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Modeling physiological processes in plankton on enzyme kinetic principles*

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SUMMARY: Many ecologically important chemical transformations in the ocean are controlled by biochemical enzyme reactions in plankton. Nitrogenase regulates the transformation of N_2 to ammonium in some cyanobacteria and serves as the entryway for N_2 into the ocean biosphere. Nitrate reductase controls the reduction of NO_3 to NO_2 and hence new production in phytoplankton. The respiratory electron transfer system in all organisms links the carbon oxidation reactions of intermediary metabolism with the reduction of oxygen in respiration. Rubisco controls the fixation of CO_2 into organic matter in phytoplankton and thus is the major entry point of carbon into the oceanic biosphere. In addition to these, there are the enzymes that control CO_2 production, NH_4 excretion and the fluxes of phosphate. Some of these enzymes have been recognized and researched by marine scientists in the last thirty years. However, until recently the kinetic principles of enzyme control have not been exploited to formulate accurate mathematical equations of the controlling physiological expressions. Were such expressions available they would increase our power to predict the rates of chemical transformations in the extracellular environment of microbial populations whether this extracellular environment is culture media or the ocean. Here we formulate from the principles of bisubstrate enzyme kinetics, mathematical expressions for the processes of NO_3 reduction, O_2 consumption, N_2 fixation, total nitrogen uptake,

Key words: allometry, electron transport, ETS, glutamine synthase, nitrate reductase, nitrogen fixation, nitrogenase.

RESUMEN: MODELADO DE PROCESOS FISIOLÓGICOS EN EL PLANCTON BASADOS EN PRINCIPIOS DE CINÉTICA ENZIMÁTICA. – En el océano, muchas transformaciones químicas importantes desde el punto de vista ecológico son controladas por reacciones bioquímicas enzimáticas en el plancton. La nitrogenasa regula la transformación de N_2 a amonio en algunas cianobacterias y sirve como vía de entrada para el N_2 en la biosfera oceánica. La nitrato reductasa controla la reducción de NO_3 a NO_2 y, con ello, la producción nueva en el fitoplancton. El sistema respiratorio de transferencia de electrones en todos los organismos conecta las reacciones de oxidación del carbono del metabolismo intermediario con la reducción del oxígeno en la respiración. La rubisco controla la fijación de CO_2 en materia orgánica en el fitoplancton, y así es el principal punto de entrada del carbono en la biosfera oceánica. Además de estos enzimas, están los que controlan la producción de CO_2, la excreción de NH_4 y los flujos de fosfato. Algunos de tales enzimas han sido reconocidos e investigados por los científicos del mar en los últimos treinta años. Sin embargo, hasta fecha reciente los principios cinéticos del control enzimático no han sido explotados para formular ecuaciones matemáticas precisas de las expresiones fisiológicas de control. Si se dispusiera de tales ecuaciones, aumentaría nuestra capacidad de predecir las tasas de las transformaciones químicas en el ambiente extracelular de las poblaciones microbianas, ya fuera este ambiente extracelular los medios de cultivo o el océano. En este trabajo formulamos, a partir de los principios de la cinética enzimática bisustrato, expresiones matemáticas para los procesos de la reducción de NO_3, el consumo de O_2, la fijación de N_2 y la absorción de nitrógeno total.

Palabras clave: alometría, transporte de electrones, ETS, glutamina sintasa, nitrato reductasa, fijación de nitrógeno, nitrogenasa.

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INTRODUCTION

Oceanic biogeochemical cycles of nitrogen, carbon, and oxygen are greatly impacted, if not controlled, by a few essential enzymes and enzyme systems. Among these, rubisco, the CO_2 fixing enzyme in photosynthesis, is the most abundant enzyme and cytochrome oxidase, the oxygen-reducing enzyme in respiration, is the most widespread enzyme on the planet. Rubisco will be found in all the algae and higher plants while cytochrome oxidase will be found in all the animals, protozoa, most microbes, as well as in all the plants and algae in the ocean. In addition to these two essential enzymes, three others, nitrate reductase, glutamine synthase, and nitrogenase will command the gateways to the oceanic biosphere for the mineral forms of nitrogen, NO_3^-, NH_4^+ and N_2. Nitrate reductase will be found in all algae, the phytoplankton. Glutamine synthase will be found in both autotrophs and heterotrophs, as is cytochrome oxidase. Nitrogenase will be found in the nitrogen fixing cyanobacteria that live in the oligotrophic gyres of the oceans.

The objective here is to present kinetic models of oceanographically significant enzymes and the physiological processes they control. The enzyme/physiological rate pairs considered are: (1) nitrate reductase/phytoplankton NO_3 uptake; (2) respiratory electron transfer system/respiratory O_2 consumption (universal); (3) nitrogenase/cyanobacterial N_2 fixation; and (4) glutamine synthase/ total nitrogen assimilation (universal). Each physiological rate is difficult to measure in situ and each lacks a first-principle-based mathematical description required for its calculation in ocean space.

For some of these processes, respiration, for example, we have statistically-based allometric equations to facilitate calculation from body size, body surface, or biomass (Weibel, 2002). However, we do not have comparably accurate equations based on fundamental principles, but they could be developed. Equations for physiological processes based on biochemical principles and properties such as enzyme activities and substrate concentrations are not unreasonably difficult to conceive. Such equations would provide a means for calculating physiological rates when direct measurements are impractical. This practice is followed in other scientific disciplines (Mohr and Taylor, 2001) and would greatly facilitate oceanographic research as well as ecosystem modeling. The biochemical models presented here are designed to advance this approach by introducing equations based on multisubstrate enzyme kinetics. These models follow the equations designed to calculate phytoplankton nitrate uptake (Packard *et al*., 1971a), and bacterial respiration (Packard *et al*., 1996a; 1996b; Roy and Packard, 2001) and are conceptually similar to Farquhar's photosynthetic model (Farquhar *et al*., 1980).

Our objective parallels that of the *in silico* movement (Taubes, 2002) at Stanford University (USA), but on a smaller scale. The *in silico* objective is to simulate, via nested algorithms on the computer, the integrated biology of a cell. Such a simulation would integrate all physiological processes and enzyme reactions of a cell! Here we limit the focus to only a few of these processes. However, just as the *in silico* research will facilitate computer experimentation with new pharmaceutical products and medical procedures before experimentation with animals, our algorithms will facilitate computer modeling of oceanic ecosystems before undertaking expensive experimentation at sea. The approach requires laboratory experimentation to determine essential biochemical kinetic constants and intracellular substrate concentrations, but as a recent article on modeling cellular processes from the Molecular Science Institute at Berkeley, California argues, there is a great need for such measurements (Endy and Brent, 2001).

CONCEPT

To derive quantitative expressions of physiological processes based on first principles we start by identifying certain assumptions and boundaries that become the foundation for our equation development.

1. We limit our attention to those physiological rate processes that are controlled by a measurable enzyme system such as nitrate uptake and assimilation, respiratory electron transfer activity, and nitrogen fixation. We do not try to work with rubisco and photosynthesis because rubisco's CO_2 fixing activity is complicated by its oxygen binding activity. (For every 3 molecules of CO_2 that rubisco fixes to ribulose 1,5 bisphosphate, it fixes one molecule of O_2.) Furthermore, we do not attempt to include the more complex processes such as growth, ingestion, or grazing at this time, because rate-limiting chemical reactions for these processes have yet to be identified.

2. The studied processes are considered the direct result of intracellular reactions that follow a definable stoichiometry, and are catalyzed by specific enzymes. The following relationships are examples:

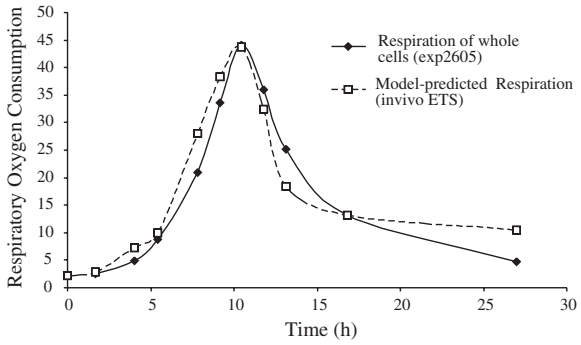

FIG. 1. – Changes in respiratory oxygen consumption as a bacteria culture (*Pseudomonas nautica*) passes from substrate-unlimited exponential growth (5-11 h) through substrate limitation (13-15h) to senescence (15-26 h). The measured and modeled respiration (μmol O_2 min^{-1} L^{-1}) are closely correlated ($r^2 = 0.921$). The modeled respiration is based on equations No. 5 and 6. (Redrawn from Packard *et al*., 1996a).

nitrogenase-N_2 fixation; isocitrate dehydrogenase-CO_2 production; and glutamine synthase-total nitrogen assimilation.

3. We consider that the regulation of these reactions, and hence the corresponding physiological processes, is controlled by a key enzyme as in the case of nitrate reductase for phytoplankton NO_3 reduction or glutamine synthase in the case of nitrogen assimilation.

4. Finally, we consider these enzymatic reactions to obey the rules of enzyme kinetics.

Here is a list of processes to which this approach could be applied: N_2 fixation, NO_3 reduction, NO_2 reduction, nitrification, denitrification, total N assimilation, NH_4 excretion, respiratory O_2 consumption, respiratory CO_2 production, respiratory ATP production, photosynthetic O_2 production, photosynthetic CO_2 consumption, photosynthetic ATP production, and photosynthetic NADPH production. All are of major importance in modeling ocean processes.

Previous application of this concept

Although this concept has been applied to photosynthesis (Farquhar *et al*., 1980), its development here is based on the models of nitrate reduction in phytoplankton (Packard *et al*., 1971a), respiratory oxygen consumption (Packard *et al*., 1996a) and respiratory carbon dioxide production (Packard *et al*., 1996b; Roy and Packard, 2001). In one of the earlier applications, the physiological rate of O_2 consumption was calculated from respiratory electron transfer activity without the benefit of intracellular substrate measurements and Michaelis constants (Packard *et al*., 1996a). Nevertheless, the respiration rates were calculated from a bisubstrate kinetic model and agreed substantially with the measured rates (Fig. 1). In the most recent application (Roy and Packard, 2001), the physiological rate of CO_2 production was calculated from a bisubstrate kinetic model using isocitrate dehydrogenase (IDH) activity, measurements of intracellular isocitrate and $NADP^+$, the IDH substrates, and the Michaelis constants. The agreement of the calculated CO_2-production-rate time courses with the measured time courses (Fig. 2) added support to the idea that physiolog-

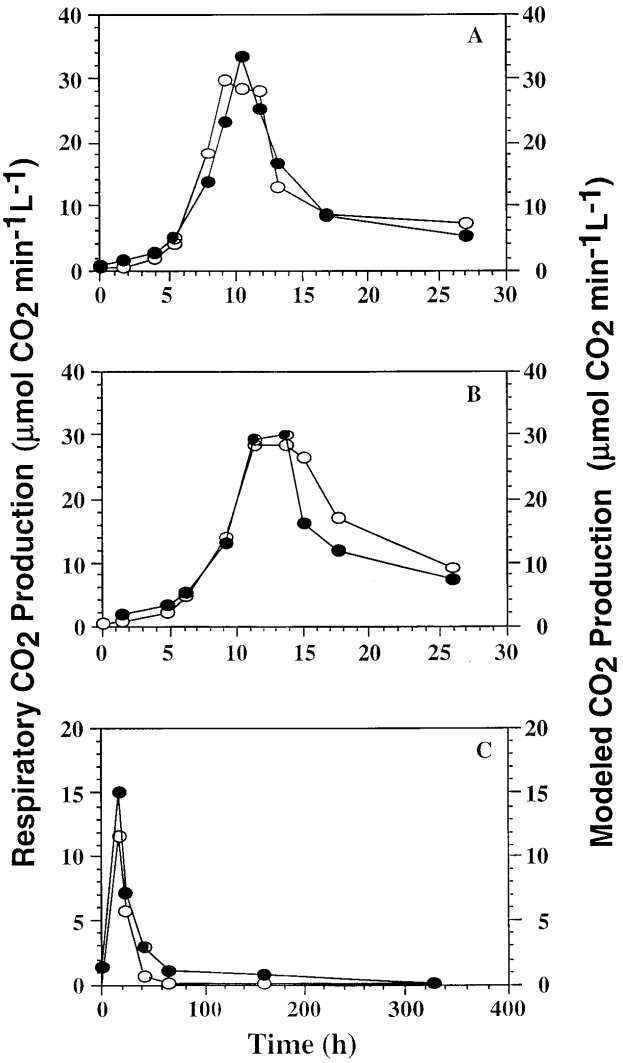

FIG. 2. – Changes in respiratory CO_2 production as three *Pseudomonas nautica* cultures pass from substrate-unlimited exponential growth to substrate limitation and senescence. Correspondence between the modeled respiratory CO_2 production rate (open circles) and the measured rate (full circles) for replicates of a 1 day time course experiment (A and B) and a 14 day time course experiment (C). The modeled CO_2 production rates are based on equations similar to No. 5 and 6, but for isocitrate dehydrogenase, $NADP^+$, and isocitrate rather than the respiratory ETS, NADH, and NADPH (Roy and Packard, 2001). The measured and modeled respiration (μmol CO_2 min^{-1} L^{-1}) in the three experiments are closely correlated. For experiments A, B, and C the r^2 equaled 0.88, 0.89, and 0.98. (Redrawn from Roy and Packard, 2001).

ical rates could be calculated from first principle models of their controlling enzyme kinetics (Roy and Packard, 2001). These results were sufficiently successful to encourage applying this approach to other physiological processes and their controlling enzyme systems. Hence comes this paper.

Future application of this concept

Using the above assumptions we will give quantitative expressions based on the enzyme reactions that control physiology rather than on the statistical relationship between the rate process and some causally unrelated parameter such as size or weight. We will apply the above precepts first to the processes of phytoplankton nitrate reduction and respiratory oxygen consumption in bacterioplankton. Then, we will address cyanobacterial nitrogen fixation and phytoplankton total nitrogen assimilation. Other processes will not be addressed here. For example, ammonium production by zooplankton would require a much more complicated kinetic model than we are using; an allosteric activator and repressor are involved in the reactions that control this process. In addition, photosynthetic energy conversion requires an assessment of cyclic and noncyclic electron flow between photosystem 1 and 2 as well as an assessment of NADPH production by ferredoxin: $NADP^+$ oxidoreductase. Thus, the mathematical model would be more complicated than the one we are proposing.

Nitrate reductase and assimilatory nitrate reduction

The time (t) dependent rate of nitrate reduction ($\partial[NO_3]/\partial t$) in a phytoplankton cell is catalyzed by the enzyme, nitrate reductase (NR), reacting with intracellular nitrate [NO_3] and the reduced pyridine nucleotide, [NADH]. The reaction is:

$$NO_3^- + H^+ + NADH \rightarrow NO_2^- + NAD^+ + H_2O \quad (1)$$

As a first approximation, one could develop a simple equation,

$$(-\partial[NO_3]/\partial t) = [NR_v] = C\ \{NR\}, \qquad (2)$$

where $[NR_v]$ is the in vivo enzyme activity of NR, {NR} is the in vitro enzyme activity of NR, and C is an empirically derived constant. Under certain conditions, such as exponential growth, C may approach a constant; however, under most field and laboratory conditions C is not a constant. As a result, this conceptual model has limited use (Blasco *et al*., 1984). Considering what we know about enzyme reactions and previous studies of isocitrate dehydrogenase (IDH) and CO_2 production in bacteria (Packard *et al*., 1996b; Roy and Packard, 2001), we propose a more realistic mathematical and chemical representation of nitrate uptake. Rather than considering only nitrate limitation as we did in our original model (Packard *et al*., 1971a), the proposed model would consist of an enzyme kinetic expression based on bisubstrate limitation by [NADH] as well as [NO_3]. In the parlance of enzyme kinetics (Mahler and Cordes, 1971; Segel, 1993) this can be expressed as:

$$(-\partial[NO_3]/\partial t) = [NR_v] = \\ =\{NR\}[NO_3^-][NADH]/(K_\beta + [NO_3^-][NADH]) \quad (3)$$

where

$$K_\beta = (K_{NADH})(K_{i\alpha}) + (K_{NO3})[NADH] + (K_{NADH})[NO_3^-] \quad (4)$$

Here, K_{NADH} and K_{NO3} are the bisubstrate-Michaelis constants for NADH and NO_3^-. K_{ia} is the dissociation constant for the enzyme-NO_3^- complex. Note that [NADH] and [NO_3^-] are the intracellular NR substrate concentrations, that $-\partial[NO_3]/\partial t$ is the physiological rate of nitrate reduction in the living phytoplankton cell and {NR} is the measurement of maximum in vitro activity (V_m) of the enzyme, nitrate reductase. The protons for the reduction process (Eq. 1) are not considered limiting and thus are not needed in the equation (Segel, 1993). Note also, that the constant, C, in equation 2 is replaced by the expression, $[NO_3^-][NADH]/(K_\beta + [NO_3^-][NADH])$, in Eq. 3.

To verify Eq. 3, the Michaelis constants, the dissociation constant, and the intracellular reactant concentrations [NO_3^-] and [NADH] need to be measured throughout different growth phases of the algal cell. Very likely the constants will be independent of time and growth phase, but it is not certain. The intercellular concentrations will vary with time and growth phase and can likely be calculated from external nitrate and ambient light, but as with the constants, experimental verification is needed.

Respiratory electron transfer system and respiration

Another process for which a mathematical model can be constructed is the respiratory oxygen consumption that occurs in all aerobes. This process is

controlled by the enzymatic respiratory electron transfer system (ETS), and the two electron transfer reactants, NADH and nicotinamide adenine dinucleotide phosphate (NADPH). (The omission of the succinate dehydrogenase is largely explained by its relatively minor role as compared to the roles of the dehydrogenases associated with the two pyridine nucleotides (Savenkoff *et al*., 1995). The reaction (Packard *et al*., 1996a) is:

$$(-\partial[O_2]/\partial t) = [ETS_v] = \{ETS\}[NADPH][NADH]/(K_\beta + [NADPH][NADH]) \quad (5)$$

$$K_\beta = (K_{NADH})(K_{i\alpha})+(K_{NADPH})[NADH]+(K_{NADH})[NADPH] \quad (6)$$

Here, K_{NADH} and K_{NADPH} are bisubstrate-Michaelis constants for NADH and NADPH. $K_{i\alpha}$ is the dissociation constant for the enzyme-NADH complex and [NADH] and [NADPH] are the intracellular ETS substrate concentrations (Savenkoff *et al*., 1995). $[ETS_v]$ is the in vivo enzyme activity of respiratory ETS, the equivalent of the physiological oxygen consumption rate in the living cell. {ETS} is the measurement of maximum activity (in vitro) of the respiratory ETS. Note that the expression, $[NADPH][NADH]/(K_\beta + [NADPH][NADH])$, in Eq. 5 replaces the ratio, R/ETS, discussed in the early literature on ETS in marine plankton(Packard *et al*., 1971b; Packard, 1985a, 1985b). As with the case of nitrate reductase, to verify this equation, the constants and the reactant concentrations will need to be measured experimentally throughout different growth phases.

Nitrogen fixation

We have seen how equations could be developed for respiratory oxygen consumption and phytoplankton nitrate uptake. We will now develop equations for nitrogen fixation by cyanobacteria. This process is wide spread in the tropical oligotrophic ocean. The time dependent rate of the process ($-\partial[N_2]/\partial t$) is carried out by the enzyme, dinitrogenase (dNRg), reacting with dissolved dinitrogen gas $[N_2]$, protons $[H^+]$, ATP, and reducing equivalents (e^-) transferred via the enzyme dinitrogenase reductase. The overall reaction is:

$$N_2 + 16ATP + 10H^+ + 8e^- \rightarrow \rightarrow 16ADP + 2\,NH_4^+ + 16PO_4 + H_2 \quad (7)$$

Building on our model of nitrate uptake, we can develop an enzyme kinetic expression based on limitation by [ATP] and $[e^-]$. The availability of protons and nitrogen gas can be considered unlimited. In this model $(-\partial[N_2]/\partial t) = [dNRg_v] = f\{dNRg\}$, where $[dNRg_v]$ is the in vivo dinitrogenase activity and {dNRg} is the in vitro dinitrogenase activity. As before, our equation takes the form:

$$(-\partial[N_2]/\partial t) = [dNRg_v] = = \{dNRg\}[e^-][ATP]/(K_\beta + [e^-][ATP]) \quad (8)$$

where

$$K_\beta = (K_{ATP})(K_{i\alpha}) + (K_e)[ATP] + (K_{ATP})[e^-] \quad (9)$$

Here, K_{ATP} and K_e are the bisubstrate-Michaelis constants for ATP and e^-. K_{ia} is the dissociation constant for the enzyme complex. [ATP] and $[e^-]$ are intracellular dNRg substrate concentrations, $-\partial[N_2]/\partial t$ is the physiological rate of nitrogen fixation in the living cyanobacterial cell and {dNRg} is the measurement of maximum in vitro activity (V_m) of the dinitrogenase.

Total nitrogen assimilation

This process in phytoplankton includes nitrogen fixation, nitrate uptake, ammonium uptake, and urea uptake. The enzyme responsible for this universal process is glutamine synthase (GS). The time dependent rate of this process ($-\partial[N]/\partial t$) is carried out by GS, reacting with glutamate, ATP, and dissolved ammonium $[NH_4^+]$. The overall reaction is:

$$ATP + glutamate + NH_4^- \rightarrow \rightarrow ADP + H^+ + PO_4 + glutamine \quad (10)$$

An enzyme kinetic expression for $-\partial[N]/\partial t$ can be based on limitation by [ATP] and $[NH_4^-]$. The availability of glutamate can be considered unlimited because in the cell this reaction is coupled with the glutamate synthase reaction that recycles glutamine back to glutamate. This condition can be simulated in an enzyme assay by adding excess glutamate to the reaction mixture. In this model for total nitrogen assimilation $(-\partial[N]/\partial t) = [GS_v] = f\{GS\}$, where $[GS_v]$ is the in vivo glutamine synthase activity and {GS} is the in vitro glutamine synthase activity. Our equation takes the form:

$$(-\partial[N]/\partial t) = [GS_v] = = \{GS\}[NH_4^+][ATP]/(K_\beta + [NH_4^+][ATP]) \quad (11)$$

where

$$K_{\beta} = (K_{ATP})(K_{ia}) + (K_{NH4+})[ATP] + (K_{ATP})[NH4^{+}] \quad (12)$$

Here, K_{ATP} and K_{NH4+} are the bisubstrate-Michaelis constants for ATP and NH_4^+. K_{ia} is the dissociation constant for the enzyme complex. [ATP] and $[NH_4^+]$ are intracellular GS substrate concentrations, $-\partial[N]/\partial t$ is the physiological rate of nitrogen assimilation in cell and {GS} is the measurement of maximum in vitro activity (V_m) of the glutamine synthase. Note that the application of this approach to whole plankton samples would be complicated by the fact that bacteria, protozoans, and zooplankton also have GS.

These mathematical models (Eqs. 3, 5, 8, and 11) are potential algorithms that could be nested in future ecosystems models. They should be considered as prototypes, preliminary drafts of models to be confirmed or modified by well-controlled and replicated laboratory research.

DISCUSSION

The rationale for using the terms, "first principles" and "meaningful equations," is based on the premise that "knowledge of the underlying causal dynamics of a phenomenon generally improves the prediction of events that fall outside the boundary conditions of available observations". This rationale can be illustrated by presenting two examples outside physiology. In addition, we will show how these equations could be used in a recent ecosystem model to improve the model's predictive capacity.

First principles

As stated in the introduction above, we seek to develop mathematical expressions that are based on first principles to represent physiological processes. To many this expression, "first principles", is not intuitively obvious. They would ask, what do we mean by that? Our immediate response would be "Oh, Newton's laws of motion, such as F = ma, Maxwell's equations for electromagnetism, or the ideal gas law." Now, it is true that since the work of Einstein and Karl Popper we know that even these fundamental laws are relative and can be supplanted by even more accurate laws and equations. However, for the present, they provide the most reliable description of the natural phenomenon about us. Still, what makes these laws or equations so fundamental?

Three characteristics stand out with equations based on first principles. First, they equate properties that are closely related by cause and effect. Second, the properties on the right side of the equation are difficult to divide or reduce further. Quantities such as time, mass, volume, and length fall into this indivisible category. Third, the predictive qualities of an equation based on first principles are superior to other equations or other modes of prediction. For some of the first principles of physics, the predictions from their equations are so close to the observations that it is easy to forget that the equation is simply a "best fit" model of the physical process and not the process itself.

Physics and chemistry have been successful in developing mathematical models of natural phenomena; geology and biology have been less successful. This is because biological and geological phenomena are much more complex; ecology is even more complex. For example, developing a predictive mathematical model of ocean productivity requires accurate equations to describe the advective, diffusive, thermal, and illumination properties of the ocean (physics); the atmospheric input of trace elements and gases (meteorology); fluxes in the nutrients, carbon, and oxygen (ocean chemistry); photosynthesis, respiration, nitrogen uptake, nitrogen fixation, nitrification, nitrogen excretion, carbon excretion, regeneration of nutrients, grazing, growth of the bacteria, phytoplankton, protozoans, zooplankton (biology); and sedimentation rates (geology). Given this kind of complexity, it is small wonder that plankton models can not achieve the predictive capacity that a model of an asteroid's trajectory around the sun can achieve. Nevertheless, improvement will come by developing more fundamental, descriptive, and accurate equations for physiological processes.

Meaningful equations

It is clear from the previous discussion that the equations we developed above are more complex than the common allometric equation for respiration (R), $R = aW^b$, where a and b are constants, W is biomass (Riisgård, 1998). However, we are questing for causality rather than simplicity. Kinetic equations are more complex, but they are based on the principles of cause and effect and, from a heuristic point of view, are preferable to what we will call proxy-based equations. At this point, the skeptic would say, "Show me, I'm not convinced! After all, in the study

of solution chemistry we calculate the rate of a reaction from the product of a first order rate constant (k) and the concentration of a substance in solution (c). Why is the allometric equation so different?" If the allometric equation were based on the concentration of the enzyme responsible for the process, we would have a parallel situation to solution chemical kinetics. In fact, this is close to the concept on which the original use of the ETS assay was based (Packard *et al*., 1971b). An enzyme assay that assesses the V_{max} of a reaction is also a measure of the enzyme's concentration. The ratio, R/ETS, is analogous to the first order rate constant, k. An allometric equation ignores the biochemical basis of physiology and seeks to relate physiology to more easily measured parameters, such as biomass, size, or surface area (Enquist *et al*., 1999). These are simply proxies of the true controlling parameters such as the number of mitochondria and the enzymes that regulate ATP production, oxygen consumption, and CO_2 production. Proxy parameters do correlate with many biological processes, but physiological state, activity level, nutritional state, and many other variables interfere with this correlation. Equations based on first principles minimize this interference.

Examples of meaningful equations

Below we describe two cases in which newer more meaningful mathematical models replaced older empirical proxy-based ones. The replacement of the Secchi disk equation is an example with which oceanographers are familiar. In this case, the equation relating the Secchi disk depth (D) with the extinction coefficient of light $\chi(\chi = 1.7/D)$ has been replaced by the Beer-Lambert law ($I = I_0\, e^{-\chi lc}$) for the absorption of light in a water column. Clearly, the equation based on the Beer-Lambert law is more complex. However, it is based on the principles of light being absorbed in a logarithmic fashion. Furthermore, this absorption is determined by the chemical characteristics of the light absorbing substances in the water (the extinction coefficient, χ), the concentration of these substances (c), and the distance the light passes through a solution of these substances (l). Consequently, it is preferable.

Another example is one of Newton's laws of motion, F = ma. Note that when a small object collides with a wall, the force transferred to the wall (F) is equated to the acceleration of the object times its mass and not its weight. Why? Because weight is not a fundamental property; it is easily measured and so is a common proxy for mass, but weight is really the product of the mass times the acceleration of gravity (g), W = mg. Thus, weight is low at great distances from a large body (i.e., Earth) and large when close to a large body. The mass, for practical purposes, is constant. Thus F = ma is a preferable expression to F = Wa/g because it is independent of gravity and hence more general or more basic. In a similar fashion, phytoplankton nitrate reduction is theoretically more closely related to the activity of the enzyme, NR, than it is to the nitrogen content of the cell. Likewise, a copepod's respiration is theoretically more closely related to its ETS activity or its IDH activity than it is to its carbon content. In both cases, the nitrogen and carbon are associated with many processes. The enzymes are dedicated to individual processes.

Enzyme activities and ecosystem models

We would like to argue that developing equations for physiological processes based on enzyme kinetics need not be justified by specific potential applications. We assume that we only need to be reminded of the enormous benefits to modern society of the engineering applications of Newton's laws and Maxwell's equations. All of which were largely unforeseen at the time these laws and equations were formulated. Furthermore, we would like to argue that although direct measurements are preferable, much of science is based on indirect measurements. For example, many of the fundamental constants such as the charge of an electron, are based on indirect measurements. In fact, "the best values of the fundamental constants can rarely be determined by direct measurement. Instead, they are usually found at the end of a chain of experimental observations and theoretical relationships" (Mohr and Taylor, 2001). The equations that we propose here provide such indirect, but theoretically justifiable rate measurements.

However, these arguments alone are not enough and it is useful to describe applications that we foresee after the initial modeling has been done in the laboratory. Here, we will show how enzyme based equations could be used to improve ecosystem models. Recently, such an ecosystem model for the Gulf of St. Lawrence, Canada calculates planktonic rate processes as either multiples of biomass or other rate processes (Tian *et al*., 2000). For example, respiration is simply considered a constant loss in growth efficiency in the different pools of bacteria, phytoplankton and zooplankton. Nitrate and ammonium

uptake rates are calculated as a variable fraction of the phytoplankton growth rate. The growth rate is considered a multiple of the biomass. In a nod to first principles, the variability in the growth rate is calculated using a half saturation constant in an expression resembling a Michaelis-Menten equation. Ammonium excretion is a multiple of bacteria and zooplankton biomasses. We argue that rate equations for such models be based on enzyme activities and intracellular metabolites controlling the rates. Tian's model (Tian *et al*., 2000) calculates large-phytoplankton NO_3 uptake as $\partial NO_3 / \partial t = \mu\mu_l P_l NO_3/(NO_3 + H_{ln})$. In this equation, NO_3 is seawater nitrate concentration (μM), μ is phytoplankton growth rate (day^{-1}), μ_l is growth efficiency coefficient for large phytoplankton (0.7), P_l is biomass of phytoplankton larger than 5 μm ($mgCm^{-3}$), and H_{ln} is the half-saturation constant for NO_3 (1 μM). The growth rate of both large and small phytoplankton (Tian *et al*., 2000), is a function of photosynthetic available radiation and maximum phytoplankton growth rate (Platt *et al*., 1980). This equation could be improved by replacement with one based on NR activity, i.e., Equations 3 and 4 here. Similarly we would replace their assumption of respiration as a constant factor with either of the equations based on ETS (given earlier and in Packard *et al*., 1996a), or based on IDH (Roy and Packard, 2001). Obviously, this approach would entail making field measurements of NR and ETS activities in addition to other currently measured variables, but the added effort would be compensated by improved model accuracy. It is important to point out here that much laboratory research into the enzyme kinetics and their relationships with whole-cell physiology over time is a prerequisite to any application of this approach to fieldwork.

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