consumption of oxygen. Here, ETS complex I, the complex that oxidizes the pyridine nucleotides, regulates the electron flux to O_2 and hence the rate of O_2 consumption. This flux sets the respiratory potential, the V_{max} of respiration. Using this V_{max} as a predictor of respiration, rather than biomass of the organism (M), alone yields a mechanistic understanding of respiration. However, the predictive capability becomes even greater upon realizing that, because V_{max} is a reaction rate, one can apply the theories of both Michaelis–Menten, to resource limitation (substrates), and Arrhenius, to predict temperature dependence. With this understanding, it becomes possible to write a first-principle-based model of respiration as in Equation (2).

Therefore, is Equation (2) a good first-principles model of zooplankton respiration from potential respiration, enzyme kinetics, and the Arrhenius equation? Because the dominant part of the EKM is the relationship between the respiration and the potential respiration and because this relationship, $R = f(\Phi) = 0.466^{*}\Phi - 1.560$ ($r^2 = 0.9846$) or $R = 0.54^{*}(\Phi)^{0.9414}$ ($r^2 = 0.9811$), describes the respiration rate in zooplankton so well over five orders of magnitude, the EKM has a good chance of being successful. Add to this, the probability that the successful use of Michaelis–Menten kinetics to describe the variations of respiration during different physiological states in bacteria (Figure 6) can be repeated in zooplankton, and the chance of the EKM being successful becomes even greater. Furthermore, Sizer (1942), Moore (1955), Pauling (1958), Eggers *et al.* (1964), Fruton and Simmonds (1958), Giese (1963), Prosser and Brown (1961), Mahler and Cordes (1971), Segel (1976), Lehninger *et al.* (1993), and Hochachka and Somero (2002) have explained to generations of students how, via the Arrhenius equation, temperature determines the rate of chemical reactions, biochemical reactions, enzymatic reactions, and the physiological processes built on these reactions. Consequently, the use of Equation (2) with the Arrhenius equation embedded in it should describe respiratory temperature dependence well.

Conclusions

(i) The MTE and Kleiber's law describe respiration over a large range of body sizes, but do not describe respiration on short time-scales or in different physiological states (Figures 2a and 4).

(ii) The Arrhenius equation describes the impact of temperature on the organism's respiration within the normal temperature range of an organism's habitat.

(iii) The EKM argues that predictions of respiration could be improved, as in Figure 6, if the respiration model were based on potential respiration and not an organism's biomass.

(iv) Finally, the EKM states that, at any temperature, respiration is the product of potential respiration and the relative availability of the substrates that modulate the potential respiration [Equation (3)]. On this basis, the model can explain and accurately predict respiration on short time-scales (Figure 6).

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References

Angelova, B., Avramova, T., Stefanova, L., and Mutafov, S. 2007. Temperature effect on bacterial azo bond reduction kinetics: an Arrhenius plot analysis. Biodegradation. DOI 10.1007/s10532–007–9144–4.

Arrhenius, S. 1889. Uber die Reaktionsgeschwindigkeit bei der Inversion von Rohrzucker durch Sauren. Zeitschrift für Physikalische Chemie Stochiometrie und Verwandtschaftslehre, 4: 226–248.

Arrhenius, S. 1915. Quantitative Laws in Biological Chemistry. Bell and Son, London. 164 pp.

Berdalet, E., Packard, T. T., Lagacé, B., Roy, S., St-Amand, L., and Gagné, J. P. 1995. CO_2 production, O_2 consumption, isocitrate dehydrogenase and electron transport system activities in the marine bacterium *Pseudomonas nautica*. Aquatic Microbial Ecology, 9: 211–217.

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., and West, G. B. 2004. Towards a metabolic theory of ecology. Ecology, 85: 1771–1789.

Eggers, D. F., Gregory, N. W., Halsey, G. D., and Rabinovitch, B. S. 1964. Physical Chemistry. John Wiley and Sons, Inc., New York. 783 pp.

Enquist, B. J., Brown, J. H., and West, G. B. 1998. Allometric scaling of plant energetics and population density. Nature, 395: 163–165.

Feynman, R. R. 1997. Six not-so-easy pieces: Einstein's relativity, symmetry, and space–time. Perseus Books, Cambridge, MA. 152 pp.

Fruton, J. S., and Simmonds, S. 1958. General Biochemistry. John Wiley and Sons, Inc., New York. 1077 pp.

Giese, A. C. 1963. Cell Physiology. Saunders and Co., Philadelphia. 592 pp.

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., and Charnov, E. L. 2001. Effects of size and temperature on metabolic rate. Science, 293: 2248–2251.

Gillooly, J. F., Charnov, E. L., West, G. B., Savage, V. M., and Brown, J. H. 2002. Effects of size and temperature on developmental time. Nature, 417: 70–73.

Gómez, M., Torres, S., and Hernández-León, S. 1996. Modification of the electron transport system (ETS) method for routine measurements of respiratory rates of zooplankton. South African Journal of Marine Science, 17: 15–20.

Hochachka, P. W., and Somero, G. N. 2002. Biochemical Adaptation. *In* Mechanism and Process in Physiological Evolution. Oxford University Press, New York. 466 pp.

Ikeda, T., Torres, J. J., Hernández-León, S., and Geiger, S. P. 2000. Metabolism. *In* ICES Zooplankton Methodology Manual, pp. 455–532. Ed. by R. Harris, P. H. Wiebe, J. Lenz, H. R. Skojoldal, and M. Hintley. Academic Press, London. 684 pp.

Keilin, D. 1966. The History of Cell Respiration and Cytochrome. Cambridge University Press, London. 416 pp.

King, F. D., and Packard, T. T. 1975. Respiration and the activity of the respiratory electron transport system in marine zooplankton. Limnology and Oceanography, 20: 849–854.

Kleiber, M. 1932. Body size and metabolism. Hilgardia, 6: 315–332.

Lehninger, A. L., Nelson, D. L., and Cox, M. M. 1993. Principles of Biochemistry. Worth Publishers, New York. 1013 pp.

López-Urrutia, Á., San Martín, E., Harris, R., and Irigoien, X. 2006. Scaling the metabolic balance of the oceans. Proceedings of the National Academy of Science of the United States of America, 103: 8739–8744.

Mahler, H. R., and Cordes, E. H. 1971. Biological Chemistry. Harper and Row, New York. 1009 pp.

Martínez, R., and Estrada, M. 1992. Respiratory electron transport activity of microplankton in the Weddell Sea during early spring: influence of the ice cover and the ice edge. Polar Biology, 12: 275–282.

Moore, W. J. 1955. Physical Chemistry. Prentice-Hall Chemistry Series. Prentice-Hall, Inc., Englewood Cliffs, NJ. 633 pp.

Nelson, D. L., and Cox, M. M. 2005. Principles of Biochemistry. Freeman and Co., New York. 1119 pp.

Owens, T. G., and King, F. D. 1975. The measurements of respiratory electron-transport-system activity in marine zooplankton. Marine Biology, 30: 27–36.

Oxtoby, D. W., Gillis, H. P., and Nachtrieb, N. H. 1999. Principles of Modern Chemistry. Saunders College Publishing, Fort Worth, TX. 876 pp. plus appendices.

Packard, T. T., Berdalet, E., Blasco, D., Roy, S., St-Amand, L., Legacé, B., Lee, K., *et al.* 1996b. Oxygen consumption in the marine bacterium, *Pseudomonas nautica* predicted from ETS activity and bisubstrate kinetics. Journal of Plankton Research, 18: 1819–1835.

Packard, T. T., Berdalet, E., Roy, S., Blasco, D., Lee, K., and Gagné, J. P. 1996a. CO_2 production predicted from isocitrate dehydrogenase activity and bisubstrate enzyme kinetics in the marine bacterium, *Pseudomonas nautica*. Aquatic Marine Ecology, 11: 11–19.

Packard, T. T., Blasco, D., and Estrada, M. 2004. Modeling physiological processes in plankton on enzyme kinetic principles. *In* Biological Oceanography at the Turn of the Millenium. Ed. by J. M. Gili, J. L. Pretus, and T. T. Packard. Scientia Marina, 68(Suppl. 1): 49–56.

Packard, T. T., Devol, A. H., and King, F. D. 1975. The effect of temperature on the respiratory electron transport system in marine plankton. Deep Sea Research, 22: 237–249.

Packard, T. T., Harmon, D., and Boucher, J. 1974. Respiratory electron transport activity in plankton from upwelling waters. Téthys, 6: 213–222.

Packard, T. T., Healy, M. L., and Richards, F. A. 1971. Vertical distribution of the activity of the respiratory electron transport system in marine plankton. Limnology and Oceanography, 16: 60–70.

Packard, T. T., Williams, P. J., and Le, B. 1981. Rates of respiratory oxygen consumption and electron transport in surface seawater from the northwest Atlantic Ocean. Oceanologica Acta, 4: 351–358.

Pauling, L. 1958. General Chemistry (An Introduction to Descriptive Chemistry and Modern Chemical Theory). A Series of Chemistry texts. W. H. Freeman and Co., San Francisco. 710 pp.

Prosser, C. L., and Brown, F. A. 1961. Comparative Animal Physiology. W. B. Saunders Co., Philadelphia. 688 pp.

Ramirez, T., Liger, E., Mercado, J. M., Cortes, D., Vargas-Yañez, M., Sabastián, M., Reul, A., *et al.* 2006. Respiratory ETS activity of plankton in the northwestern Alboran Sea: seasonal variability and relationship with hydrological and biological features. Journal of Plankton Research, 28: 629–641.

Roy, S. O., and Packard, T. T. 1998. NADP-isocitrate dehydrogenase from *Pseudomonas nautica*: kinetic constants determination and carbon limitation effects on the pool of intracellular substrates. Applied and Environmental Microbiology, 64: 4858–4964.

Roy, S. O., and Packard, T. T. 2001. CO_2 production rate predicted from isocitrate dehydrogenase activity, intracellular substrate concentrations and kinetic constants in the marine bacterium, *Pseudomonas nautica*. Marine Biology, 138: 1251–1258.

Savenkoff, C., Packard, T. T., Rodier, M., Gérino, M., Lefèvre, D., and Denis, M. 1995. Relative contribution of dehydrogenases to respiratory ETS activity in some marine organisms. Journal of Plankton Research, 17: 1593–1604.

Segel, I. H. 1976. Biochemical Calculations: How to Solve Mathematical Problems in General Biochemistry. John Wiley and Sons, New York. 441 pp.

Seiwell, H. R. 1937. Consumption of oxygen in seawater under controlled laboratory conditions. Nature, 140: 506–507.

Sizer, I. W. 1942. Effects of temperature on enzyme kinetics. Advances in Enzymology, 3: 35–62.

Treacy, J., Curley, M., Wenger, J., and Sidebottom, H. 1997. Determination of Arrhenius parameters for the reactions of ozone with cycloalkenes. Journal of the Chemical Society, Faraday Transactions, 93: 2877–2881.

West, G. B., Brown, J. H., and Enquist, B. J. 1997. A general model for the origin of allometric scaling laws in biology. Science, 276: 122–126.

West, G. B., Brown, J. H., and Enquist, B. J. 2000. The origin of universal scaling laws in biology. *In* Scaling in Biology, pp. 87–112. Ed. by J. H. Brown, and G. B. West. Oxford University Press, Oxford, UK.

Whitfield, J. 2006. In the Beat of a Heart: Life, Energy, and the Unity of Nature. Joseph Henry Press. 261 pp.

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