

Chapter 3

STOICHIOMETRY

Michael Kaspari

SUMMARY

1 Metabolism is the use of energy and materials by life to perform work. Most of that work is applied chemistry, and so metabolic rates should covary in interesting ways with the availability and utility of chemical elements.

2 I explore the questions underlying ecological stoichiometry (ES). The 25 or so chemical elements essential to life play a variety of roles – as energy sources, structure, electrochemicals, and catalysts – and are used in rough proportion to their environmental availability. Life both tracks and creates biogeochemical gradients.

3 There are two theoretical underpinnings to ES. The first is Droop's Law, describing a positive saturating relationship between metabolic performance and availability of an element. The second is Liebig's Law of the Minimum, which posits that the element

with the highest ratio of utility to availability is likely to limit metabolic performance. However, multiple nutrients are likely to limit any large-scale or complex ecological process.

4 The metabolic theory of ecology (MTE) has components related to the supply and function of essential elements. As body mass increases, so does fasting endurance; and some elements are more easily stored than others. Droop's Law predicts normalization constants that are positive decelerating functions of element supply rate. Activation energy may be a function of the availability of enzymes, and, in turn, the metal cofactors they require.

5 I conclude by addressing some particularly ripe topics for the ES component of metabolic ecology, highlighting the need for field experiments that address issues of aggregation and scale.

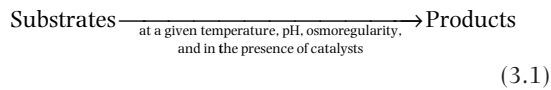
3.1 INTRODUCTION

Metabolism is the use of energy by organisms for the transformation of "stuff." At its most basic, metabolism is a measure of work: the conversion of glucose to ATP, CO₂, and H₂O by a cell; the transformation of materials and tissues for survival and reproduction; the breakdown of cellulose to CO₂ + H₂O in an ecosystem. Stuff may be thought of as "energy" (Stephens and Krebs

1986), or a mix of carbohydrates, protein, and lipids (Simpson and Raubenheimer 2001). Ultimately, the stuff of life can be cleanly decomposed into 25 or so chemical elements. These elements are continually rearranged to build the fuel, structure, and molecular machines of life. Ecological stoichiometry (ES) explores how the availability and balance of chemical elements guides and constrains metabolism from organisms to whole ecosystems (Lotka, 1925).

Metabolic Ecology: A Scaling Approach, First Edition. Edited by Richard M. Sibly, James H. Brown, Astrid Kodric-Brown.
© 2012 John Wiley & Sons, Ltd. Published 2012 by John Wiley & Sons, Ltd.

Like MTE, ES is reductionist and integrative, testing just how far one can predict the behavior of organisms and ecosystems by knowing the inner workings of cells (Brown and Sibly, Chapter 2). To do this, ES relies on Lavoisier's conservation of mass, which says that in the generic chemical reaction



elements are neither destroyed nor created (e.g., all the carbon in CO_2 at the outset of photosynthesis winds up as carbohydrate). The premise of ES is that, with a little imagination, all work performed by life can be cast as equation 3.1. The implication is that the rate (represented by the arrow) is a function of the availability of substrates, and the conditions in which those substrates are combined. The substrates are the chemical compounds, each with their elemental formula, made

available in given ratios in an aqueous medium. As we shall see, the conditions for these reactions are key, and include the temperature and the presence of catalysts.

3.2 BUILDING ORGANISMS USING ENERGY AND STUFF

There is a broad similarity in the chemical composition of life. Life has a recipe. For example, in human tissue, for every million or so P atoms, there are 38 600 Cu atoms and 1 Co atom (Fig. 3.1, Table 3.1). When we take into account mass, we also note that about 99% of life consists of just four elements: H (62.8%), O (25.4%), C (9.4%), and N (1.4%); the next seven (Na, K, Ca, Mg, P, S, and Cl) make up another 0.9%; and another 10 (Mn, Fe, Co, Ni, Cu, Zn, Mo, Bo, Si, and Se) comprise 0.1% of human mass. All are essential – shortfalls in any cause pathology.

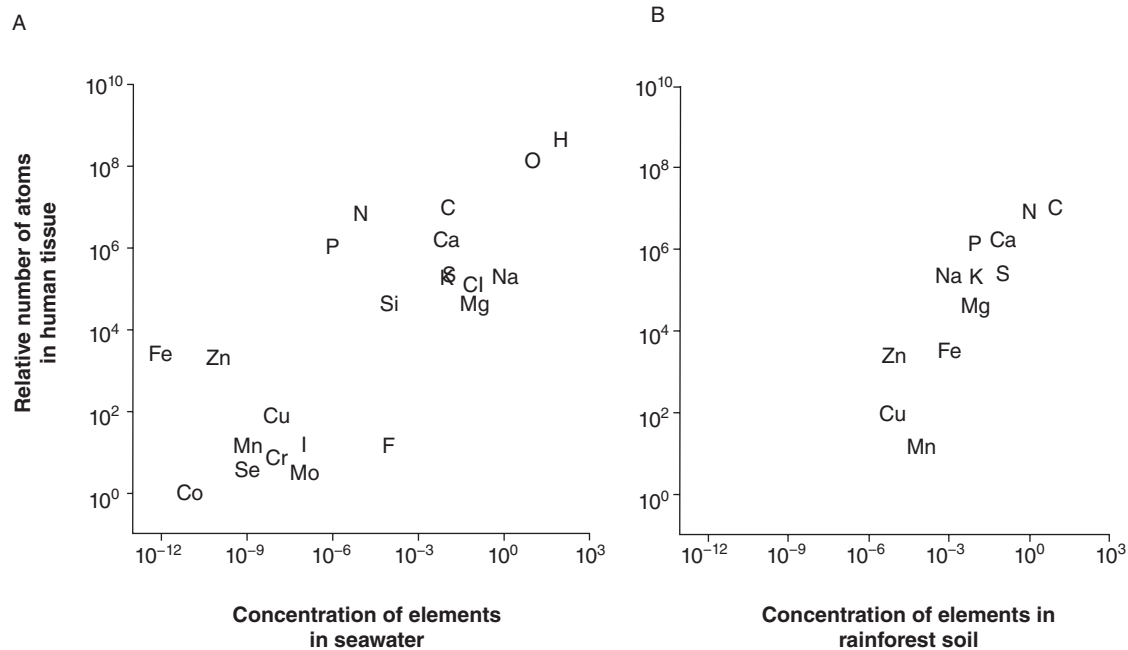


Figure 3.1 The chemical composition of human tissue correlated to that of seawater and soil. The stoichiometry of human tissue is described as relative numbers of atoms (e.g., there are 375 000 000 H atoms for every Co atom) (Sternler and Elser 2002). (A) The concentration of elements in seawater is related as mass percentage (Frausto da Silva and Williams 1991). (B) The concentration of elements in sandy soil from a Peruvian rainforest is related as mass percent dry weight, and lacks quantities of some trace metals (Kaspri and Yanoviak 2009).

Table 3.1 The 22 elements required to build a human body, their proportionate number (e.g., there are in the order of 100 000 000 H atoms for every Co atom), and some notes on their function. Data from Sterner and Elser (2002).

	Name	Number	Functions
H	Hydrogen	10 ⁸	Solvent, energy
O	Oxygen	10 ⁸	Solvent, energy
N	Nitrogen	10 ⁶	Structure
C	Carbon	10 ⁶	Energy, structure
Ca	Calcium	10 ⁶	Structure, electrochemistry, signals
P	Phosphorus	10 ⁶	Energy, structure
S	Sulfur	10 ⁵	Structure
Na	Sodium	10 ⁵	Electrochemistry
K	Potassium	10 ⁵	Electrochemistry
Cl	Chlorine	10 ⁵	Electrochemistry
Mg	Magnesium	10 ⁴	Catalyst
Si	Silicon	10 ⁴	?
Fe	Iron	10 ³	Catalyst, transport O ₂
Zn	Zinc	10 ³	Catalyst
Cu	Copper	10 ¹	Catalyst, transport O ₂
I	Iodine	10 ¹	Catalyst, signals
Mn	Manganese	10 ¹	Catalyst
F	Fluorine	10 ¹	Catalyst
Cr	Chromium	10 ⁰	Catalyst
Se	Selenium	10 ⁰	Catalyst
Mo	Molybdenum	10 ⁰	Catalyst
Co	Cobalt	10 ⁰	Catalyst

Since life evolved by natural selection, we might surmise that life is assembled from common elements that are easy and cheap to find. Indeed, life's chemical formula mimics in broad strokes the chemical proportions found in seawater and soil (Frausto da Silva and Williams 2001; Fig. 3.1). At the same time, the pumps and channels that stud cell membranes selectively acquire some elements (e.g., the C, H, and O of carbohydrates) and eliminate others (e.g., arsenic). Organisms spend considerable energy maintaining this chemistry within precise levels of tolerance (NRC 2005; Salt et al. 2008). The resulting homeostasis of intra- and intercellular environments – the concentra-

tions of substrates, structures, and the pH and osmoregularity of abiotic environments – fosters metabolic work.

3.3 WHAT DO ALL THESE ELEMENTS DO?

Metabolic ecologists include diverse processes under the descriptor “metabolic rate.” These include the synthesis of protein within a cell, the wholesale breakdown of glucose in an individual, and the generation of glucose by a hectare of forest. All share a dependence on the co-availability of essential elements – as catalysts, electrochemical ions, structures, and energy sources. Table 3.1 comprises one classification of the functional roles of elements.

3.3.1 Solvent

Most of living mass consists of liquid water, H₂O. The metabolism of most living things correspondingly occurs within the range of temperatures in which water is aqueous with a pH of 7, allowing elements to be available in a soluble form.

3.3.2 Energy

Covalent chemical bonds, when broken, release useful energy. Carbohydrates (or CHOs, for carbon, hydrogen, and oxygen) are the primary energy source of life; ATP with its high-energy phosphate bond is the universal molecule for biological energy transformation. While MTE has focused thus far on photosynthesis and cellular respiration (the breakdown of long-chain organic carbon and running the resulting sugars through the Krebs cycle), bacteria and archaea offer a variety of alternatives (Okie, Chapter 12).

3.3.3 Structure

Elements can be stored in, and make up large quantities of, hard and soft tissue. N is a big component of proteins and nucleic acids; P is common in nucleic acids, ribosomes, and phospholipid membranes. C and Si reinforce plant tissue and are found in the skeletons

of many animals. The S in the amino acids methionine and cysteine helps proteins fold into their ultimate, functional shape and provides thermal stability.

3.3.4 Electrochemicals

The charged ions of Na, Cl, K, and Ca are continually fluxed across life's membranes. These fluxes involve osmoregulation of intra- and extracellular fluids; they also maintain the electron potentials across the membranes. All serve to maintain the chemical gradients that allow critical substrates to accumulate and toxins to be eliminated. Some electrochemicals, such as Na, are not easily stored, are constantly excreted, and require continual uptake.

3.3.5 Catalysts

A variety of relatively rare metal atoms help enzymes combine with, orient, and bring together substrates. These atoms (often called cofactors) are functional parts of up to one-third of approximately 4000 known enzymes (Bairoch 2000; Waldron and Robinson 2009) where they rest in the pocket of the protein's active site (Fig. 3.2). There they aggressively catalyze reactions

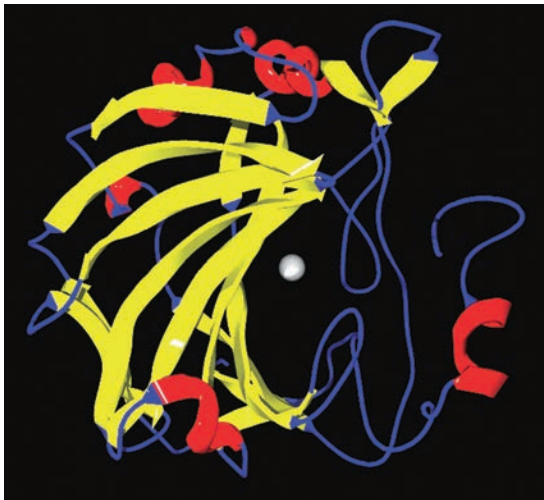


Figure 3.2 A metallomic enzyme, carbonic anhydrase, from the chloroplast, requires a single atom of Zn (center) to function.

involving the ubiquitous small molecules key to metabolism (e.g., O_2 , H_2S , and CH_4). In doing so they enhance the rate of reactions beyond those resulting from random encounters of substrates in solvent. Enzymes make life thermodynamically possible.¹

Two other functional roles of elements bear mentioning. The Fe in heme metalloproteins serves to *transport* oxygen in vertebrates; the hemocyanin in insects serves the same function but instead uses two Cu atoms. Ca binds to and activates enzymes, acting as a *signal* away from the binding site (Clapham 2007). Another metabolic signal, thyroid hormone, contains iodine (Cavalieri 1997).

3.4 HOW ARE CHEMICAL ELEMENTS DISTRIBUTED?

Elements are arrayed along a number of gradients in space and time (Table 3.2). It would seem a safe bet that life's metabolic processes must ultimately track these gradients. But things are not that simple. Metabolism both follows and generates biogeochemistry. The most telling example starts 2.5 billion years ago, when Earth's atmosphere consisted of CH_4 , SO_2 , NH_3 , and CO_2 , much of it belched up by volcanoes. This atmosphere prevented the formation of O_2 . However, as CO_2 and H_2O accrued, the stage was set for photosynthesis, the metabolic pathway that used CO_2 as a carbon source and photons as an energy source. As O_2 – a byproduct of photosynthesis – accumulated, the oxidizing atmosphere opened the door for the now ubiquitous, and remarkably efficient, aerobic respiration that runs much of life. At the same time, Fe solubility plummeted and the Fe^{3+} not bound up by the organic ligands manufactured by marine bacteria (Weaver et al. 2003; Hassler et al. 2011) sank to the dark ocean floor, away from the photosynthesizers. More on that soon (section 3.6.4).

Redfield's ratio (Redfield 1958) is another example of life shaping biogeochemistry. Redfield found that marine plankton and seawater share the formula $C_{106}N_{16}P_1$. His insight was that plankton weren't merely tracking the availability of these three essential

¹ Consider triose phosphate isomerase, one of the 10 enzymes in the glycolysis pathway. It is classified as "catalytically perfect" – allowing its reaction to proceed, regardless of temperature, at the rate substrates diffuse to and from the reaction site (Albery and Knowles 1976).

Table 3.2 Some gradients of elemental availability in time and space.

Driver	Elemental gradient
Sunlight and H₂O (Rosenzweig 1968)	Net primary productivity (NPP, gC/m ² /y) reflects the rate of CHO production by plants. NPP tends to be highest where it is sunlit and wet (in tropical rainforests and surface waters)
Ecosystem age (Walker and Syers 1976; Wardle et al. 2004)	Glaciers and volcanoes, expose new, mineral-rich bedrock, which weathers over time (see next) even as N-fixing plants accumulate and convert inert N ₂ into soil nitrates
Wind and gravity (Chadwick et al. 1999; Smil 2000)	Soils erode, depositing their nutrients at the bottom of the ocean; the churning of the ocean lifts Na, S, and other elements into the air and overland, while dust blown from continents transports P and Fe to the oceans (Fig. 3.4)
Food webs (Luoma and Rainbow 2005)	Herbivores convert tissue with a C:N ratio >0:1 to tissue with a C:N ratio >10:1, concentrating other nutrients along the way. Predators are less likely to be nutrient stressed than herbivores

elements. There instead was an equilibrium – caused by plankton’s uptake and growth and then death and release of these elements – that reflected a “global circulatory system” (Sterner and Elser 2002). P likely set the stage as most limiting, but then restricted the demand for N and C. As ecologists learned of Earth’s evolving atmosphere and Redfield’s ratio, it transformed the view of life from a passive, to an active, player in global biogeochemistry.

3.5 MODELS AND APPLICATIONS OF STOICHIOMETRY TO METABOLISM

Let’s now explore how ES models the fluxes and stores of an element and selects *which* elements on which to focus.

3.5.1 The Droop equation

The Droop equation (Droop 1974) allows us to rigorously define *nutrient limitation*. A limiting nutrient is

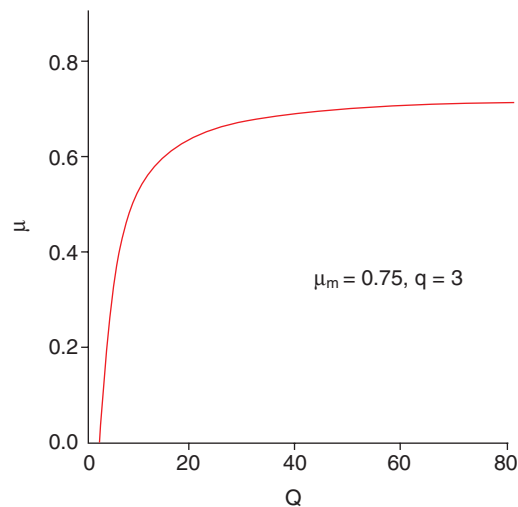


Figure 3.3 The Droop equation describes the growth rate of a cell, μ , as a function of the quota, Q , of a nutrient within the cell. Key parameters are the minimum quota to just support life, q , and the maximum growth rate, μ_m .

one which, when added to a system, increases the rate of metabolism, of growth or respiration, at the individual or ecosystem level. The Droop equation relates the elemental concentration (or quota) within the cell, to growth rate

$$\mu = \mu_m(1 - q/Q) \quad (3.2)$$

where μ is the biomass growth rate (day⁻¹); μ_m is the maximum growth rate at theoretically infinite quota; q is the minimum quota necessary to just support life (moles per cell at zero growth); and Q is the observed quota (moles per cell, Fig. 3.3). The Droop equation is a simple model that captures a basic reality – over a range of availability, the effect of a nutrient on growth saturates. In other words, as quota increases, the part of the equation in parentheses converges on 1, giving you the maximum growth rate. According to the Droop equation, there is not a *single* magnitude of nutrient limitation; it accelerates (and growth rate decelerates) as nutrient supplies decline.² Droop’s equation should hold for any element or biochemical essential for cell

²A similar equation, Monod’s Law, or the Michaelis–Menten equation, describes a similar process, but explores how a cell grows as a function of resource concentration *outside* the cell.

growth (at least across the range of Q before high concentrations become harmful).

3.5.2 Liebig's Law of the Minimum

The concept of 25 Droop equations to predict a metabolic rate (one for each essential element and daisy-chained together) is almost too much to bear. The eponymous Liebig's Law of the Minimum (LLM) was the first to deal with this chemical logjam (Liebig 1855). For a set of bioavailable nutrients, he reasoned, *only* the one in shortest supply relative to demand (that is, with the largest ratio of utility to availability) will limit yield. In Liebig's Law there can be only one limiting nutrient at a time: its supply rate becomes the metabolic rate-limiting step.

Placed in the context of Droop's Law, LLM has two parts. First, elements are essential for specific functions – any concentration less than μ_{∞} moves an organism leftward and downward on the Droop curve. Second, cells and organisms expend energy and resources to harvest elements, and these costs diminish as availability increases. Putting LLM into practice, ES practitioners often start with two likely candidates for the limitation of some metabolic rate. For each element, they estimate (1) the nutrient demand when growing at Droop's μ_{∞} , and (2) the supply rate of those nutrients. Most often, the pair of elements are C and another resource "R," where R is either N or P (White 1978, 1993; Sterner 2008; Kay 2002). For example, consider a population of planktonic crustacean, *Daphnia*, growing at their maximum rate. The baseline ratio C:P in their tissue can be used to predict how an organism will perform on diets, or in environments, of varying C:P. In lakes with low C:P, carbon-rich foods maximize growth; in lakes with relatively little P, the lack of P-rich foods limits growth (Sterner 1997).

LLM is a simple rule for finding likely candidates for nutrient limitation; it is one of ecology's most successful concepts.³ It requires that metabolic processes are limited in a strictly hierarchical way (Sterner and Elser 2002).

³Liebig's aim was modest: to advise farmers on the best choice of fertilizer to maximize crop yield. He likened the problem to an oaken bucket with vertical staves of varying lengths. To increase the capacity of the bucket it wasn't necessary to increase the length of each stave; it was only necessary to increase the length of the shortest stave. This metaphor sold lots of fertilizer (Brock 2002).

3.6 A FEW APPLICATIONS OF LIEBIG LOGIC

3.6.1 Energy

Models of optimal diet selection in animals were some of the first applications of ES. Most focused on C as the limiting element, assuming that individuals maximizing the rate of energy harvested left more offspring (Stephens and Krebs 1986). There were a number of good reasons why energy maximization models were useful. First, in a classic case of Liebig logic, energy must be expended to maintain homeostasis and search for food; in low NPP environments, organisms that don't harvest enough carbohydrates (CHOs) do not have the opportunity to be N, P, or S limited (Sterner 1997).

Optimal foraging theory was initially tested with taxa like bumblebees and hummingbirds, as their small size, thermogenesis, expensive locomotion, and high metabolic rates all suggested energy limitation (Pyke 1978; Hodges and Wolf 1981; Stephens and Krebs 1986). More recently, aggression and spite have been recognized as energetically expensive (Kaspari and Stevenson 2008). For example, colonies of the ant *Linepithema*, when fed a high C:P diet, were more aggressive to competitors and grew more quickly, suggesting that energy limits growth and defense of colonies (Kay et al. 2010).

3.6.2 Structural

N and P predominate in studies of structural elements. If one were to search for an ecosystem in an N-limited environment, you couldn't find a better site than the Cedar Creek research station in the north temperate USA. Its grasslands combine a recent glaciation with sandy, impoverished soils. Fertilization experiments at Cedar Creek consistently show increases in biomass production with added NH_4NO_3 (Tilman 1988). Moreover, an early experiment in which plots were fertilized with a combination of other candidate elements (P, K, Ca, Mg, S, and trace metals) yielded no more plant biomass than controls (Tilman 1987), reinforcing LLM's prediction that N is the one element at Cedar Creek limiting plant production.

The *growth rate hypothesis* (Elser et al. 1996) is an elegant example of Liebig logic integrating from cellular metabolism to ecosystem respiration. It starts with the observation, across a variety of invertebrates,

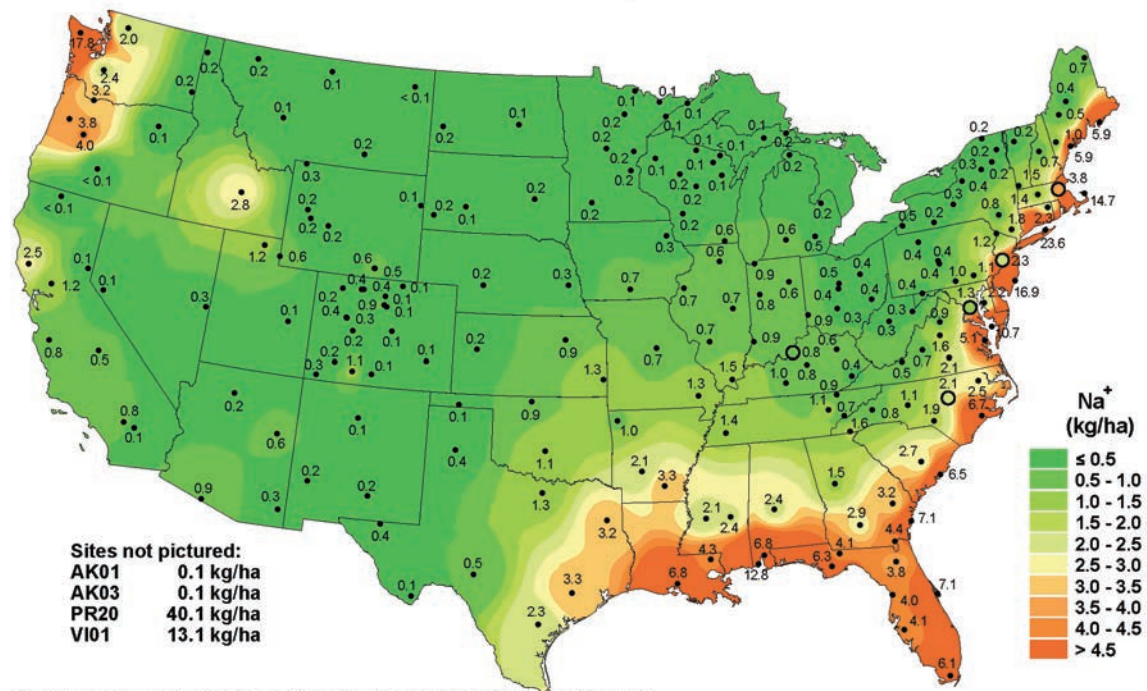
than an organism's growth rate was closely correlated with RNA content (Sutcliffe 1970). Now add the fact that P is a component of RNA, which in turn is a major constituent of ribosomes, the cellular machines that build proteins and thus underlie growth. The growth rate hypothesis predicts that P scarcity should decrease growth rates via decreases in ribosomal RNA, a pattern subsequently shown across a variety of invertebrates (Elser et al. 2003). It has also been applied to plants. Temperate ecosystems tend to have more available P than those in the tropics (Table 3.2). Trees from the temperate zone also tend to have higher tissue P, and grow faster, than their tropical counterparts (Kerkhoff et al. 2005; Lovelock et al. 2007).

3.6.3 Electrochemical

Many ionic elements are both common and tightly regulated. For example, plants typically don't use Na, but the herbivores and detritivores that eat plants do. This poses a dietary challenge to basal consumers, particularly in inland ecosystems where deposition and recharge of Na from oceanic aerosols is low (Fig. 3.4). There is growing evidence that Na shortage limits activity, population growth, and ecosystem respiration in the large swaths of the terrestrial biosphere >100 km from an ocean (Botkin et al. 1973). When my colleagues and I assayed ant use of salt, NaCl, and sucrose from coastal Florida to interior Amazon rainforests, we found that ants increasingly craved NaCl further inland

A

Sodium ion wet deposition, 2009



National Atmospheric Deposition Program/National Trends Network
<http://nadp.sws.uiuc.edu>

Figure 3.4 Mass transfer of elements at a geographic scale. (A) A map of Na deposition from oceanic aerosols, drawn by the US National Atmospheric Deposition Program. (B) The transfer of dust from the African Sahara to the mid-Atlantic ocean on February 26, 2000. (SeaWiFS satellite image courtesy of NASA.)

B

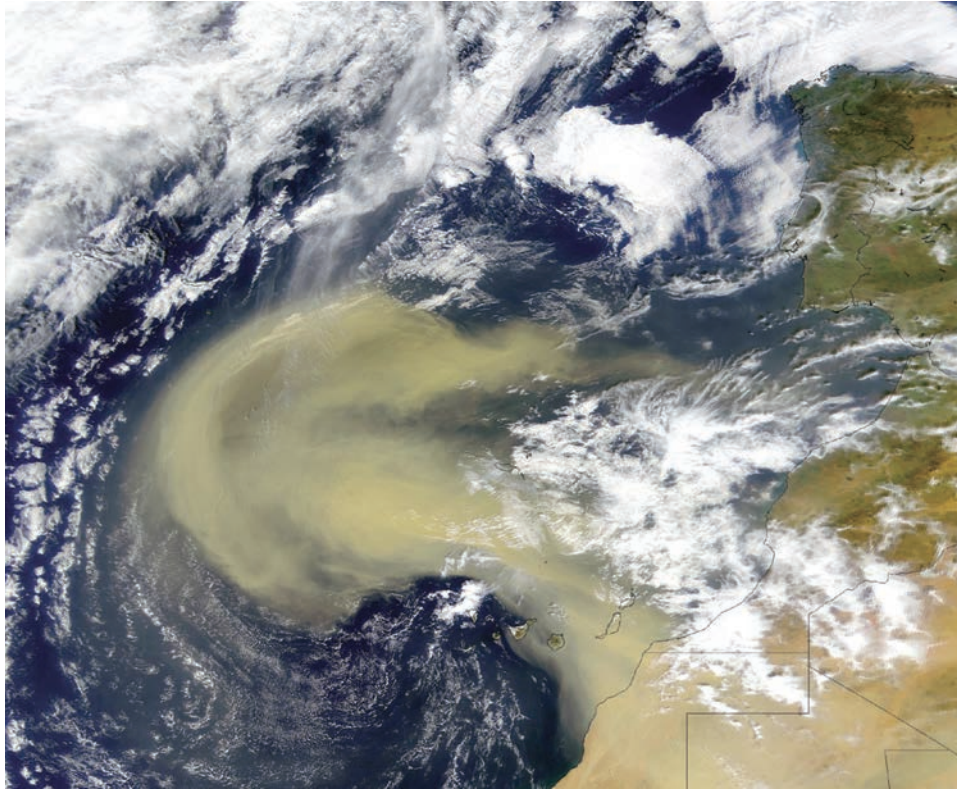


Figure 3.4 *Continued*

(Kaspari et al. 2008b). This Na limitation has ecosystem consequences: in Peruvian rainforests, termites and other decomposers accumulated on plots fertilized with NaCl, and decomposition rates were 50% higher (Kaspari et al. 2009).

3.6.4 Catalytic

Earth's evolution of an O_2 -rich atmosphere had a built-in negative feedback. Fe is a cofactor for chlorophyll, and Fe supply can limit rates of photosynthesis in oceans (Sunda and Huntsman 1997). Now recall that as the O_2 accumulated, Fe oxidized and sank to the ocean floor. Ever since, ocean algae (and ocean food webs) have depended on dust blowing in from the continental interior to resupply that Fe (Fig. 3.4). This has global, in fact epochal, implications (Martin 1990). Imagine a warm CO_2 -rich Earth: as droughts increase, inland winds fertilize the oceans with Fe-rich dust;

rates of photosynthesis increase and scrub the atmosphere of CO_2 . The Earth cools; droughts abate, as do Fe supply rates, and CO_2 accumulates again. In a spectacular "field" test of this hypothesis (Boyd et al. 2000) 50 km^2 of open ocean was fertilized with $3813\text{ kg FeSO}_4 \cdot \text{H}_2\text{O}$. The resulting 100 parts *per trillion* increase in Fe temporarily generated a 6-fold increase in chlorophyll and doubling of algae productivity. Unfortunately for the rest of the food web, those algae produced domoic acid, a potent neurotoxin (Trick et al. 2010).

3.7 BEYOND LIEBIG: WHEN METABOLISM IS LIMITED BY MULTIPLE ELEMENTS

The above tests show the utility of LLM in identifying potential limiting nutrients.

Let's consider briefly some ways (often complementary; Saito et al. 2008) in which more than one element may simultaneously limit metabolic rates.

3.7.1 Organisms deplete multiple elements, equalizing utility:availability

LLM relies on one element unambiguously maximizing the ratio of utility to availability. But natural selection, in favoring the use of common elements to build ubiquitous structures, tends to *equalize* these ratios across the range of essential elements. Consider the challenge to an algal cell growing in the open ocean. Essential ions are roughly available at the following molar concentrations (Frausto da Silva and Williams 2001):

K^+ , Na^+	$10^{-1} M$
Mg^{2+} , Ca^{2+}	$10^{-3} M$
Zn^{2+}	$10^{-9} M$
Cu^{2+}	$10^{-12} M$
Fe^{3+}	$10^{-17} M$

Fe^{3+} may be limiting because every ion of Fe^{3+} used to run photosynthesis requires an investment in ligands (CN-based proteins and sugars that snare the Fe^{3+} ions before they oxidize and disappear to the ocean's depths or are harvested by a competitor). But consider Zn^{2+} . Even though Zn^{2+} ions are 1 000 000 times more common in the environment, they are also metabolically ubiquitous, acting as cofactors of every functional group of enzyme (Frausto da Silva and Williams 2001).

If the ratios of utility:availability governing a given metabolic rate are converging toward a common value, there will never be the clear, enduring maximum assumed by LLM. Instead, a handful of elements may succeed one another in rapid succession, depending on local vagaries of supply and demand. In an elegant thought experiment, Saito et al. (2008) compiled data for the stoichiometry of marine phytoplankton and the biogeochemistry of three oceans. In each they found plausible cases where some combination of six elements (C, N, P, Fe, Co, and Zn), although available in widely different concentrations, could be hypothesized to limit productivity.

3.7.2 Scale and aggregation effects

A second way of reaching multi-nutrient limitation – that complements the first – happens when ecologists measure metabolic rates over larger areas, longer time-spans, and more populations (Levin 1992). This is easiest to imagine at the ecosystem scale.

Consider a process like decomposition. In a square meter of forest floor, thousands of species and billions of individual bacteria, fungi, and invertebrates break down leaf litter (Swift et al. 1979). Scattered within the plot may be three kinds of substrate. Two represent the same starting point but differing periods of time: a freshly fallen leaf and a decayed leaf that is little more than lignin and cellulose. A third substrate may be a butterfly chrysalis fallen from the forest canopy. Bacteria and fungal decomposers secrete different enzymes to catalyze the breakdown of different substrates: the protein-rich fresh leaf, the lignin-rich old leaf, and the chitin-rich chrysalis. Perhaps one-third of those enzymes (or the enzymes needed to synthesize them) require a metal cofactor. With this in mind, a group of colleagues compared decomposition rates in $0.25 m^2$ plots embedded in a $40 \times 40 m$ plot in a Panama rainforest (Kaspari et al. 2008a). These plots were fertilized with N, P, K, and a mix of micronutrients (including B, Ca, Cu, Fe, Mg, Mn, Mo, S, Zn). Decomposition increased on all but the +N plots, suggesting that at least three elements limited decomposition. Given the diversity of the leaf litter, and the duration of the study (48 days), multiple suites of metallo-enzymes were likely upregulated by the microbial community toward the conversion of tropical detritus to CO_2 , H_2O , and minerals.

3.7.3 Limitation cascades

Metabolic pathways tend to occur in networks. What happens in one part of the network ramifies “downstream.” If one part of the network governs the uptake of a limiting element, the lack of a metal cofactor can set up a limitation cascade: the lack of one enzyme can hinder the ability to harvest another.

One of the best examples of limitation cascades involves N fixation. Shortages of N have repeatedly been shown to limit population growth (White 1993) and ecosystem respiration (Horner et al. 1988). This puts a premium on processes that import N to ecosystems in a usable form. N_2 represents 72% of Earth's

atmosphere but it is chemically inert, with both N atoms held in place by a powerful triple bond. Biological N fixation into the biologically usable form of NH_4 or NO_3 accounts for the importation of up to 120Tg ($1\text{Tg} = 10^{12}\text{g}$), 92% of ecosystem N (lightning accounts for the rest; Vitousek 1994). But N fixation is costly. It requires ample carbohydrate as fuel. It also requires supplies of Fe and Mo to build nitrogenase enzymes. A shortage of C (via deficits of sunlight or moisture), Fe, or Mo can thus generate a limitation cascade hindering the input of N into the ecosystem. In the dark (low-C) understory of a Panama rainforest, fertilization with Mo generated 2- to 3-fold increases in N fixation by heterotrophic microbes compared to control plots (Barron et al. 2008).⁴ And in an era of increasing CO_2 , the resulting surplus of C should increase plant demand for N, N-fixation, and the relatively small stocks of soil Mo and Fe (Hungate et al. 2004; Barron et al. 2008).

3.8 LINKING ES AND MTE MODELS OF METABOLIC ECOLOGY

“Organic synthesis and metabolic rate are limited by the supply rate of essential elements.” (Reiners 1986)

Now that the case has been made for the relevance of ES to metabolic ecology, how do we more thoroughly integrate it into MTE? One way is to look for shared metabolic subunits. A second is to look for stoichiometric underpinnings of the MTE itself.

3.8.1 When metabolic components have a distinct stoichiometry

MTE models assume mitochondria, ribosomes, and chloroplasts are “invariant subunits”: they process

⁴Barron and colleagues also found increases in N-fixation on plots fertilized with P. Subsequent experiments showed this was due to Mo contamination of the phosphate (which is mined from rock). Such is a cautionary tale for one exploring the ecology of trace metals; their concentrations (at 10^{-6} the molar quantities of N and P) are difficult to measure accurately, and often difficult to manipulate without contamination.

energy and materials the same way regardless of the organisms that contain them (Brown et al. 2004; Allen and Gillooly 2007, 2009; Okie, Chapter 12). If so, the metabolic rates of an organism should correlate with the tissue density of these subunits. Since these subunits can have distinctive stoichiometries (Niklas and Enquist 2001; Gillooly et al. 2005a; Allen and Gillooly 2009; Elser et al. 2010), an organism’s metabolic rate should be associated with its stoichiometry.

Gillooly and colleagues (2005a) used this logic to explore the 100-fold variation in [P] (phosphorus concentration) observed across organisms. They started by linking rates of protein synthesis to the chemical mechanics of RNA and ATP. As predicted, the [RNA] of a variety of taxa tended to decrease as $M^{-0.25}$ (Fig. 3.5A). Moreover, whole-body [P] decreased with M , but in a decelerating fashion, due to the increase in non-metabolic pools of P (like vertebrate skeletons) in larger organisms (Fig. 3.5B). The “invariant subunit” approach also allowed whole-body [P] to account for much of the variation in temperature-corrected growth rates among eight species of zooplankton (Allen and Gillooly 2009).

3.8.2 Stoichiometric underpinnings of the MTE equation

A second way to build a more synthetic metabolic ecology is to show how the parameters of MTE are themselves a function of ES (Brown and Sibly, Chapter 2). Recall that in the MTE

$$B = B_0 M^\alpha e^{-E/kT} \quad (3.3)$$

B_0 is the normalization constant, M is mass, α is an allometric scaling exponent, E is the activation energy, k is Boltzmann’s constant, and T is temperature (K).

3.8.2.1 Mass

Not all mass is metabolically active. A large store of an organism’s mass may be metabolically inert: surplus N can be stored intracellularly as amino acids and proteins; surplus P can be stored as polyphosphate and bone (Rhee 1978; Taiz and Zeiger 1998); surplus Fe is bound up in proteins called ferritins (Harrison and Arosio 1996). Moreover, an organism’s storage capacity scales with its volume. If metabolic rates scale as

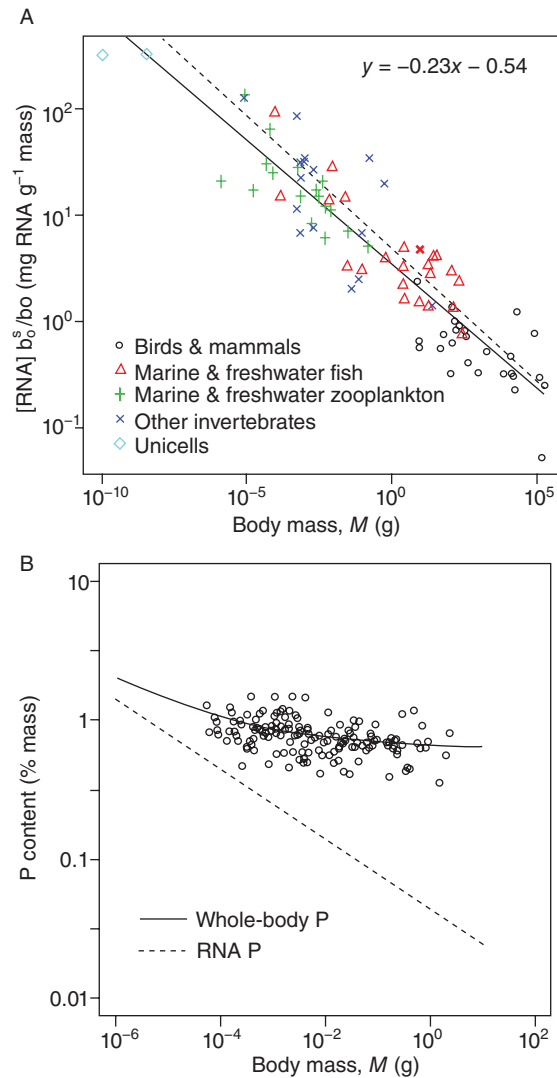


Figure 3.5 Syntheses combining WBE and ES components of metabolic ecology predicting whole-body RNA content (A) and P content (B) as a function of dry mass (Allen and Gillooly 2009).

$M^{3/4}$ and storage scales as M^1 , then fasting time should increase as $M^1/M^{3/4} = M^{1-3/4} = M^{1/4}$ (Peters 1983).

This is of particular interest in environments in which resource supply (and hence the need for fasting) varies over time. Imagine a series of environments all with the same annual NPP. However, some are aseasonal

(with that NPP evenly portioned out from January to December). Others are seasonal, with rich productive summers and barren winters. In the seasonal environments, smaller organisms with fewer fat reserves should be less able to withstand the resulting periods of resource scarcity. The starvation resistance hypothesis predicts that variable environments (in supplies of any storable essential element, not just C) will support communities of larger organisms, with consequently lower rates of ecosystem productivity and respiration (Lindstedt and Boyce 1985; Kaspari and Vargo 1995). However, not all elements are easily stored. Many of the electrochemicals (e.g., Na, Cl, and K) and elements sequestered by gut flora (e.g., Co) are more or less constantly excreted (Milewski and Diamond 2000) with excretion rates scaling as $M^{3/4}$ (Peters 1983). Periodic shortfalls of Na or K may harm small and large organisms alike (Jones and Hanson 1985).

3.8.2.2 Normalization constant

In MTE (equation 3.3) the normalization constant accounts for variability in a metabolic rate independent of the effects generated by mass scaling and the Arrhenius equation. For reasons of simple parsimony, the normalization constant is one of the first places to look for ES effects; they should show up as residuals to plots of metabolic rate vs. mass-corrected T , and temperature-corrected M (for example, in Figure 3.5A, for a given body size, there remains 100-fold variability in whole-body [RNA]).

A theory of normalization constants, based on Droop's Law, seems a good place to begin fusing ES and MTE models of metabolic ecology. For example, imagine growing size-matched plants at a fixed temperature in a common garden. Fertilize the subplots with a gradient of increasing R (where R is a nutrient) while you monitor growth rate (Tilman 1987). Normalization constants should increase in a positive decelerating fashion as one moves up [R].

3.8.2.3 Mass exponent

There remains a spirited debate as to the value(s) of the mass exponent. In at least one experiment, however, diet has been shown to influence the mass exponent (Jeyasingh 2007). Four species of *Daphnia* varying 10-fold in size were fed two diets – one diet matching

Daphnia stoichiometry (C:P c. 150) and an imbalanced, carbon-rich diet (C:P c. 800). On the balanced diet, metabolism scaled as $M^{0.83}$; on the C-saturated diet, it scaled as $M^{0.67}$. The decreased exponent resulted from a larger increase in metabolic rate in the small *Daphnia* species under P-stress. Jeyasingh suggested that the higher mass-specific requirement for P in the small species placed a premium on eliminating excess C. These small *Daphnia* effectively “burned off carbon” by upregulating C-disposal genes (Jeyasingh et al. 2011).

Jeyasingh’s work is a good example of how metabolic challenges vary with the way you eat (Sternner and Elser 2002). Bacteria and fungi eat by secreting enzymes into the environment and using transport proteins to selectively absorb individual nutrients from the resulting soup. Metazoa, on the other hand, consume prey as packages, and frequently eat foods that differ stoichiometrically from themselves (e.g., herbivores have lower C:N than their host plants). Metazoa thus often need to dispose of or store nutrients when prey stoichiometry deviates from the optimum. Experiments like the one above using synthetic diets (Cohen 2004) have much potential for exploring the metabolic consequence of this postprandial processing.

3.8.2.4 Activation energy

The metabolic rates most often measured by ecologists (respiration, photosynthesis, growth, and production) integrate over hundreds of chemical reactions in organisms and ecosystems. The activation energy (and its cousin, Q_{10}) describes the temperature sensitivity of those metabolic rates. Activation energies for a given metabolic process show a central tendency (e.g., aerobic respiration averages about 0.65 eV; Brown and Sibly, Chapter 2); one study has also revealed substantial variation around the mean (Dell et al., unpublished). One step toward a theory of activation energy focuses on the availability of enzymes that catalyze chemical reactions.

Recall that perhaps 30% of enzymes require at least one metal atom to function properly (Frausto da Silva and Williams 2001). If the quotas of these metal cofactors drop below μ_* , those shortfalls should decrease metabolic performance, especially at low temperatures. If so, metabolic rates under sufficient metal scarcity should be lower with higher temperature sensitivity (i.e., higher activation energies).

In nature, there are at least two avenues toward metal scarcity (Table 3.2). The weathered soils of older ecosystems are nutrient poor (Wardle et al. 2004). Metals also bioaccumulate: detritivores and herbivores should be more likely than their predators to consume food poor in essential metals (Jones and Hanson 1985; Milewski and Diamond 2000). As a consequence, activation energies of detritivores and herbivores should be higher than those of predators in the same environment, a disparity exaggerated in nutrient-poor ecosystems.

3.9 OPPORTUNITIES

Sternner and Elser’s (2002) volume laid the groundwork for a productive decade developing the stoichiometric basis for metabolic ecology. Here are some suggestions for the next decade.

3.9.1 Describing the chemical recipe of life and its consequences

There are still plenty of holes in our understanding of the biological function of essential elements (Saito et al. 2008; Sternner 2008; Kaspari and Yanoviak 2009). This essentially descriptive enterprise is a key first step toward understanding how biogeochemical gradients shape, and are shaped by, life. Proteomics and metabolomics (Silver 1998; Bragg et al. 2006; Lupez-Barea and Gumez-Ariza 2006) and the extensive applied literature of nutrition, agronomy, and wildlife management (Jones and Hanson 1985; NRC 2000, 2005) are excellent venues for exploitation by enterprising ecologists. One continuous source of ideas is Frausto da Silva and Williams’s opus *The Biological Chemistry of the Elements* (2001).

As ES databases grow, investigators are discovering that about half of the globally observed variability in stoichiometry exists among species in a given community (Kraft et al. 2008; Hillebrand et al. 2009; Elser et al. 2010). Some of these differences in ES reflect niche differences linked to metabolic subunits – high densities of ribosomes (and high titers of P) are found in bacterial taxa characterized by rapid growth (Stevenson and Schmidt 2004). To what extent do gradients of biogeochemistry undergird coexistence in communities (Tilman 1982) and what are the

functional traits that provide a mechanism for these population interactions (McGill et al. 2006)?

3.9.2 Mapping availability at multiple scales

For ES to be predictive we need better maps of biogeochemistry at scales relevant to our study organisms. While ecologists are increasingly adept at measuring C availability (e.g., through remote sensing; Turner et al. 2006), direct measures of nutrient availability at the landscape scale (John et al. 2007) are still rare. Maps of mineral “licks” – deposits rich in elements like Na, Mg, and Ca (Jones and Hanson 1985) – allow for the study of mineral deficiency using the comparative approach.

Measuring absolute quantities of P, Zn, or Fe may not be enough (Weaver et al. 2003; Saito et al. 2003, 2008). Ligands – organic molecules produced by microbes to bind to and sequester metals – distinguish between an element’s mere presence and its “bioavailability.” Much remains to be discovered about their abundance, diversity, and function in oceans and soils.

3.9.3 Experiments: measuring performance curves for a variety of elements

Experiments remain the definitive test of nutrient limitation. With 25 or more candidate elements, the prospect of a complete screening is a little daunting. Moreover, to quantify the Droop curve, you want to measure metabolic performance across at least three levels of availability. Now imagine you wish to explore multi-nutrient space. One can begin to see one’s lab bench space decrease geometrically even as one’s budget increases exponentially.

At this point the reader is likely considering returning to her first love, the theater. But all is not lost. First and foremost, nothing thins out the list of possible experiments like a good set of hypotheses based on a combination of realism (e.g., testing for the effects of doubling the extant environmental concentration of R) and rules of thumb (e.g., Liebig’s Law of the Minimum). Figure 3.1, comparing the composition of a vertebrate endotherm to seawater, suggests that N, P, Fe, and Zn would be worth a look as limiting elements. Comparing that same stoichiometry to the forest soils

of Peru, we see that P, Na, and Zn show LLM’s requisite high ratio of use to availability.

Four elements seem especially promising for future studies. Na and I are candidates for limitation of herbivores and detritivores in inland and weathered ecosystems (Milewski and Diamond 2000; Kaspari et al. 2008b, 2009), which support a considerable proportion of the world’s biota. Two macronutrients – Mg, a cofactor in numerous enzymes in the glycolytic pathway, and S, a component of two amino acids critical for protein folding – are both essential elements that are patchily distributed across landscapes and continents (Jones and Hanson 1985; Frausto da Silva and Williams 2001).

A second reason for optimism is the proliferation of useful molecular methods. You can now count and compare the number of gene copies for transport proteins for a given ion (Silver 1998). Microarrays like the Geochip (Zhou et al. 2008) quantify the expression of thousands of substrate-specific enzymes. Stress experiments detect the upregulation of stress-response genes (Webb et al. 2001) or whole metabolic cascades (Fauchon et al. 2002; Salt et al. 2008). All of these can provide valuable insights to the field ecologist provided you learn the right techniques or find the right collaborator.

3.9.4 Scaling up to communities and ecosystems

Metabolic ecology is transforming ecosystem-level models from the statistical and descriptive (e.g., Gholz et al. 2000; Mahecha et al. 2010) to those based on first principles of energetics and stoichiometry (Andersen-Teixeira and Vitousek, Chapter 9). Challenges remain. A recent review of herbivory patterns suggests one reason why (Hillebrand et al. 2009). The authors compared the ability of mass, temperature, and stoichiometric deficits to predict patterns of herbivory at the individual and population level. Consumption rates of herbivores scaled well to mass across 11 orders of magnitude of body size, but mean body size was ineffective at predicting the population consumption rate (since body size and population size were inversely related). In contrast, the stoichiometric mismatch of herbivore NP to plant NP was the best predictor of herbivory rates at the population level and within feeding guilds. We need more such studies

contrasting the utility of MTE, ES, and synthetic models toward predicting metabolism at differing levels of aggregation.

Accurate ecosystem and global models are ever more important. To what extent, however, do they predict the maximum possible rates of ecosystem respiration? How do these maxima compare to a world with nutrient shortfalls across broad, predictable sections of the globe (Martin 1990; Chadwick et al. 1999;

Kaspari et al. 2009)? Are organisms from inland communities, denied Na and I, slower and (given iodine's importance in brain development) dumber (Milewski and Diamond 2000)?

A key goal of this volume is to weave together models of biochemistry and energetics and by doing so inspire the next generation of metabolic ecologists. If we succeed, and I suspect we will, I predict that, as Frank Sinatra once suggested, "The best is yet to come."