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Allometric scaling and metabolic ecology of microorganisms and major evolutionary transitions

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ALLOMETRIC SCALING AND METABOLIC ECOLOGY OF MICROORGANISMS AND MAJOR EVOLUTIONARY TRANSITIONS

by

JORDAN G. OKIE

B.A., Biology, Carleton College

DISSERTATION

Submitted in Partial Fulfillment of the
Requirements for the Degree of

Doctor of Philosophy
Biology

The University of New Mexico
Albuquerque, New Mexico

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ABSTRACT

My dissertation centers around investigating big-picture questions related to understanding the consequences of metabolism and energetics on the evolution, ecology, and physiology of life.

The evolutionary transitions from prokaryotes to unicellular eukaryotes to multicellular organisms were accompanied by major innovations in metabolic design. In my first chapter, I show that the scaling of metabolic rate, population growth rate, and production efficiency with body size have changed across these transitions. Metabolic rate scales with body mass superlinearly in prokaryotes, linearly in protists, and

sublinearly in metazoans, so Kleiber's 3/4 power scaling law does not apply universally across organisms. This means that major changes in metabolic processes during the early evolution of life overcame existing physical constraints, exploited new opportunities, and imposed new constraints on organism physiology.

Surface areas of physiological structures of organisms impose fundamental constraints on metabolic rate. In my second chapter, I demonstrate that organisms have a variety of options for increasing the scaling of the area of their metabolic surfaces with body sizes. I develop models and examples illustrating the role of cell membrane elaborations, mitochondria, vacuoles, vesicles, inclusions, and shape-shifting in the architectural design, evolution, and ecology of unicellular microbes. I demonstrate how these surface-area scaling adaptations have played important roles in the evolution of major biological designs of cells and the physiological ecology of organisms.

In my third and final chapter, I integrate and synthesize findings from the previous two chapters with important developments in geochemistry, microbiology, and astrobiology in order to identify the fundamental physical and biological dimensions that characterize a metabolic theory of ecology of microorganisms. These dimensions are thermodynamics, chemical kinetics, physiological harshness, cell size, and levels of biological organization. I show how addressing these dimensions can inform understanding of the physical and biological factors governing the metabolic rate, growth rate, and geographic distribution of cells. I propose a unifying theory to understand how the major ecological and evolutionary transitions that led to increases in levels of organization of life, such as endosymbiosis, multicellularity, eusociality, and multi-

domain complexes, influences the metabolism and growth and the metabolic scaling of these complexes.

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CHAPTER 1: Introduction

Life is a state of orderliness that can only be maintained by a flux of energy from the environment. All organisms require energy and materials to build and maintain their complex structures far from thermodynamic equilibrium. Life has evolved enzymes to harness energy from a variety of sources—sunlight, organic carbon, and energy yielding (exergonic) geochemical substrates.

Metabolism is the uptake, transformation, allocation, and excretion of energy and materials. Whole-organism metabolic rate is the total rate at which energy and materials are processed by an organism. Because all organisms require energy and materials to reproduce, metabolism has direct consequences for biological fitness. Organisms with higher metabolic rates per unit of body mass (mass-specific metabolic rate) have higher fitness. In fact, all else being equal, the reproductive rate of an organism, e.g., the maximum population growth rate, is proportional to mass-specific metabolic rate (Brown and Sibly 2006). Because many traits of organisms are mediated by their metabolic rate and whole ecosystem metabolism reflects the metabolic rates of the individuals in the ecosystem, it is a fundamentally important variable in ecology and evolution. Rates of evolution and mutation, organismal and ecosystem biomass production, organism lifespan, rates of interspecific interactions, and many other variables are functions of metabolic rate (e.g., see Brown et al. 2004 and subsequent papers).

Given the fundamental nature of metabolic rate, investigating the constraints and factors governing metabolic rate is an important area of research in biology. Some of these variables are extrinsic physicochemical or biological variables whereas others are intrinsic biological or physicochemical attributes of an organism. Since the metabolism of

an organism emerges from the ensemble of its biochemical reactions, it is necessarily dependent on fundamental principles and variables of chemistry and physics. Temperature is one of the most fundamental physiochemical variables affecting both the rates of the reactions (kinetics) and their thermodynamic properties (whether a reaction is energy-yielding or not; see Chapter 4)). Consequently, the effects of temperature on metabolic rate have been extensively studied, both empirically and theoretically, in ecology and physiology (refs). The body mass or volume of an organism imposes constraints on the total mass and volume within which an organism's metabolic reactions proceed. Thus body mass must have important effects on metabolic rate (the focus of Chapter 2). Indeed, the study of how biological traits scale with body mass is a significant area of research known as allometry (refs) and metabolic rate scales closely with body mass. Resources are taken up and processed on the surfaces of an organism's body and its various physiological structures. Thus, the surface areas of an organism and its structures also influence an organism's metabolic rate and are essential variables in many biophysical and physiological models of metabolic rate (refs) (Chapter 3). Finally, the structural and spatial design of an organism's metabolic architecture must be considered, such as the specific location of different catabolic and anabolic processes within the cell. Through mathematical theory (Chapter 2) and macroecological approaches (Chapter 3) I investigate how metabolic design influences metabolic scaling. In particular, the evolution of eukaryotes from prokaryotes and multicellular organisms from unicellular organisms, known as major evolutionary transitions (refs), involved major changes in metabolic design. These transitions also led to overall increases in

body mass. Therefore the scaling of metabolic rate is expected to be intimately intertwined with the major evolutionary transitions.

The biological and physicochemical diversity of life is extraordinary, so it is a challenging problem to develop a unified understanding of metabolic rate. In fact, although a unicellular organism is in some regards the simplest of organisms, it is as difficult as macro-organisms, if not more so, to develop an integrative understanding of the factors constraining their metabolic rates. First, cells are not just 'bags of cytoplasm' consisting of randomly diffusing enzymes, as is often assumed in ecological and physiological models of cells (refs). Instead, their cytoplasm is complex in structure and highly organized in function at the molecular level (Hartwell et al. 1999, Hochachka 1999, Aon et al. 2004, Gitai 2005, Spitzer and Poolman 2005, Grima and Schnell 2006, Gallos et al. 2007). The cytoplasm is crowded with macromolecules. As a result, much if not all of the cytoplasm is in a gel-like rather than a liquid state, and reactions are localized and channeled through heterogeneous spaces. The cell membranes of prokaryotes can be riddled with invaginations. Eukaryote cells have a particularly complex network of structures and organelles with highly active and dynamic cytoskeletons and vesicles, many of which play still poorly understood roles in the rapid movement of materials along highly organized pathways by means of active transport and molecular motors. However, unlike animals and plants, due to the size, short timescales, and spatially dynamic nature of their networks, it is more technologically, methodologically, and analytically difficult to map out, quantify, and study the structure and dynamics of these distribution networks and their fluxes. Second, unicellular organisms, in particular prokaryotes, also exploit the greatest diversity of energy-yielding

metabolic pathways. Thus, for example, theory based on aerobic respiration will necessarily be of limited theoretical and applied relevance in prokaryotes unless it can also be generalized to other metabolic lifestyles. Third, many species of microorganism live in syntrophy. Therefore they are difficult to culture and study in the lab. And studying the properties of an individual can be misleading when that individual must in fact live as part of a group or community. Because of these challenges, a metabolic theory of microorganisms is arguably the least complete and arguably the greatest potential lies in developing metabolic theory for these organisms. Advances in microscopy, microbiology, theoretical geochemistry, and network modeling can provide the data and necessary theoretical and analytical techniques to study the metabolic theory of unicellular organisms.

This dissertation centers around investigating the effects of body size and shape, thermodynamics, kinetics, and the major evolutionary and ecological transitions on the metabolic rate and metabolic ecology of organisms. Although the dissertation focuses on unicellular organisms, by comparing and contrasting unicellular organisms with multicellular living systems, I am able to gain further insights into the metabolic theory of both unicellular and multicellular systems. The overarching goal of the dissertation is to unify perspectives from evolutionary biology, geometry, chemistry, and physiology in order to develop an integrative understanding of metabolism—understanding that should help resolve controversies and inform new understanding of metabolic theory. Specifically, I evaluate theory and empirical support for Klieber's Law, the scaling of metabolic rate with body mass as a power law with an exponent of ¾, showing that ¾ scaling does not apply to all organisms and that in fact metabolic scaling exponents shift

across the major evolutionary transitions from heterotrophic unicellular prokaryotes to unicellular protists to metazoans. I develop theory that shows that organisms have numerous strategies that they can use on ecological and evolutionary timescales in order to increases the scaling of their surface area with volume, and consequently their metabolic scaling relations, to an exponent greater than 2/3, which is the null hypothesis based on geometric principles . The theory demonstrates the pervasive importance of considering the surface areas of organisms in order to understand the ecology, evolution, and physiology of organisms. Integrating these previous findings with developments in geochemistry, biochemistry, and microbiology, I identify the major physical and biological dimensions that must be considered in order to develop a metabolic theory of the ecology of microorganisms.

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CHAPTER 2: Shifts in metabolic scaling, production, and efficiency across major evolutionary transitions of life

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Abstract

The diversification of life involved enormous increases in size and complexity. The evolutionary transitions from prokaryotes to unicellular eukaryotes to metazoans were accompanied by major innovations in metabolic design. Here we show that the scaling of metabolic rate, population growth rate, and production efficiency with body size have changed across the evolutionary transitions. Metabolic rate scales with body mass superlinearly in prokaryotes, linearly in protists, and sublinearly in metazoans, so Kleiber's ¾ power law does not apply universally across organisms. The scaling of maximum population growth rate shifts from positive in prokaryotes to negative in protists and metazoans, and the efficiency of production declines across these groups. Major changes in metabolic processes during the early evolution of life overcame existing constraints, exploited new opportunities, and imposed new constraints.

Introduction

The 3.5 billion year history of life on earth was characterized by dramatic increases in the size, complexity, and diversity of living things. The first organisms were

microbes with relatively simple body plans and metabolic networks. A few major transitions in form and function occurred during the subsequent evolution of life (1). The resulting diversity of contemporary organisms ranges from minute, relatively simple unicellular prokaryotes and archaea to giant, complex animals and plants containing multiple differentiated organelles, cells, tissues, and organs.

Two of the largest transitions were from simple prokaryotic to complex eukaryotic cells, and from unicellular to multicellular eukaryotes. Each transition required the integration of multiple individual organisms into a new higher-level unit of organization and selection (1, 2). These transitions involved dramatic changes in structure and function, and several orders of magnitude increase in body size (3). Since all organisms share a common set of molecules and biochemical reactions (4, 5), the increases in size and organizational complexity were accomplished by assembling these universal components in new ways (6). Major changes in genetic systems made these transitions possible (1, 2), and complementary changes in metabolic systems supplied the energy and materials to grow larger and support more complex morphologies and physiologies (7, 8).

Scaling relations offer powerful insights into the fundamental processes that constrain and regulate biological structure and function. Nearly all characteristics of organisms, from use of energy to the population growth it fuels, vary with body size. Most of the variation can be described by allometric equations or power functions of the form

$$Y = Y_0 M^{\alpha} \qquad (1)$$

where Y is the trait of interest, Y_0 is a normalization constant, M is body mass in grams, and α is the scaling exponent. There is a large and longstanding literature on these biological scaling relations in plants and animals but fewer focused on unicellular prokaryotes and protists. The large changes in structure and function that occurred at the major evolutionary transitions likely affected the allometric scaling of three traits that we consider below:

1) Metabolic rate: Metabolic rate, B, the rate of energy transformation within an organism, is perhaps the most fundamental biological rate. It sets the pace of life. It is statistically correlated with and functionally linked to many other traits. In the 1930s Max Kleiber (9) showed that the metabolic rate of birds and mammals scales as approximately the ¾ power of body mass. Subsequent findings of similar scalings for metabolic rates in many kinds of life forms led to the canonization of "Kleiber's law": $\alpha \approx 0.75$ was thought to apply to all organisms, including unicellular prokaryotes and eukaryotes (10 - 13). Renewed interest in biological scaling relations has led to re-evaluation of Kleiber's law, with much discussion about the exact value of α in different taxonomic and functional groups. Theoretical models have attributed 3/4-power scaling to the fractal-like designs of vascular systems of large, complicated organisms (14), while empirical studies have reported exponents >0.75 for some prokaryotes, protists, and small plants (15 - 18). Clearly, the scaling of metabolic rate with body mass in small organisms needs to be re-examined, with a focus on the evolutionary transitions that connects these disparate forms of life.

2) Population growth rate: The rate of population growth, *r*, is another trait with fundamental importance in both ecology, where it provides a standardized estimate of the population-level rate of biomass production, and evolution, where it is often taken as a measure of fitness. Maximal population growth rate under optimal conditions, r_{max}, has received considerable attention in both basic and applied studies of microorganisms. Because production of new biomass for both growth and reproduction is fuelled by metabolism, it has generally been assumed that r_{max} scales in the same way as mass-specific metabolic rate, so with an exponent of less than one and approximately -0.25, given that they follow Kleiber's law. This has generally been supported by empirical studies of large, multicellular organisms (12, 19). Although a seminal early study of r_{max} in protists reached similar conclusions (20), the scaling of r_{max} across the evolutionary transitions should be re-examined.

3) Efficiency of biomass production: Another basic characteristic of organisms is the efficiency with which they convert metabolic energy into new biomass. This efficiency, *E*, can be expressed in units of gJ^{-1} as the rate of biomass production divided by the rate of metabolism, both standardized as per unit body mass, so as $E = r_{\max} /(B/M)$. *E* is not only a fundamental biological parameter; it has important practical applications in areas such as agriculture, biotechnology, and biofuel production. So it is timely to quantify the scaling of *E* as a function of body size and across the evolutionary transitions.

Here we compile data on the scaling of these three fundamental characteristics, metabolic rate, *B*, maximum population growth rate, r_{max}, and efficiency of biomass production, *E*, in three functional groups of heterotrophic organisms: prokaryotes,

protists, and small multicellular aquatic animals (hereafter metazoans) (see SI *Data*). Application of a scaling framework is especially powerful and informative when the organisms vary in body size by many orders of magnitude in body mass. Our data include organisms spanning about 16 orders of magnitude in body size and representing the evolutionary transitions from prokaryotes to unicellular eukaryotes to multicellular animals. To control for the effects of food supply and activity, the metabolic rate data are classified into two categories according to the conditions of under which the measurements were taken: (*i*) active and fed, and (*ii*) inactive or endogenous or starved. We refer to these as active and inactive. The data include 167 and 188 species in each state, respectively. We analyze these data in the context of allometric scaling to evaluate our hypothesis that scaling of metabolic rate changed across the evolutionary transitions from small, simple prokaryotes to much larger and more complex metazoans. Using nested ANOVAs, we identify differences in scaling slopes and intercepts among groups. Our findings contradict current dogma about the scaling of metabolism and r_{max}, demonstrate how existing constraints and new innovations affected the evolutionary transitions, and raise exciting new questions about the role of energy in the diversification of life.

Results and discussion

Whole-organism metabolic rate increases with body size across prokaryotes, protists, and metazoans, but each group is characterized by a distinctive scaling relationship

that is unique to the body size range of the group (Fig. 1).

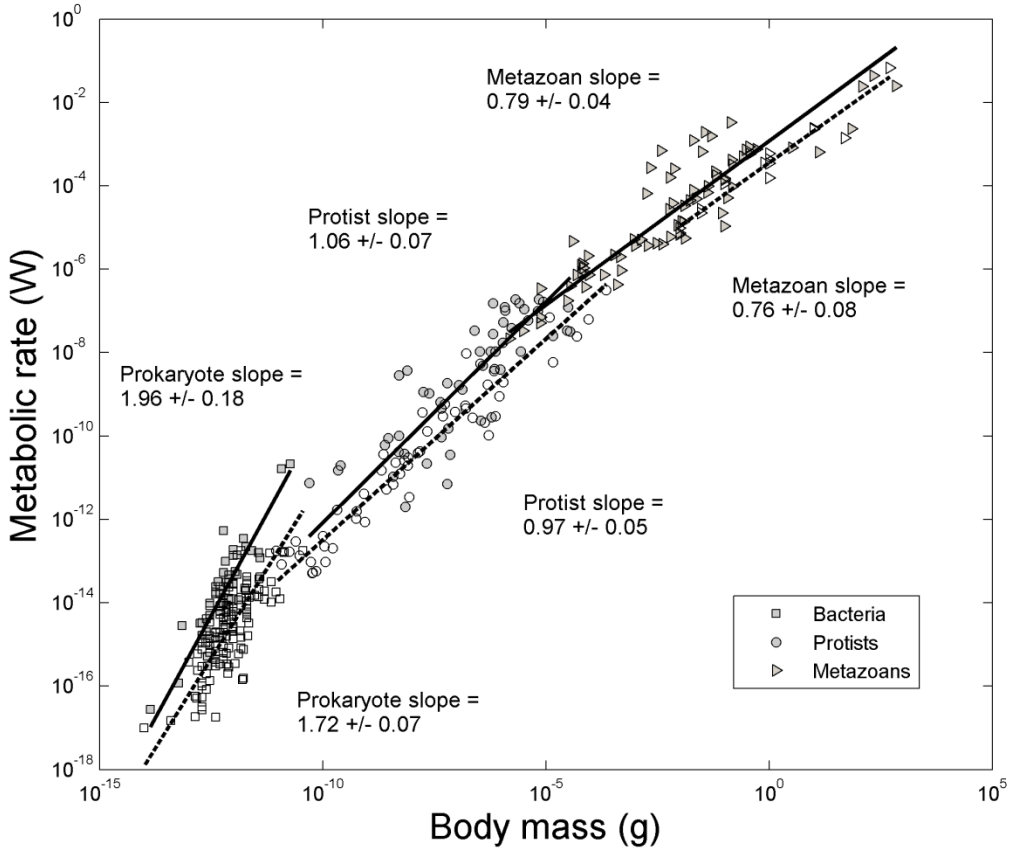

Figure 1. Relationship between whole organism metabolic rate and body mass for heterotrophic prokaryotes, protists, and metazoans plotted on logarithmic axes. Fits are RMA slopes +/- SE. Data for active (filled symbols, solid line) and inactive (unfilled symbols, dashed line) metabolic rates are shown. Differences in slopes among all groups are significant for both physiological states ($p \leq 0.05$).

Although the entire dataset for each metabolic state can be fit with a single power law that accounts for most of the variation, the relationship between body mass and metabolic rate for both active and inactive states is significantly improved by incorporating evolutionary group (ANOVA comparing a 3-line with a 1-line model; active, $F_{4,161} = 9.57$, $p < 0.0001$; inactive, $F_{4,182} = 6.07$, $p < 0.0001$). We also tested for

differences in slopes between protists and metazoans, which differ for both active and inactive rates (ANOVA comparing a 2-line with a 1-line model; active, $F_{1,119} = 3.87$, $p = 0.05$; inactive, $F_{1,63} = 3.96$, $p = 0.05$). Figure 1 shows the raw data, fits, and exponents (+/- SE) for each group. The slopes for the two physiological states are parallel. There is a pronounced shift in the scaling of both active and inactive metabolic rates, from highly super-linear ($\alpha = 1.7$-2.0) in prokaryotes, to nearly linear ($\alpha = 1.0$-1.1) in protists, to sublinear ($\alpha = 0.76$-0.79, so approximating Kleiber's law) in metazoans.

The differences across groups and the large discrepancy between the canonical $\alpha = 0.75$ and the observed, significantly larger, exponents for protists and especially for prokaryotes clearly show that Kleiber's law, long thought to extend across all living things, does not hold for single-celled organisms. These data suggest that scaling of metabolic rate is not governed by a single, overarching design principle that applies to all living things, but instead by different constraints at different body sizes and levels of structural and functional organization.

The scaling of r_{max} also changes across the evolutionary transitions. r_{max} increases with mass in prokaryotes and scales negatively in both protists and metazoans (Fig. 2A). This result contradicts previous findings that found r_{max} scaling with an exponent of approximately -0.25 across diverse taxa from prokaryotes to mammals (20). Since metabolic rate fuels biomass production and population growth, the naive expectation is that r_{max} should scale similarly to active mass-specific metabolic rate, so as $M^{\alpha-1}$. Overall, the scalings of r_{max} roughly parallel the scalings of mass-specific active metabolic rate as expected, with no significant differences in slopes (Fig. 2A, ANOVA,

$F_{3,331} = 0.13$, *NS*). This supports the interpretation that metabolism fuels biomass production.

From these parallel scalings of r_{max} and mass-specific metabolic rate, it follows that the efficiency of biomass production, measured as the ratio of these two variables, is invariant with size within groups. Indeed, the efficiency of production shows no size dependence within groups. Importantly, however, the mean efficiency decreases with each successive transition, from 23×10^{-4} gJ^{-1} for prokaryotes, to 9.2×10^{-4} for protists, and to 1.6×10^{-4} for metazoans (Fig. 2B, $p < 0.001$). Evidently, the increased whole-organism metabolic rate that accompanies the transitions occurs at the expense of decreased efficiency of conversion of metabolic energy into biomass. The mechanisms underlying this decrease in efficiency with increasing body size and complexity across the transitions warrant investigation. Larger, more complex organisms must allocate relatively more metabolic energy to acquiring and processing food resources and relatively less to biomass production. Some of this decrease may be due to changes in the organization and location of energy processing machinery. Metabolic processes are extra-cellular or localized on cell surfaces in prokaryotes, organelle-based in protists, and dependent on complex digestive, respiratory, and circulatory systems in metazoans. So, for example, oxygen is obtained by simple diffusion in unicellular organisms but taken up by gills or lungs and transported through vascular systems in large metazoans. It may not be coincidental, therefore, that each of these evolutionary transitions apparently coincided

with major increases the concentration of oxygen in the atmosphere and oceans (3).

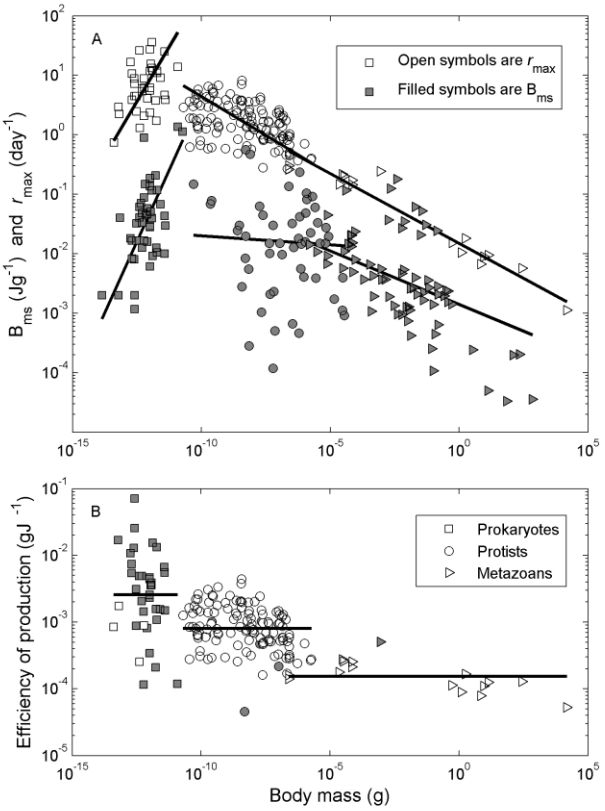

Figure 2. (A) Scaling of r_{max} (maximum rate of population growth; unfilled symbols) and mass-specific metabolic rate (B_{ms}, filled symbols) with body mass for heterotrophic prokaryotes, protists, and metazoans plotted on logarithmic axes. For r_{max}, the RMA slopes are 0.73 for prokaryotes, -0.26 for protists, and -0.23 for metazoans. The plots for mass-specific metabolic rate are approximately parallel to those for r_{max}, consistent with the hypothesis that metabolic rate fuels biomass production. (B). Efficiency of biomass production decreased more than ten-fold across the three groups. Closed symbols are for species where both r_{max} and mass-specific metabolic rates were known. Open symbols are those where r_{max} were known for species but mass-specific metabolic rateswere estimated from the regressions in Figure 1.

A first step in understanding these transitions is to identify potentially important variables that are associated with the scaling of metabolic rate with body size in each

group (Fig. 3). In an initial attempt to account for our unexpected results, we propose the following hypotheses:

1) Prokaryotes: We hypothesize that the very rapid increase in metabolic rate with increasing cell size is made possible by an increase in the number of genes. If cell size limits the number of genes and/or quantity of DNA, then larger cells can have larger genomes. In prokaryotes, larger genomes have more coding genes, which produce a larger number of different enzymes and result in larger, more complicated biochemical networks. These networks can confer increased metabolic power because cells can utilize a greater diversity of substrates as energy sources or use a given substrate more completely, thereby producing more ATP molecules per unit substrate and per unit time.

The link between cell size and metabolic network complexity is further supported by three empirical findings. First, genome size exhibits the predicted positive scaling with cell size. Fig. 4 shows that both number of genes and total genome size scale with cell size as $M^{0.35}$. The parallel scaling confirms that increasing genome size is due to increasing numbers of protein-coding genes (21). Second, the proportion of metabolism-related genes increases with genome size in prokaryotes (22). And third, limited data for the five taxa of prokaryotes in Price et al. (23) show a positive scaling relationship between the total number of metabolic reactions and genome size ($R^2 = 0.83$, $y = 12.5x^{0.62}$). These findings are at least consistent with the hypothesis that the superlinear scaling of metabolic rates in prokaryotes is due to the increase in genome size with body size. Finally, Lauro et al (24) found all three of these types of mass-dependent effects in bacteria.

Metabolic power would be expected to increase with increasing genome size only up until the prokaryotic cells have a relatively complete complement of metabolic enzymes and pathways. Indeed, the smallest eukaryotes, such as yeast, have such a complete metabolic network. Moreover, in prokaryotes, the respiratory complexes of enzymes and protein pumps used in ATP synthesis are located in the cell membranes. This would suggest that with increasing cell size, cell surface area would eventually limit metabolic rate, causing the metabolic scaling to decrease from superlinear toward sublinear. At this point, where surface area constraints take hold for prokaryotes, linear scaling in protists allows them to be more powerful and competitively superior to similarly sized bacteria (25), and therefore they begin to dominate at this size. In this way, the superlinear scaling of metabolic rate with mass gives way to linear scaling, at the precise point in size where bacteria give way to protists along the body size axis.

2) Protists: We hypothesize that the approximately linear scaling of metabolic rate in protists reflects a linear increase in the membrane bound sites of ATP synthesis located in organelles. The ancestral heterotrophic eukaryotes were able to overcome the constraints of limited ATP synthesizing sites on the cell surface by ingesting the symbiotic prokaryotes that evolved into mitochondria (26). This innovation allowed the host cell to contain many mitochondria and have a much larger number of respiratory complexes than if the enzymes and proton pumps were located in the external cell membrane. The new design would allow the total volume of respiratory complexes and the metabolic

rates of eukaryotic cells to scale linearly with size.

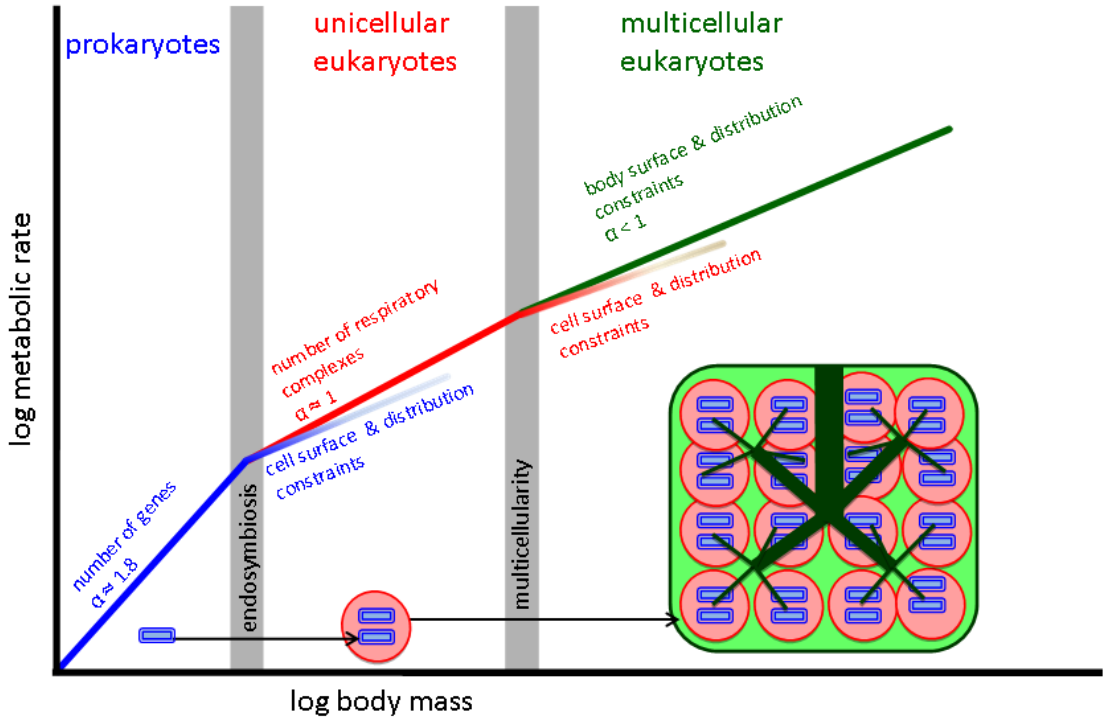

Figure 3. Schematic representation of our hypotheses to account for the scaling of metabolic rate in prokaryotes, protists, and metazoans. Scaling within each group reflects constraints on metabolic power due to the number of respiratory complexes, but geometric constraints on exchange surfaces and transport distances ultimately limit capacity to supply substrates to these active sites. Superlinear scaling in prokaryotes (solid blue line) reflects the increase in number of genes and metabolic enzymes with increasing cell size, until a new constraint (fading blue line) due to cell surface area, where the enzyme complexes and proton pumps are localized, becomes limiting, imposing sublinear scaling. Protists overcome this constraint because the respiratory complexes are in the mitochondria. Larger protists can accommodate more of these organelles, resulting in linear scaling of metabolic rate with volume of mitochondria and cell mass (solid red line), until a new geometric constraint of surface exchange or transport distance limits rate of resource supply to the mitochondria, imposing sublinear metabolic scaling (fading red line). Metabolic rates of metazoans initially tend to increase linearly with number of cells and body mass, but as vascular systems evolved to distribute resources to increasingly large bodies, geometric constraints required sublinear scaling, converging to the ¾-power scaling of Kleiber's law (green line).

This hypothesis predicts that the number or total volume of mitochondria scales linearly with cell mass, similar to the scaling of organs in metazoans. Support for this hypothesis comes from the linear relationship between mitochondrial volume and cell volume in the alga *Polytoma papillatum* (27) and yeast ***Saccharomyces cerevisiae*** (28), and the linear relationship between metabolic rate and the volume of mitochondria in cells of metazoans (29. 30).

Such linear scaling cannot be maintained indefinitely, however. As cell volume and number of mitochondria increase, capacity to supply resources to the respiratory complexes eventually becomes limiting, because cell surface area limits the diffusion and number of active sites for uptake of resources from the environment and because distance within the cell limits the diffusion or active transport of materials to the mitochondria. The consequence is a shift from linear to sublinear scaling. At this point, where surface area constraints take hold for protists, steeper scaling in metazoans allows them to be more powerful and competitively superior to similarly sized protists, and therefore they begin to dominate at this size (25). As with the shift from prokaryotes to protists, the linear scaling of metabolic rate with mass that characterizes protists gives way to sublinear scaling in metazoans, at the size where protists begin to give way to metazoans. Note, however, that there is some overlap in size and metabolic rates of the largest protists and the smallest metazoans.

3) Metazoans: The next evolutionary transition was the origin of multicellular body plans. Having multiple small cells instead of a single large one allows tiny metazoans to

evade constraints of external surface area and internal transport distances.

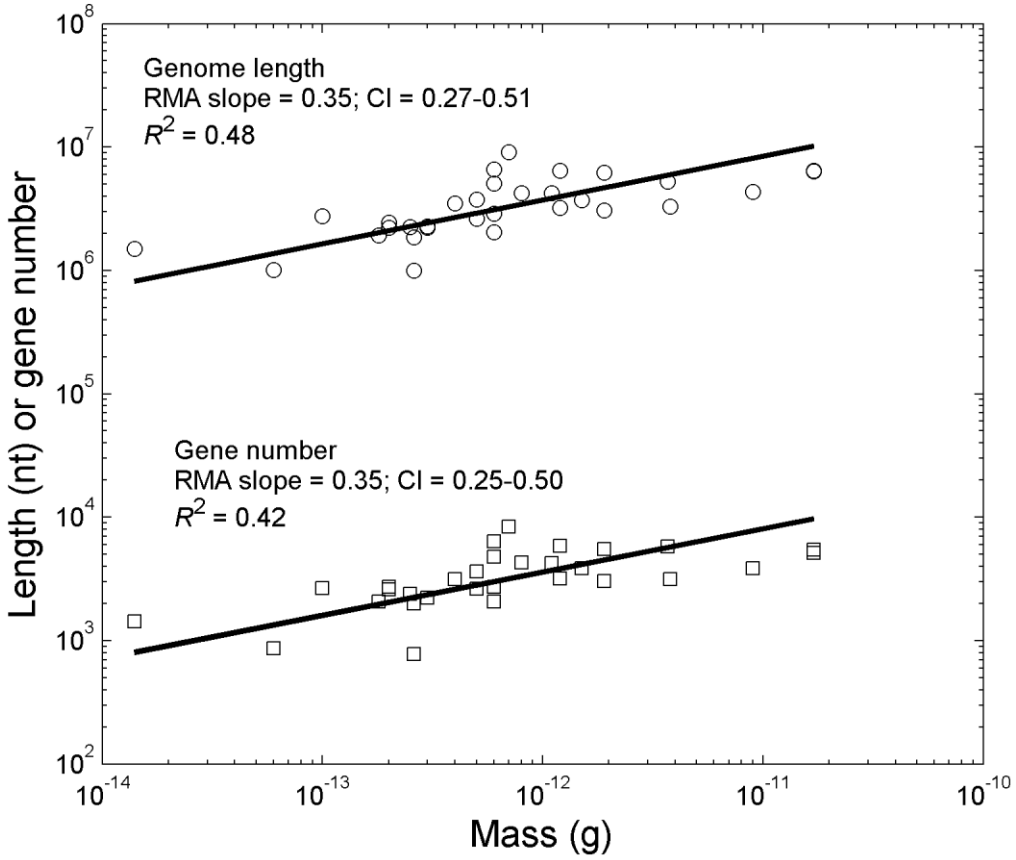

Figure 4. Scaling of genome size with cell size in prokaryotes. Total number of nucleotides (above) and number of different genes (below) scale with identical slopes of 0.35, consistent with our hypothesis that scaling of metabolic power in prokaryotes reflects the number of genes and the complexity of the biochemical network.

We hypothesize that the scaling in the smallest metazoans is initially near linear, as observed empirically in very small animals and plants (15, 16), at least in the region where there is size overlap between protists and metazoans. As body size increases, however, an increasing fraction of body mass has to be allocated to increasingly differentiated vascular and skeletal systems to provide resource supply and mechanical support. Models of resource distribution through vascular networks show the

impossibility of maintaining linear scaling of metabolic rate as body size increases, and several different models independently suggest that the maximal exponent converges to the $\alpha \approx 0.75$ of Kleiber's law (14, 31).

The transitions from prokaryotes to unicellular eukaryotes to metazoans allowed many orders of magnitude increase in body size and accompanying diversification of form and function (3). Changes in the scaling of biological energetics over the resultant 16 orders of magnitude in body size reflect the fundamental dependence of metabolic rate on: i) the number of membrane-bound respiratory complexes where proton pumping and ATP synthesis occur; and ii) geometric constraints on transport distances and surface exchanges that affect rates of resource supply. Each evolutionary group – prokaryotes, protists, and metazoans – display a distinctive scaling that reflects the particular way in which these two constraints arise. The evolutionary transitions themselves, then, can be seen as giving rise to structural and functional innovations that overcame constraints on their precursors, but imposed new constraints that governed the scaling of metabolic rate. Because metabolism fuels biomass production for growth and reproduction, differences across the transitions in scaling of metabolism are also reflected in transitions in population growth rate and production efficiency.

In conclusion, our data and analyses clearly show that the sublinear metabolic scaling and quarter-power scaling relations documented for large, multicellular animals and plants, with the $\alpha \approx 0.75$ for metabolic rate and the $\alpha \approx -0.25$ for r_{max}, do not extend to the smallest organisms. Changes in allometric scaling relations across the major

evolutionary transitions identify some of the most fundamental features of biological energetics that shaped the early evolution of life.

Methods

We combined metabolic rate data from several sources, and all data used in these analyses are available in Supplemental Data 1 and 2. Physiological state has a strong effect on metabolic rates and may influence the observed scaling of metabolic rate with mass (11, 32). We therefore separated data into active and inactive rates. Active rates were defined as rates where individuals were measured in the presence of food or, if not, had only been washed free of their food just prior to measurement. For active and inactive rates of prokaryotes, we used the data sets compiled by Makareiva et al. (17, 18), which are available as supplementary material attached to their original article. We only included prokaryotes species that are obligate heterotrophs (so we excluded species capable of phototrophy, chemotrophy, and mixotrophy, and archaea). For inactive rates of protists, we used the data from Makareiva et al. (17), and for inactive rates of small metazoans, we used the zooplankton data from Gillooly et al (33). For active metabolic rates of protists and zooplankton, we surveyed the literature and developed new data sets. All values in these new data sets were included only after consulting the original references, checking the data, and making sure that the physiological state and other conditions met our criteria for standardization. Multiple values for a species were averaged to create a data set with one mass and one metabolic rate per species. All original metabolic rate units were converted to W, and volumes and masses were converted to g. The data set for active metabolic rate included 44, 51, and 71 species or

strains of prokaryotes, protists, and metazoans, respectively, and for inactive metabolic rate it included 121, 52, and 15 species.

Since all data in this study are for ectotherms, temperature strongly influences their metabolic rates. We used the Boltzmann factor with an activation energy of 0.61 eV to correct all data to 20°C (33). This approach works well because a single correction can be applied to all data, reducing the error variance in the scaling estimates. We analyzed the uncorrected data and still found superlinear scaling in prokaryotes, linear scaling in protists, and sublinear scaling in metazoans, albeit with slightly shallower scaling exponents.

The original studies represented in the data sets used several different methods to measure body mass, including weighing single individuals, weighing large numbers of cells and dividing by the estimated number of cells, and estimation of volume from external dimensions. Body mass data were not available in some studies of protists, so we used values from Fenchel and Finlay (11). Differences in the slopes among groups were determined by ANOVA on log-transformed data, comparing models with group-by-slope interaction terms to models without these terms. As indicated above, there are several sources of error in the body masses reported in these data sets. The presence of non-negligible error in the x-axis variable means that an OLS fitting procedure is likely to produce scaling slopes that are artificially shallow. Many previous studies on the scaling of unicells have used OLS to estimate exponents, which is one of the many reasons that previous studies on the metabolic rate scaling of protists reported sublinear slopes. As advocated by Smith (34), we correct the slopes and intercepts produced by the ANOVA

analysis to the reduced major axis (RMA) equivalent. These corrected parameters are the same as what would be produced by a direct RMA regression, and we present only the corrected slopes. We report standard errors from the OLS fitting procedure, which are equal to those produced by RMA (35).

We surveyed the literature to obtain r_{max} values for prokaryotes, and used data from Caron and Rose (36) for protists and from Savage et al. (19) for metazoans. The data set included 37, 122, and 16 species or strains of prokaryotes, protists, and metazoans, respectively. We also collected data for genome size for prokaryotes from the National Center for Biotechnology Information (NCBI) genome database (http://www.ncbi.nlm.nih.gov/), matching species in our dataset with values for active metabolic rates rate (18), with species-level data in the NCBI database. For some species, multiple entries, with varying genome sizes, were present in the NCBI database. In these cases, we always used the largest genome size.

We estimated the efficiency of biomass production of each species in the r_{max} data set by dividing r_{max} by the active mass-specific metabolic rate. If the active rate was known from the metabolic rate data set, it was used. If the active rate was not known, it was estimated from the regression in figure 1. With a unit conversion of seconds to days, we obtain an efficiency expressed in units of gJ^{-1}.

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CHAPTER 3: Surface area scaling strategies: fractality, geometric dissimilitude, and internalization

Abstract

The surface areas and volumes of biological systems—from molecules to organelles, cells, and organisms—affect their rates and kinetics. The areas of surfaces constrain whole-organism metabolic rate. Understanding the scaling of the surface area, especially the metabolically relevant surface area, with body mass and volume can offer important insights into metabolic scaling and the role of surfaces in the evolution, ecology, and anatomy of living things. The naïve expectation is that the surface area and metabolic rate of an organism scale with body volume or mass as a power law with an exponent of two-thirds. However, quantitative theory and empirical examples show how a variety of adaptations allow organisms to significantly alter their surface area scaling and surface-area-to-volume ratios. These surface area scaling strategies fall into three fundamental categories: (*i*) complexity incorporating fractal-like convolutions and crinkles; (*ii*) geometric dissimilitude through elongating, flattening, fattening, and hollowing; (*iii*) and internalization of surfaces. I develop models and examples illustrating the role of cell membrane elaborations, mitochondria, vacuoles, vesicles, inclusions, and shape-shifting in the architectural design, evolution, and ecology of unicellular microbes. Multicellular organisms and other complex metabolic systems such as biofilms, colonies, and cities can adopt analogous strategies. This unifying theory at the interface of physiology, evolution, and ecology, highlights the fundamental role of body surfaces in metabolism and function of living things.

Keywords: Shape shifting, metabolic scaling, allometry, endosymbiosis, vacuoles, cell physiology, mitochondria, endomembranes, fractal dimension, organelles, Kleiber's law.

Introduction

Living systems are composed of molecules that react in highly ordered processes. Some reactions are informational processes involved in the maintenance and replication of genomes and other informational systems. Other reactions are metabolic processes involved in the uptake, transformation, and allocation of energy and materials. All such reactions involve surfaces, either within the organism or between the organism and the environment—the surfaces of molecules, organelles, and cells. The areas of these surfaces affect the rates and spatial organization of the reactions and are fundamental to anatomy, physiology, ecology, and evolution (e.g., Phillips et al. 2009). The molecular components are spatially embedded within the volumes of the living systems, so the volumes of these components and of the living thing as a whole constrain the surface areas and activities of the components. Understanding biological activities requires considering the spatial structure and functional organization of the surfaces and volumes. Metabolism influences the fitness through the allocation of energy and materials to survival, growth, and reproduction, so the surface areas of metabolic structures are anatomical, physiological, and ecological outcomes of evolution by natural selection.

Metabolic rate, the rate of uptake, transformation, and expenditure of energy and materials, is constrained by surface area at multiple levels of organization. The number and density of sites for active transport and other metabolic processes is ultimately limited by surface area. Furthermore, passive diffusion of gases and other membrane-permeable substances may also be limited by surface area. So a zeroth-order theory

predicts that the rate, B_i, of a metabolic process is proportional to the surface area A_i, of the relevant molecules, organelles, or membranes, and the total metabolic rate of an organism is proportional to the total surface area of the rate-limiting physiological structures. The total active or diffusive flux of materials between organism and environment is constrained by the surface area of the relevant exchange structures, such as the lungs and guts of animals, the cell membranes of prokaryotes and unicellular protists, and the roots and leaves of plants. Some of these fluxes allow the organism to take up resources used for maintenance metabolism and biomass production. Other exchanges may be passive fluxes that are detrimental, requiring structures and energy expenditure to exclude the substance or pump it out against a concentration gradient. Consequently, the total surface area in contact with the surrounding is of great significance to fitness.

A fundamental feature of life is the localization of many metabolic activities on membranes of organelles and cell membranes. The proton-motive force that is harnessed to synthesize ATP by photophosphorylation and oxidative phosphorylation occurs across membranes—the cell membrane in prokaryotes, and the inner membrane of mitochondria and thylakoid membrane of chloroplasts in eukaryotes. Thus the total rate of ATP synthesis is constrained by the total surface area where the critical energy-capturing reactions occur. So, given the effect of surface area on the metabolic activities and physiological homeostasis of cells and organisms, it is not surprising that organisms have evolved adaptations to optimize surface areas for particular functions and to alter surface areas in response to changing environmental conditions.

Body volume is of biological importance for several reasons. Larger organisms require more energy and materials to build and maintain their forms and functions. Also, the volume of an organism imposes limits on the total surface area of the body and physiological exchange structures, with consequences for whole-organism metabolism. The rate of energy transformation within an organism, metabolic rate, and many other characteristics of organisms scale with body volume and mass (Peters 1986; Niklas 1994b; Calder 1996; McMahon and Bonner 1983). The scaling relationship between these traits and body volume or mass is the topic of allometry and is commonly characterized by a power law: $Y = Y_0 X^b$ where Y is the trait, such as metabolic rate or growth rate, Y_0 is normalization constant, X is the size variable body mass or volume, and b is the scaling exponent. Because metabolic rate sets the pace of life, volume or mass-specific biological rates are generally proportional to metabolic rate, and biological times such as lifespan are generally inversely proportional to mass-specific metabolic rate (Brown, Gillooly, et al. 2004).

Because surface area constrains rates of metabolism, an integrative understanding of metabolic organization necessitates understanding how surface area scales with body mass or volume. The naïve expectation is that external surface area and hence metabolic rate scale with volume to the two-thirds power. This expectation rests on the assumption of geometric similitude—that shape and geometry do not change with size. And it rests on the assumption that the surfaces are smooth, Euclidian surfaces. Under these assumptions, $A \propto l^2$, where l is the characteristic length, and V is volume, $V \propto l^3$, and thus $A \propto V^{2/3}$. From the mid 1800s until the mid 1900s, animal physiologists invoked

"the surface law" - that the metabolic rate of an animal is proportional to its surface area – to explain scaling of metabolic rate with body size (Bergmann 1848; Rubner 1883; Schmidt-Nielsen 1984). Following the discovery that metabolic rate of macro-organisms scales with body size with an exponent greater than the expected two-thirds, the body surface law and the importance of surface area in allometric scaling was generally dismissed by animal physiologists (e.g., Kleiber 1947; Peters 1986; but see Niklas 1994b; West, Brown, and Enquist 1999), but still entertained by microbiologists (Lewis 1976; Patterson 1992) and biophysicists (Phillips et al. 2009). Additionally, experiments, analyses, and theory have demonstrated the influence of cell surface area on metabolic rates in unicellular organisms (Paasche 1960; Smayda 1965), gut surface areas and hence digestion and assimilation rates in mammals (Karasov and Diamond 1985), lung and gill surface areas and hence respiration rates in both aquatic and terrestrial animals (Schmidt-Nielsen 1984; Weibel 1987), and total leaf surface area and hence photosynthesis rates in higher plants (Enquist et al. 2007). Furthermore, surface area has remained a fundamental variable in models of biophysical processes and rate kinetics of cells (Phillips et al. 2009). Therefore, a re-examination of the theory relating surface area to biological scaling is timely and warranted.

Here I show how a variety of adaptive modifications of the geometry and physiological organization of surfaces can alter the allometric scaling of metabolic processes, leading to surface area scaling exponents significantly different from two-thirds and correspondingly altered surface area-volume ratios. I show that greater than two-thirds scaling of metabolic rate is not surprising, should actually be quite common, and does not undermine a reformulated surface law.

I develop the quantitative theory and examples to apply specifically to metabolism and scaling of unicellular organisms. The quantitative theory can easily be extended to cells within multicellular organisms, even though the evolutionary and ecological context of the strategies may differ. Multicellular organisms and other complex metabolic systems such as microbial consortia and cities can also follow similar strategies, so some of my results are relevant to scaling across a wide range of animals, plants, metazoans, fungi, and human metabolic systems. The zeroth-order models developed can be modified to incorporate additional details relevant to particular groups of organisms or biological structures at other levels of organization. Surface area scaling strategies elucidate fundamental processes underpinning the major evolutionary transitions of life and are a fundamental feature of cell physiology. Because these strategies play out on ecological timescales, they are also relevant for a trait-based approach to ecology. The surface area theory and examples presented here underscore the foundational relevance of surface area to many problems in evolution, ecology, physiology, and microbiology.

Fractality and the elaboration of surfaces

Theory

Increasing the convolution and roughness of surface can increase the surface-to-volume ratio and surface area scaling (Fig. 5). Such elaborated surfaces are ubiquitous in metabolic systems (e.g., G. A. Losa, Nonnenmacher, and Merlini 1998). Here, I provide a quantitative theory that models the effect of elaborations on surface area scaling. I use the mathematics of fractal geometry, a powerful mathematical approach for modeling the

non-Euclidean nature of rough and elaborated surfaces. This application does not require the assumption that biological surfaces are pure deterministic fractals exhibiting self-similarity to infinitely minute scales. Instead, it quantifies the degree to which the convolutions, folds, and textures that cause departures from smooth surfaces can affect surface area. I use the widely used box-counting dimension, which provides an intuitive characterization of the fractality of surfaces and is more empirically practical than other fractal dimensions.

A surface can be covered with N number of square boxes with an edge of length ε, the yardstick used in measuring the surface area. Decreasing ε leads to an increase in N. If the surface is Euclidian, N decreases as $N \propto \varepsilon^{-D_S}$ with $D_S = 2$, reflecting the simple two-dimensional nature of the surface. However, elaborations in the surface may lead to a departure from $N \propto \varepsilon^{-2}$. The most general representation assumes that $N \propto \varepsilon^{-D_S}$ where $2 < D_S < 3$ and D_S is the fractal box-counting dimension of the surface, indicating that the box-covering properties of the surface are similar at different yardstick lengths (Falconer 2003; Crownover 1995). Other functions for $N(\varepsilon)$ can be used for particular surfaces, but here I develop theory for the more general case of self-similarity because it is the most common spatial scaling pattern in nature (Hastings and Sugihara 1993), allows for analytically tractable solutions more clearly demonstrating the effect of fractality on surface area scaling, provides results that are robust to deviations from $N \propto \varepsilon^{-D_S}$, and builds on a vast literature on the fractality of surfaces (for examples see Literature Cited).

Organisms, like all other objects in nature, are composed of indivisible parts (molecules, protons, etc.) of invariant length $\varepsilon_{\min}$. The effective external surface area of the object, A_{ext}, is thus determined by the number of boxes needed to cover these

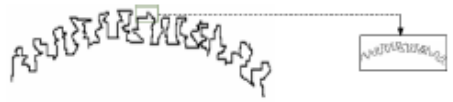
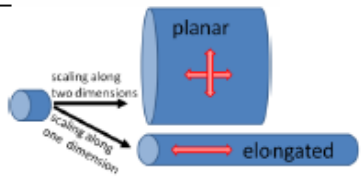
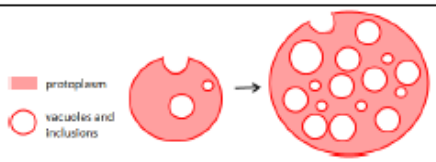
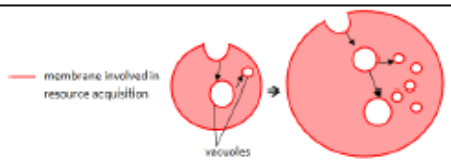
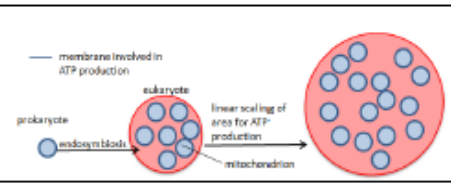
Strategy	Example	Schematic	Central equation	Scaling exponent
Surface elaborations & fractality	Increased resource uptake across the cell membrane		$A \propto V^{D_S/3}$	$2/3 - 1$
Geometric dissimilitude	Solid object shape-shifting		$A \propto V^{(1+\delta)/(1+\delta+\beta)}$	>2/3
	Increasingly "hollow" cells with vacuoles & inclusion bodies		$A = c_2(V_p + bV_p^{\sigma})^{D_S/3}$	$\geq 2/3$
Internalization	Resource uptake with vacuoles & vesicles		$a = c_1 V_{vac}^{-1/3} qV + c_2 V^{D_S/3}$	$2/3 \rightarrow 1$
	ATP synthesis on mitochondria and chloroplasts		$A_{MT} \propto V^1$	1

Figure 5. Overview of surface area scaling strategies, examples, and equations. A = surface area; a = effective surface area for resource uptake; A_{MT} = total mitochondrial surface area; V = cell volume; V_p = protoplasm volume; D_S = box-counting fractal dimension of cell surface; β and θ parameterize the level and type of geometric dissimilitude, all c symbols are shape parameters and constants.

indivisible units multiplied by the area of the boxes: $A_{ext} = N(\varepsilon_{\min})\varepsilon_{\min}^2$. The depth of the convolutions is determined by the maximum box size, $\varepsilon_{\max}$, that can cover the object. The depth of the surface roughness must be a function of the volume of the object size in

order for the roughness to affect surface area scaling, as shall be seen shortly. I make the zeroth-order assumption that $\varepsilon_{\max}$ is proportional to the object's length l, giving

$$\varepsilon_{\max} = cl, \qquad (1)$$

where c is a proportionality constant between zero and one. Assuming other positive functional relationships leads to more complex mathematics but does not change the general nature of the results—fractality increases the surface area-volume scaling exponent. Because the fractal box dimension of the surface, D_S, is equal to $\frac{\log N(\varepsilon_{\max}) - \log N(\varepsilon_{\min})}{\log \varepsilon_{\max} - \log \varepsilon_{\min}}$, substitution gives the following formula :

$$A_{ext} = N_{\min} c^{D_S} \varepsilon_{\min}^{2-D_S} l^{D_S}. \qquad (2)$$

Equation 2 shows that the surface area depends on the fractal dimension characterizing the roughness. If the maximum yardstick of this roughness were the same in both small and large objects, that is $\varepsilon_{\max} \propto l^0$, then instead $\mathrm{A}_{ext} \propto \varepsilon_{\min}^{2-D_S} l^2$ and the roughness would not influence the scaling of surface area with object size. It would, however, still influence the surface-area-to-volume ratio and thus be of great biological relevance.

Scaling of area with volume can now be derived from the scaling of volume with the length scale. For a solid object, I shall show that as size increases, the scaling of volume converges on $V \propto l^3$, giving $A_{ext} \propto V^{D_S/3}$. First consider that the volume can be partitioned into two parts: (*i*) the solid core, which is unaffected by surface convolutions and has diameter $(1-c)l$ and volume $c_V(1-c)^3 l^3$; and (*ii*) the fractal "shell", which has width cl, diameter l, and a volume F that is less than that of a Euclidian shell of the same width and diameter, because the open spaces of the surface convolutions hollow out

the volume of the Euclidian shell. Thus, *F* is some function of c and l, and is constrained to be less than $c_V l^3 - c_V(1-c)^3 l^3$. Because the surface convolutions are self-similar, the proportion of open spaces created by the surface elaborations does not change with size and $F = hcl^3$, where *h* is a constant related to the shape of the object and attributes of the surface geometry. Therefore, $V = c_V(1-c)^3 l^3 + hcl^3$ and substituting this equation into equation 2 gives

$$A \propto V^{D_S/3}. \qquad (3)$$

Equation 3 shows that increasing the dimensionality of a surface by only a small amount can substantially increase the scaling exponent to greater than two-thirds. For example, a surface fractal dimension of 2.25 leads to a surface area scaling exponent of 0.75.

Empirical observations

Roughness and convolutions in biological membrane surfaces are commonly observed. Few studies have investigated their fractality. However, the ones that have find significant levels of fractality. These studies have used microscopy, imaging techniques, and yardstick or box counting methods to determine the fractal dimensions of the perimeters of the membranes, which can be used to roughly approximate the fractal box dimension of the surface (Paumgartner, Losa, and Weibel 1981). Fractal dimensions greater than 2 have been found for the surfaces of a variety of cell types and organelles of varying sizes (Mashiah et al. 2008; Ferreira et al. 2006; Adam et al. 2006; Keough et al. 1991; Paumgartner, Losa, and Weibel 1981; G. A. Losa et al. 2000; G. A. Losa, Nonnenmacher, and Merlini 1998)(Keough et al. 1991). Graphical inspection of micrographs ranging from small cells of bacteria to large cells of protists suggests that the

depth of the fractality often increases with cell size so that the fractality influences the surface area scaling exponent in addition to its effect on the surface-area-to-volume ratio. Although I am not aware of any studies that have investigated this topic in unicellular organisms, several empirical observations support the importance of surface elaborations. The bacterium *Epulopiscium fishelsoni*, one of the largest known prokaryotes, contains many invaginations in its inner membrane (Young 2006) and membrane convolutions are pervasive in many other prokaryotes, such the thylakoids of cyanobacteria. Many protists such as diatoms and foraminifera have incredibly intricate shapes and surfaces.

Geometric dissimilitude and shape-shifting

Theory

Organisms can alter their surface area scaling by increasing the lengths of their bodies along one spatial dimension more than the other spatial dimensions or by increasing the hollowness of their body with increasing size. This phenomenon is referred to here and often in the literature as geometric dissimilitude or dissimilarity—changing shape as size changes. Geometric dissimilitude can be sustained over several orders of magnitude. It is in some ways an underappreciated phenomenon in the literature on allometric scaling, despite being an important adaptive strategy in bacteria (Young 2006) and eukaryote micro- and macro-organisms (Niklas 1994b; Niklas 1994a; McMahon and Bonner 1983). Organism can also alter surface areas by shape-shifting without changing volumes, thereby reducing their area-to-volume ratios.

Solid objects. If an object is solid (i.e., not hollow), then the only option for geometric dissimilitude is to become narrower, flatter, or rounder as size changes,

thereby increasingly approaching or departing from the geometry of a sphere, the shape with the lowest surface area-volume ratio. Here I develop quantitative theory to show how geometric dissimilitude can affect the surface area scaling of solid objects, in addition to the more obvious and already appreciated effects on surface-area-to-volume ratios.

The volume and surface area of organisms is some function of the length scales that parameterize their shapes. A minimum of three characteristic length scales, l_1, l_2 and l_3, which are the longest straight lengths that measure an object, are necessary in order to determine the general shapes of simple three-dimensional objects. More length scales can be used as desired to increase the precision of the quantitative description of more complicated shapes. For simplicity of presentation, I use three characteristic length scales to determine effects of geometric dissimilitude on the scaling of surface area with volume (see Appendix for mathematical details for objects characterized by more than three length scales). As size increases, the functional relationships between l_3, l_2, and l_1 determine how shape changes with scale. If the three lengths are linearly related, organisms exhibit geometric similitude or isometry and smooth surface area scales to the two-thirds power of volume. Alternatively, organisms can maximize geometric dissimilitude by allowing l_1 to increase while allowing l_3 and/or l_2 to remain constant or decrease. In this case, surface area scales linearly with volume if l_3 and l_2 remain constant, and the scaling can even be superlinear (exponent greater than one) if l_3 and l_2 decrease. Organisms can adopt intermediate scaling strategies that result in scaling exponents greater than two-thirds but less than one. Power laws describing the

relationship between the three lengths allow for the simplest and most general characterization of geometric dissimilitude across many orders of magnitude variation in size, so I develop scaling theory according to such scaling relations:

$$l_2 \propto l_1^{\theta} \text{ and } l_3 \propto l_1^{\beta}, \quad (4)$$

where the following relation is defined: $\beta \leq \theta \leq 1$. Other functions can be readily used to describe other more specific kinds of geometric dissimilitude and their resulting surface area scaling relations.

In many geometries, volume scales exactly as $V \propto l_1 l_2 l_3$ and area scales exactly as $A \propto l_1 l_2$. In all other solid objects, based on dimensional analysis, volume and area must approach the scaling of $V \propto l_1 l_2 l_3$ and $A \propto l_1 l_2$ as the ratio of one length to the other lengths increases. For example, for a cylinder with $l_1/l_3 = 2$, the local scaling slope between log *A* and log $l_1 l_2$ is 0.92, not 1 as predicted by $A \propto l_1 l_2$; however, for $l_1/l_3 = 10$, the local scaling slope between log A and log $l_1 l_2$ is 0.97, very close to the predicted $A \propto l_1 l_2$. So as the area-to-volume ratio increases, it follows from the substitution of the above equations that the scaling of area with volume approaches

$$A \propto V^{(1+\theta)/(1+\theta+\beta)}. \quad (5)$$

For example, considering a cylinder scaling with θ = 1 and β = 2/3 and $l_1/l_3 = 10$ the numerical computation of the local area-volume slope is 0.73, not far from the theoretical slope of ¾ predicted in the limit by equation 5. This scaling theory shows that geometric dissimilitude can increase the surface area scaling by biologically significant amounts.

Geometric dissimilitude falls into three canonical categories of scaling: planar, string, and hybrid (Fig. 6). In planar scaling, $\beta < \theta = 1$ and the object becomes sheet-like

in the limit of increasing size—its morphometric transformation is a "stretch" in two dimensions. In elongated scaling, $\beta = \theta < 1$ and the object becomes longer and thinner in the limit of increasing size—it is stretched in one-dimension. If $\beta < \theta < 1$, a hybrid form of geometric dissimilitude occurs that exhibits properties of both sheet and elongated scaling, and the object is stretched in two dimensions. This geometric dissimilitude theory holds for more complex shapes, like star-shaped objects and organisms with appendages, in which case the change in shape of the largest appendage ultimately governs the surface area scaling behavior.

Although dimensional analysis identifies the scaling relation in the limit of increasing size, it is important to note that for particular values of the shape and scaling parameters the scaling may in fact have a different exponent over a particular range in size. In other words, equation 5 is the simplification of a mixed-power law function having one, two, or three scaling regimes with differing exponents. If $C_I < C_{II}$, where C_I, C_{II}, and , C_t are groupings of the scaling and dissimilitude parameters (see Appendix for details), then surface area scales with volume according to three different scaling regimes of consecutively greater exponents (see Appendix and Fig. 9):

$$A \propto \begin{cases} V^{(\beta+\theta)/(\theta+\beta+1)}, V < C_I & \text{phase } i \\ V^{(\beta+1)/(\theta+\beta+1)}, C_I < V < C_{II} & \text{phase } ii \\ V^{(\theta+1)/(\theta+\beta+1)}, V > C_{II} & \text{phase } iii \end{cases} \qquad (6)$$

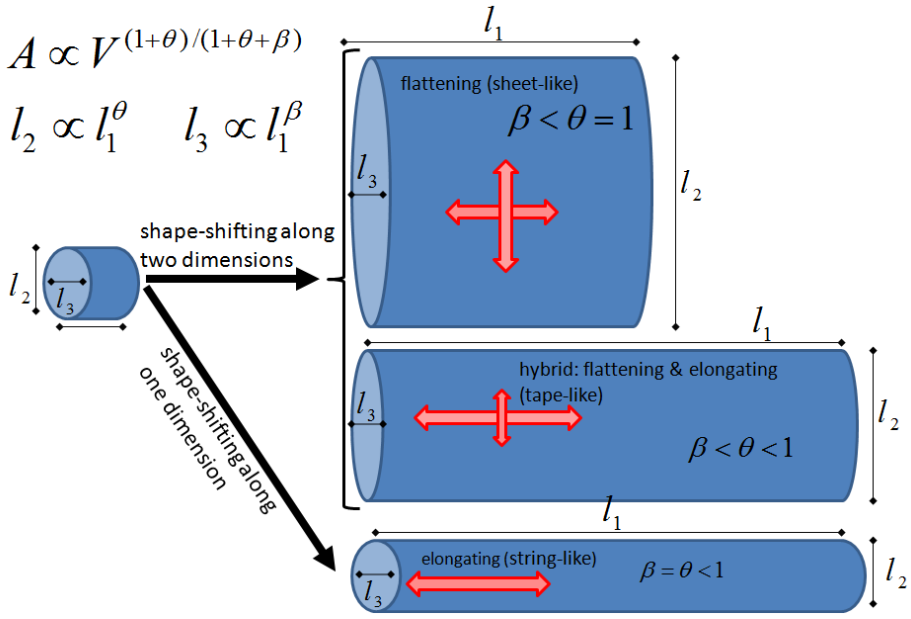


Figure 6. Schematic of solid-object geometric dissimilitude as a strategy for augmenting the scaling of surface area A with volume V of organisms to an exponent different from two-thirds. $\boldsymbol{l_1}$, $\boldsymbol{l_2}$, and $\boldsymbol{l_3}$ are the three major length scales used to characterize the shape of the object, and β and θ quantify the level and kind of dissimilarity observed as the organism increases in volume.

The combination of parameters affects the range in size over which each scaling regime is observed. For some combinations of parameter values, the middle scaling regime will occur across a very limited range of volume. So the result can range from what appears to be simply some slight curvature to a distinctive three-phase pattern. If instead $C_I > C_{II}$, then the scaling behavior has two scaling regimes:

$$A \propto \begin{cases} V^{(\beta+\theta)/(\theta+\beta+1)}, V < C_t & \text{phase } i \\ V^{(\theta+1)/(\theta+\beta+1)}, V > C_t & \text{phase } iii \end{cases} \qquad (7)$$

A rule of thumb is that if $b_2 \ll b_3$ (where b_2 and b_3 are the aspect ratio parameters from $l_2 = b_2 l_1^{\theta}$ and $l_3 = b_3 l_1^{\beta}$) and $\beta \ll \theta$, there are three possible phases of scaling; otherwise, there are essentially two phases. In both cases, the first phase of scaling results from the object increasing in length along its shortest dimensions. Thus, aspect ratios approach 1, the surface-area-to-volume ratio decreases, and the surface area scaling exponent is < 2/3. If lengths continue to increase along these dimensions, they will eventually become longer than the other length scales and the will begin to converge on the last scaling regime with a surface area scaling exponent > 2/3: $A \propto V^{(\theta+1)/(\theta+\beta+1)}$. It is noteworthy that a range in volumes near the critical scaling shift can exhibit curvature in log-log space and a regression line fit to this range of volumes would have a scaling exponent between the exponents of the two relevant scaling regimes (Fig. 7A). Observing the three phases of scaling will require a wide range in volumes and therefore not be observable in many data sets.

Hollow objects: vacuoles and nonliving inclusions. Vacuoles, vesicles, inclusion bodies, granules, and other ergastic substances can lead to cells effectively exhibiting geometric dissimilitude. Cells with these organelles and substances are no longer solid

objects of protoplasm, because the interior of the vacuoles and inclusions can be metabolically inactive and in the case of vacuoles and vesicles can be similar to the outside environment. These vacuoles and inclusions are pockets of non-living material within the living cell, leading to cells being like "swiss cheese" when several vacuoles exist or like a hollow ball when just one large vacuole occupies the inside of the cell. Such "swiss cheese" is extremely common in eukaryotes (Raven 1987a), which rely extensively on bilipid membrane bound vacuoles and vesicles. It can also be found in prokaryotes, which possess protein-shelled gas vacuoles/vesicles and inert inclusion bodies (Young 2006). So, the volume that does not include these vacuoles and inclusions, which is known as the protoplasmic volume V_p, can be considered the quantity of greatest biological relevance for measuring cell size; and the scaling of surface area with protoplasmic volume V_p is the scaling relation that should be considered.

As before, I choose to model the geometric dissimilitude in the simplest way in order to demonstrate the essence of the scaling effects (Fig. 7B). So, I choose a power function to characterize the geometric dissimilitude: $V_{VAC} = bV_p^{\sigma}$, where V_{VAC} is the total volume of non-living bodies, such as vacuoles. Because $V_c = V_p + V_{VAC}$ and $A = c_2 V_c^{D_S/3}$,

$$A = c_2\left(V_p + bV_p^{\sigma}\right)^{D_S/3} . \quad (8)$$

If the vacuoles and cells are spheres, $c_2 = \pi(6/\pi)^{2/3} = 4.84$. When the proportion of total vacuolar volume increases with cell volume ($\sigma > 1$), the scaling converges on

$$A \propto V_p^{D_S\sigma/3}. \quad (9)$$

The transition in scaling from $D_S/3$ to $D_S\sigma/3$ occurs when the protoplasmic volume equals $b^{D_S/(D_S-\sigma D_S)}$, which is when the cell volume is twice the protoplasmic volume:

$V_t = 2b^{Ds/(Ds-\sigma Ds)}$. The apparent scaling as measured by fitting a linear regression model in log-log space is still steeper than $D_S/3$ for ranges of size that are several orders of magnitude smaller than the transition point V_t.

Empirical observations

Solid-shape geometric dissimilitude and shape shifting is supported by numerous observations. There are many examples of shape-shifting in microorganisms where species respond to changing environmental conditions by altering their shapes. For example, under nutrient poor conditions, some strains of the bacterium *Escherichia coli* increase their lengths and volumes while keeping their diameters constant, thereby leading to an increased surface area without the concomitant decrease in the surface-area-to-volume ratio that would result from geometric similarity (Begg and Donachie 1978). Similar phenomena have been observed in several other species of bacteria (Young 2006; Steinberger et al. 2002) and in protists (Sommer 1998). Because of biomechanical problems with maintaining sufficiently strong structures that are highly elongated or planar, significant solid-shape geometric dissimilitude across very wide ranges in body volume is less common (Niklas 1994a).

Hollow shape geometric dissimilitude is commonly observed in microbes. The relevance of vacuoles to area-volume ratios in eukaryotic algae was originally pointed out by Raven (Raven 1987a), although he did not explain its relevance to scaling. I compiled a data set of vacuole and cell volumes in unicellular protists. Total vacuole volume per cell scales with protoplasm volume with an exponent of 1.35 across over nine orders of magnitude variation in cell volume (R^2=0.98, Fig. 8). Assuming a Euclidian cell

membrane, this geometric dissimilitude leads to a surface area scaling exponent of 0.90. The sublinear scaling of carbon mass (carbon cell quota) with cell volume in unicellular eukaryotes (Niklas 1994b; Strathmann 1967) also likely reflects the observed increase in vacuolation with increasing size in protists. In giant bacteria, a large proportion of the volume is occupied by protein-shelled vacuoles, gas vesicles, and mineral inclusions (Otte et al. 1999; Head et al. 2000; Young 2006; in one species a vacuole even occupies 98% of the cell: Schulz et al. 1999), suggesting that similar levels of hollow-shape geometric dissimilitude can also occur in prokaryotes.

Internalization of surface processes with organelles

Theory

Cells can use organelles to increase the surface areas involved in the uptake, transformation, and allocation of resources. This biological design allows cells to vary the scaling of metabolic surface areas. Here I develop scaling theory showing how mitochondria and vacuoles can increase surface-area scaling.

Vacuoles and vesicles. Vacuoles and vesicles also allow cells to increase the effective surface area involved in the exchange of materials with the environment. Vesicles and vacuoles in eukaryotes are enclosed by bilipid membranes. Endocytosis is an important mechanism by which many eukaryote cells ingest resources from the external environment and store the resource in vacuoles. In prokaryotes, endocytosis is unlikely to be common, but it has been reported (Lonhienne et al. 2010), and large bacteria and Archaea typically have large protein-shelled vacuoles containing stored metabolites (Young 2006; Schulz et al. 1999). In many cases, the composition and

function of the interiors of vacuoles are more or less similar to the exteriors of the cells, allowing cells to take up resources from the environment both across vacuolar and external cell membranes (Raven 1987a; Raven 1997). Thus the effective surface area relevant to resource uptake, a, is often equal to the total membrane area of all the vacuoles in the cell that have resources from the environment, A_{VAC}, plus the external cell membrane area, A_{ext}. I assume that the shape and size of vacuoles is invariant with cell size. Similar to mitochondria, A_{VAC} can scale linearly with cell volume, because $A_{VAC} = c_1 V_{vac}^{D_{vac}/3} N_{vac}$ and $N_{vac} = qV / V_{vac}$, where q is the proportion of cell volume occupied by vacuoles, V_{vac} is vacuole volume, and D_{vac} is the fractal dimension of the vacuole surfaces. Thus,

$$a = c_2 V^{D_S/3} + \left(c_1 V_{vac}^{(D_{vac}-3)/3} q\right) V^1 \qquad (10)$$

where c_1 and c_2 again are the shape normalization constants for the vacuoles and cell, respectively. Equation 10 predicts a mixed power function that transitions from sublinear scaling to linear scaling at $V_t = \left(V_{vac}^{1/3} c_2 / q c_1\right)^{3/(3-D_S)}$. If the vacuoles and cells have the same shapes and smooth surfaces, $V_t = V_{vac} q^{-3}$. So, as expected, increasing the proportion of vacuolar volume or decreasing the size of vacuoles causes the transition to linear scaling to occur at smaller volumes. In ranges of cell sizes covering this transition point, the scaling will appear to have an exponent between $D_S / 3$ and 1 and therefore will be greater than two-thirds. But it will be difficult to empirically distinguish between the mixed power law and a pure power law, even for data with orders of magnitude variation in size (Fig. 7C).

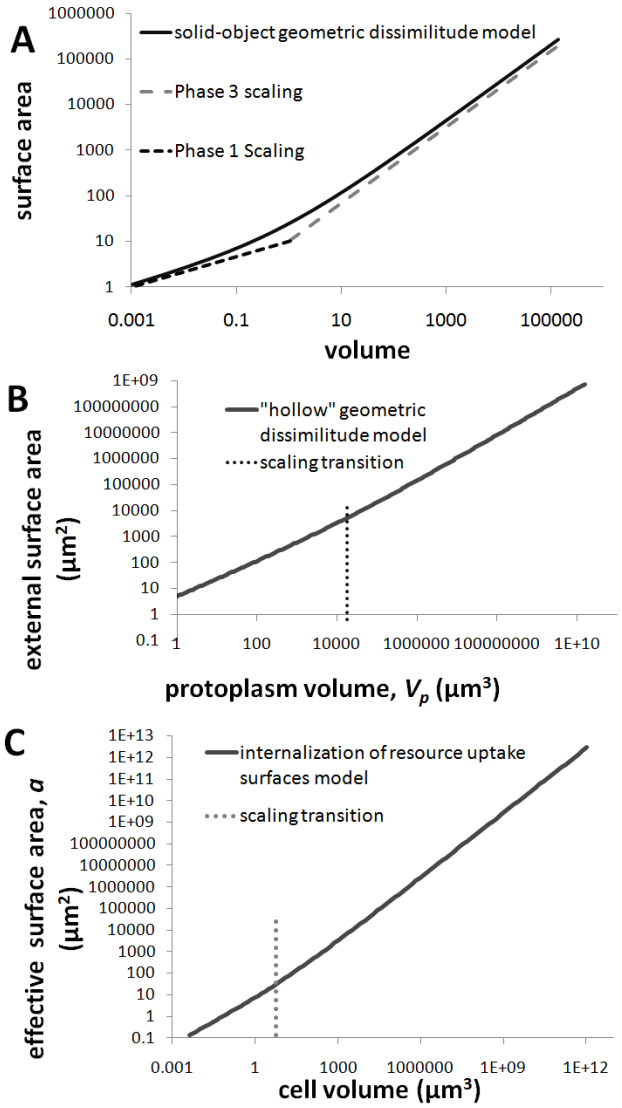


Figure 7. Theoretical examples of different surface area scaling strategies that result in mixed-power law functions. (A) Solid-object geometric dissimilitude for an object elongating with increasing volume by shape-shifting along one dimension (scaling shifts from an exponent of 1/3 to 5/6; β=0.25, θ=0.25, b_2=1, b_3=10; in the plot, l_1 increases from .002 to 100, l_2 increases from 0.2 to 1000, and l_3 increases from 2 to 6). (B) Hollow geometric dissimilitude in cells resulting from an increasing proportion of vacuoles and non-living inclusion bodies with increasing cell size. Model parameterized according to the observed increase in total volume fraction of vacuoles (see Fig. 8). (C) The effect of vacuoles and vesicles on the scaling of the effective surface area involved in resource uptake in cells. The scaling shifts from two-thirds scaling to linear scaling with increasing cell volume. Parameters based on data from Atkinson et al. (1987b) on the alga Chlorella fusca.

Mitochondria. By internalizing the membranes involved in ATP synthesis, eukaryotic cells have the potential for the ATP-producing surface area to scale linearly with cell volume. They can also have greater ATP-producing surface area and higher rates of aerobic respiration than prokaryotic cells of the same size, where the ATP-producing reactions are on the external cell membranes. The total surface area of the mitochondria per cell, A_{MT}, is equal to the number of mitochondria, N_{mt}, times the average surface area of a mitochondrion, A_{mt}: $A_{MT} \propto N_{mt}A_{mt}$. A_{mt} scales with V_{mt}. So when it is advantageous to maximize aerobic metabolic power, this can be accomplished by maximizing the scaling of total mitochondrial volume $N_{mt}V_{mt}$with cell volume. Superlinear scaling of total mitochondrial volume cannot be sustained over orders of magnitude changes in cell volume. For instance, if the mitochondrial volume scales as $V_c^{4/3}$, and occupies 10% of the volume in a small cell, after three orders of magnitude increase in cell size total mitochondrial volume would occupy 100% of the cell, leaving no space for the cytosol and essential organelles. So, the zeroth-order theory is that $N_{mt}V_{mt} = qV$, where q is the mitochondria volume fraction. Assuming the size and shape of mitochondria are invariant with cell size, as supported by recently compiled data (Okie et al. *in prep)*, the following result is obtained:

$$A_{MT} \propto V^1. \qquad (11)$$

In fact, so long as the number of mitochondria increases with cell volume and q does not decrease with increasing size, the total mitochondria surface area will scale with volume with an exponent greater than two-thirds. Mathematically, this is shown by considering the following scaling relations, $A_{MT} \propto N_{mt}A_{mt}$, $N_{mt}V_{mt} \propto V^Z$, $N_{mt} \propto V^{Z_1}$, $V_{mt} \propto V^{Z_2}$,

and $A_{mt} \propto V_{mt}^{2/3}$, where Z, V^{Z_1}, and Z_2 are the scaling exponents. Substituting, we obtain $A_{MT} \propto V^{Z_1+(2/3)Z_2}$ and $z = z_1 + z_2$, giving $A_{MT} \propto V^{Z_1+(2/3)(Z-Z_1)}$. If $Z = 1$ and $Z_1 > 0$, $Z_1 + (2/3)(Z - Z_1) > 2/3$ and so $A_{MT} \propto V^{\epsilon}$, where $\in > 2/3$.

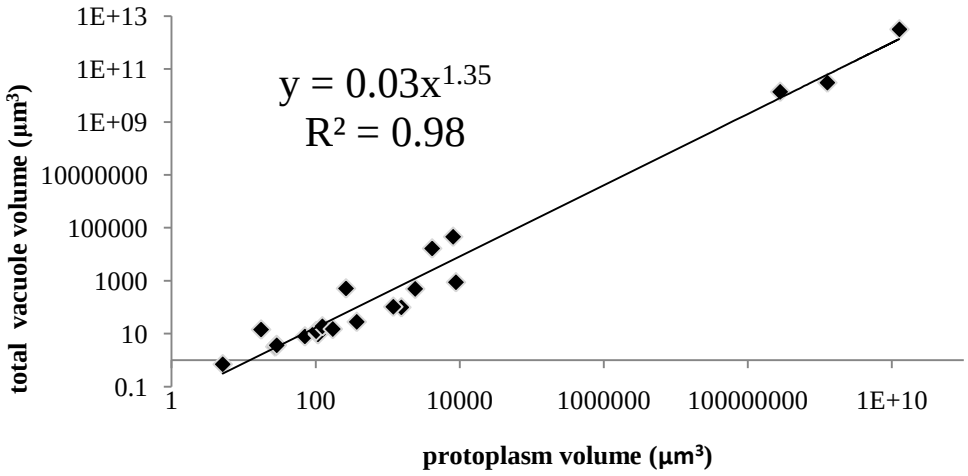


Figure 8. Scaling of total vacuole volume with protoplasm volume in eukaryote cells. The superlinear scaling relation demonstrate the presence of "hollowness" geometric dissimilitude in these cells: larger cells are more hollow than small cells, allowing cells to have greater than two-thirds scaling of surface area with protoplasm volume. Note that a fit that excludes the three large species still has an exponent greater than 1, demonstrating robust geometric dissimilitude. Data are from Raven (1987b).

The following example demonstrates the viability of this strategy for increasing the total surface area available for ATP production. The endomembrane scaling strategy can be estimated quantitatively by considering a spherical cell of radius r_c and spherical mitochondria or endosymbionts of radius r_{mt} that are packed within the cell. As few as six mitochondria occupying 43% of the host cell volume are required in order for the total surface area of the mitochondria to exceed the surface area of the host cell (based on straightforward calculations from simulation results in Birgin and Sobral 2008; see also

Aste and Di Matteo 2005). This may be a conservative estimate, because the internal membranes of mitochondria are not smooth but instead have higher fractal dimensions (Aon, Cortassa, and O'Rourke 2004; Paumgartner, Losa, and Weibel 1981).

Empirical observations

Because not all vacuoles within a cell may function as surfaces for resource uptake, it is problematic to use data from the literature to determine where species fall with respect to theoretical scaling exponents. However, empirically supported parameter values suggest local slopes for effective surface area are often between 2/3 and 1. For example, in a mature protist algal cell (G_2 Interphase) of the species *Chlorella fusca*, the parameter values are $q = 0.08$, $V = 188\ \mu m^3$, $c_2 = 4.8$, $c_1 = 4.8$, $V_{vac} = 0.24\ \mu m^3$, and $N_{vac} = 63$ vacuoles, so V_t is predicted to be $5.6\ \mu m^3$ and the local scaling exponent at *C. fusca*'s volume of $188\ \mu m^3$ is 0.92, (conservatively assuming two-third scaling of cell external surface area with cell volume; data from Atkinson, John, and Gunning 1974). The origin of mitochondria via endosymbiosis was a crucial event in the major evolutionary transition of eukaryogenesis. The scaling theory developed above quantitatively demonstrates the metabolic advantage of this endosymbiosis, elucidating underlying causes of this important transition in the evolution of early life from prokaryotes to eukaryotes that allowed for the diversification of animals and plants. The scaling theory also explains why the evolution of mitochondria may have been a necessary innovation to allow for the evolution of the orders of magnitude increase in cell size that accompanied the evolutionary transition from prokaryotes to unicellular eukaryotes (Payne et al. 2009).

Other combinations of scaling strategies

The three three strategies—fractality, geometric dissimilitude, and surface internalization—are not mutually exclusive, and all may be used to modify the surface area scaling relations.

If the vacuole and external cell membrane have a fractal dimension *D*, then combining the effects of surface fractality and the effects of hollow-object geometric dissimilitude result in the total effective surface area for resource uptake scaling according to the following formula:

$$a = kbV_p^{\sigma} + kV_p + c_2\left(V_p + bV_p^{\sigma}\right)^{Ds/3}, \qquad (12)$$

and with increasing size, this converges on $a \propto V_p^{\sigma}$, where σ is again the scaling exponent from the power law relationship between total vacuole volume and protoplasm volume. So, vacuole volume can have an even greater effect than presented earlier.

Simultaneous effects of surface fractality and solid-object geometric dissimilitude may also be combined into one model. Unfortunately, it is a much more complex mathematical problem—no complete and general analytical model can likely be obtained and complicated computer simulation modeling will probably be necessary. However, for cases of non-extreme geometric dissimilitude, an approximation theory can be derived to elucidate the scaling.

The fractal dimension determines the effect of fractality on the surface area of an object with volume V_0 compared to if that object were a Euclidian object with a smooth surface but the same volume. Substituting the equation for the fractal area of the object, $A \propto V_0^{Ds/3}$, with the Euclidian area, $A_{eu} \propto V_0^{2/3}$, the following relation between the fractal and Euclidian area of an object is obtained: $A \propto A_{eu}^{Ds/2}$. Substituting, equations

6 and 7, previously obtained for the surface area scaling of geometrically dissimilar Euclidian objects, gives $A \propto V^{\omega D_S/2}$, where ω is the area-volume scaling exponent that would occur in absence of fractality. For example, in the limit of increasing size, $\omega = (\theta + 1)/(\theta + \beta + 1)$. However, because the assumption for the fractal convolutions, $\varepsilon_{\max} = cl$, is necessarily violated by geometric dissimilitude, the approximation $A \propto V^{\omega D_S/2}$ provides an upper bounds on the surface area scaling exponent. Most importantly, surface fractality by its very nature cannot have an effect on surface area scaling exponents that are > 1. Therefore, an improved approximation is

$$A \propto \begin{cases} V^{\omega D_S/2}, \text{ for } \omega D_S/2 \leq 1 \\ V^{1}, \text{ for } 2/D_S < \omega < 1 \\ V^{\omega}, \text{ for } \omega > 1 \end{cases}. \qquad (13)$$

Discussion and synthesis

The unifying theory developed here demonstrates that unicellular organisms can adopt numerous strategies that alter surface area scaling exponents and surface-area-to-volume ratios for surfaces involved in metabolic processes. This theory also has broader implications for understanding microbial ecology, metabolic scaling, plant and animal physiology, the metabolic theory of ecology (sensu Brown, Gillooly, et al. 2004 and subsequent papers), and the role of metabolism in evolution.

General implications for allometric scaling. The surface area scaling theory presented above shows that there are several ways that unicellular organisms, as well as biological systems at other levels of organization, can attain surface area scaling exponents greater than two-thirds for surfaces and their processes that potentially limit metabolic rate. This result has important implications for metabolic scaling in

microorganisms and organisms in general, and it reaffirms the underlying importance of the surface law.

First, for a given body size and all else being equal, a higher metabolic rate translates into higher fitness by allowing more energy to be allocated to reproduction (Savage, Gillooly, et al. 2004; Brown and Sibly 2006). Thus there is reason to expect that organisms will tend to exhibit scaling of exchange surface areas and consequently metabolic rate so that exponents are >2/3, as is most commonly observed (Peters 1986; Savage, Gillooly, et al. 2004).

Second, interpretation of metabolic scaling relations within the context of this surface area scaling theory can help in the identification of fundamental processes underpinning metabolic scaling relations. For example, the surface internalization strategy shows how heterotrophic unicellular protists may be able to achieve linear scaling of the total rate of resource uptake and ATP synthesis with cell mass. Indeed, linear metabolic scaling has been observed in heterotrophic unicellular protists (DeLong et al. 2010). This suggests that the distribution of resources to the mitochondria via diffusion and active transport does not ultimately constrain metabolic scaling across the wide size range of most heterotrophic protists. A combination of surface area scaling strategies may be essential for heterotrophic bacteria to attain suplerlinear metabolic scaling, as has been recently observed (DeLong et al. 2010). Although the scaling theory presented here underscores the importance of surface area in understanding metabolic scaling, it does not preclude a role for within-organism distribution networks to also constrain metabolic scaling, especially in large multicellular animals and plants, which have evolved vascular systems to distribute resources within the body (West, Brown, and

Enquist 1997; Banavar et al. 2010). Rather, it shows the need to consider both surface and within organism processes in tandem in order to develop an integrative understanding of allometric scaling that more explicitly recognizes the symmorphosis of physiological structures (for one successful example of this integration see Hou et al. 2008).

Third, surface area scaling theory often predicts mixed power laws having one or more scaling regimes and exhibiting curvature in log-log space at volumes near the transition points. This suggests that shifts in scaling exponents and biologically-meaningful curvature may be common, and the identification of such curvature and scaling shifts in allometric data can offer powerful insights into processes that regulate metabolic structure and function. Indeed, the application of such a perspective in recent analyses of metabolic scaling data has resulted in important insights into scaling of basal metabolic rate (DeLong et al. 2010; Kolokotrones, Van Savage, and Fontana 2010; Mori et al. 2010) and assimilation rate during ontogeny in animals (Hou et al. 2008).

Fourth, the substantial effect of the "hollowness" of organisms on scaling invokes a fundamental question underlying allometry: what is the appropriate measure of body size that should be used in comparative studies? We have seen that protoplasm volume is one appropriate measure of cell volume, because it excludes the metabolically inactive volumes inside vacuoles and non-living inclusions. Because these vacuoles and inclusions have low densities of organic materials (and lower densities of solid materials in general in vacuoles), they affect the scaling of whole-cell carbon, nitrogen, and phosphorous content with cell volume or mass. Thus the observed disproportionate increase in total vacuole volume with cell volume (Fig. 8) should translate into a sublinear scaling of carbon, phosphorous, and nitrogen mass with cell volume or mass.

Indeed, whole-organism carbon and nitrogen content have been observed to scale sublinearly with cell volume in protists (Mullin, Sloan, and Eppley 1966; Menden-Deuer and Lessard 2000; Johnson et al. 2009) and also in plant cells, in which the sublinear scaling in carbon mass was linked to increasing total vacuole volume fraction (Niklas 1994b). Given the biochemical universality of carbon, total carbon mass is also an appropriate measure of size. Indeed, protist ecologists have often focused as much on the scaling of traits with cell carbon content, as with cell volume or cell mass (e.g., Moloney and Field 1989; Johnson et al. 2009).

Similar considerations are also of concern in the scaling of multicellular organisms. For example, in many soft-bodied aquatic and marine invertebrates, much of the body weight is water that functions as a hydrostatic skeleton. In such cases dry weight usually is a more appropriate measure of size than wet weight. Another example is the dead or relatively metabolically inert material providing the mechanical support and/or protective shield for organisms: the bones of vertebrates, the dead tissues of the xylem and bark in trees, and the mineralized shells and exoskeletons of many animals. Since most of these tissues scale allometrically with body mass (Peters 1986; Niklas 1994b), they necessarily have to be considered in developing a complete and integrated understanding of metabolic scaling.

Scaling of organelles and of cells of multicellular organisms. Scaling of surface area also has important implications for understanding scaling of cells within multicellular organisms and allometric scaling at other levels of biological organization. Bodies of multicellular organisms are composed of cells that vary widely in both size and shape, and all of the living cells have metabolism: they take up resources across their cell

membranes and synthesize ATP. Therefore such cells face similar scaling problems as unicellular organisms. Just as the metabolic rate of a unicellular organism is constrained by cell size and the availability of resources, the metabolic rate of a cell within an animal or plant must be affected by its size and the supply of resources. Allometry traditionally has focused on how the body size of a multicellular organism affects the total flow of resources to cells, in other words the whole-organism metabolic rate. However, in order to understand the physiology of the cell, its biological design and scaling properties must also be considered. For example, geometric dissimilitude has been observed in cells of the green alga *Chara*, which elongate and increase in total vacuole volume fraction with increasing cell size (Niklas 1994b).

This surface area scaling theory also applies to the bodies and organs of multicellular organisms and to the surface areas of biofilms and microbial mats. The guts and lungs of animals are surfaces that allow the organism to increase the effective surface area involved in the exchange of resources and wastes (oxygen, carbon dioxide, food) with the environment. These organs are highly convoluted and fractal, with high fractal dimensions (Weibel 1991), resulting in enormous surface areas that scale with body mass, typically with exponents between 3/4 and 1 (Weibel 1987; Peters 1986). Some animals actually increase the surface area of the gut after feeding so as as to assimilate food at a greater rate (Karasov 1996). Multicellular organisms without vascular systems employ solid object and hollow object geometric dissimilitude that increases surface area for the exchange of oxygen and nutrients. Here are some examples: parasitic worms tend to have highly elongated shapes and many invertebrates exhibit solid-object geometric dissimilitude (Niklas 1994a); some primitive multicellular organisms are hollow balls,

which gives 0.76 surface area scaling exponents in *Volvox* (Niklas 1994b); the body cavities in primitive coelomates and pseudomates increase the surface area available for nutrient absorption—in fact, the evolution of the coelom is recognized to be a critical step in the evolution of higher animals.

These surface area scaling principles are also of relevance to understanding the form and function of organelles. The high fractal box-counting dimensions of the inner mitochondria membrane and endoplasmic reticulum membranes, 2.54 and 2.72, respectively (Paumgartner, Losa, and Weibel 1981), reflect the known function of these organelles—maximizing synthesis of ATP, lipids, and protein, which are surface-dependent processes that occur across the mitochondria inner membrane and on the endoplasmic reticulum. In contrast, the function of some membranes and other structures is to efficiently compartmentalize chemicals, so these structures should have low fractal dimensions and rounded shapes approaching spherical geometries. Indeed, the outer mitochondrial membrane functions to contain free radicals that are produced by oxidative respiration and cause damage in parts of the cell. It has a smooth surface with a low fractal dimension of 2.09 (Paumgartner, Losa, and Weibel 1981) and the typical mitochondrion has the external shape of a short rod. Likewise, one of the important functions of protein micro-compartments such as carboxysomes is to compartmentalize and concentrate enzymes, volatile compounds, and gases within their protein shells, chemicals that may be harmful to the rest of the cell, essential for metabolic reactions like photosynthesis, and readily diffuse through bi-lipid membranes but not the more selectively permeable protein shells (Yeates, Thompson, and Bobik 2011; Bobik 2007). Therefore, the observed sphere-like shapes of these organelles maximizes the amounts

and concentrations of enzymes and resources that can be sequestered per shell surface area by reducing the surface-area-to-volume ratio as much as possible

Concluding remarks. Unicellular organisms can adopt numerous strategies to alter surface area scaling exponents and surface area to volume ratios for structures involved in metabolic processes. Some of these strategies require major evolutionary innovations that alter the fundamental metabolic design, such as the evolution of mitochondria. Other strategies require simple phenotypic changes such as the lengthening, flattening, or increasing vacuolation of a cell and the complexifying of the cell membrane, and may be elicited by changing ecological conditions. The examples presented here highlight the fundamental importance of integrating the geometry of surface structures and processes to obtain a more synthetic understanding of organismal form and function at the interface of physiology, evolution, and ecology.

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Appendix: Mathematical details of solid-body geometric dissimilitude

The surface area *A* and volume *V* of a solid object are functions of the length scales. At least three characteristic lengths are necessary in order to determine the general shapes of a variety of objects: l_1, l_2, l_3, additional length scales, l_4, l_5, ... , may be used as desired to increase the precision of the quantitative description of the object’s shape and size. For dimensional reasons,

$$V = l_1 l_2 l_3 f_V\left(\frac{l_3}{l_1}, \frac{l_3}{l_2}, \ldots\right) \text{ and} \qquad (1)$$

$$A = l_1 l_2 f_A\left(\frac{l_3}{l_1}, \frac{l_3}{l_2}, \ldots\right) \quad (2)$$

where f_V and f_A are functions of the ratios of the various length scales. Power functions provide general descriptions of the relationships between length scales across a range of sizes of the object:

$$l_2 = b_2 l_1^{\theta} \text{ and } l_3 = b_3 l_1^{\beta}, \quad (3)$$

where by definition the following relationship exists between exponents : $\beta \leq \theta \leq 1$ (such a definition simplifies the presentation of the analysis without altering the general results in any way). Substituting equation 3 into 1 and 2, area can be written as a function of volume:

$$A \propto V^{(\theta+1)/(\theta+\beta+1)} \quad (4)$$

Although dimensional analysis identifies the scaling relation in the limit of increasing size, it is important to note that for particular values of the shape and scaling parameters the scaling may in fact exhibit a different exponent over a limited range in size. In other words, equation 4 is in fact the simplification of a mixed-power law function. This mixed-power law is readily revealed by considering the scaling of a box: $A = l_1 l_2 + l_2 l_3 + l_1 l_3$ and $V = l_1 l_2 l_3$. Substituting in these equations with $l_2 = b_2 l_1^{\theta}$ and $l_3 = b_3 l_1^{\beta}$, we obtain the function $A = b_2 V^{(\theta+1)/(\theta+\beta+1)} + b_3 V^{(\beta+1)/(\theta+\beta+1)} + b_2 b_3 V^{(\beta+\theta)/(\theta+\beta+1)}$. We obtain the following critical groupings of parameters that determine the volumes at which the scaling regime switches to another scaling regime: $C_I = b_2^{(\theta+\beta+1)/(1-\theta)}$, $C_{II} = (b_3/b_2)^{(\theta+\beta+1)/(\theta-\beta)}$, $C_t = b_3^{(\theta+\beta+1)/(1-\beta)}$. If $C_I < C_{II}$, then surface area scales with volume according to three different scaling regimes of consecutively greater slopes:

$$A \propto \begin{cases} V^{(\beta+\theta)/(\theta+\beta+1)}, V < C_I \\ V^{(\beta+1)/(\theta+\beta+1)}, C_I < V < C_{II} \\ V^{(\theta+1)/(\theta+\beta+1)}, V > C_{II} \end{cases} \quad (5)$$

For some combinations of parameter values, the middle scaling regime will occur across a very limited range in volume and thus exhibit quite noticeable curvature in log-log space. If instead $C_I < C_{II}$, then the scaling behavior has two scaling regimes:

$$A \propto \begin{cases} V^{(\beta+\theta)/(\theta+\beta+1)}, V < C_t \\ V^{(\theta+1)/(\theta+\beta+1)}, V > C_t \end{cases} \quad (6)$$

We find from this analysis and as suggested by logic, that a rule of thumb is that if $b_2 \ll b_3$ and B $\ll$ θ, there are three phases of scaling. Otherwise, there are one or two phases of scaling. The scaling for a range in volume that encompasses or is near the critical volume at which the scaling shifts can exhibit curvature in log-log space and a regression line fit to the this range of volumes would have a scaling exponent falling between the scaling exponents of the two relevant scaling regimes.

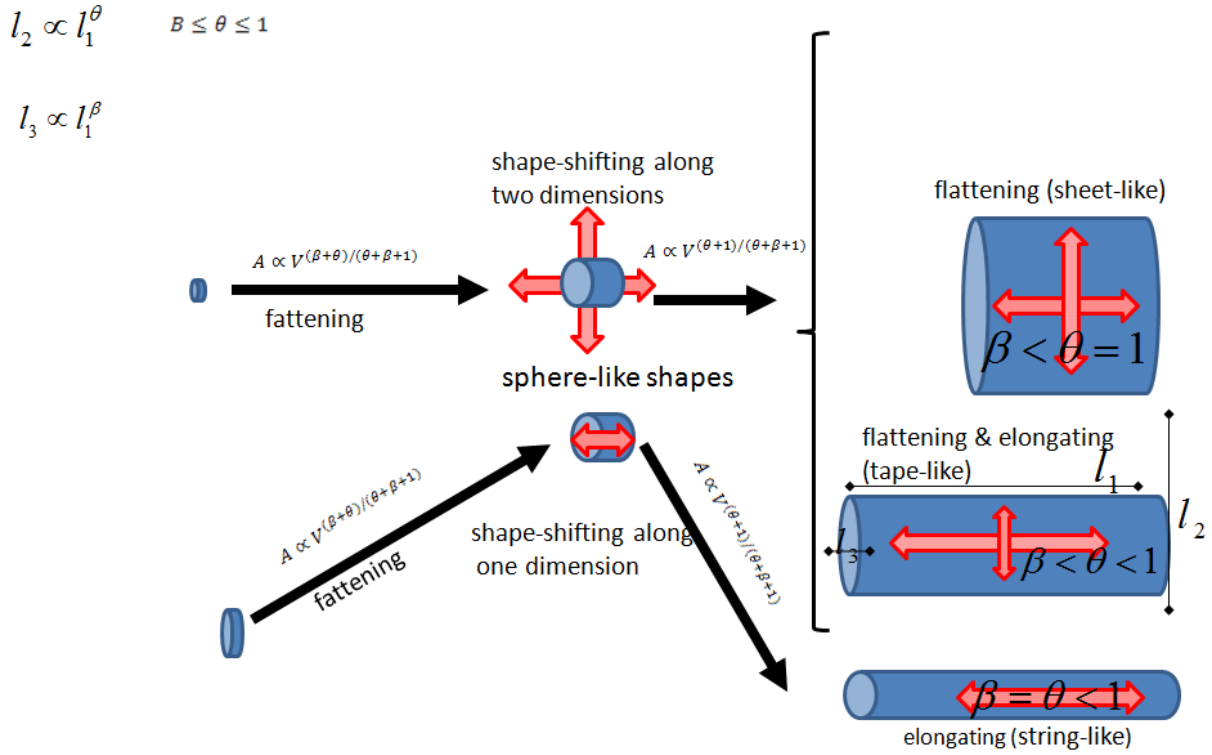

Figure 9. Mixed-power law scaling resulting from solid-object geometric dissimilitude over a wide range in size.

CHAPTER 4: Towards a metabolic theory of ecology for microorganisms

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Summary

1. The biological activity and diversity of prokaryotes and unicellular eukaryotes is extraordinary.
2. The metabolic ecology of these microorganisms is governed by five fundamental physical and biological dimensions of life:
    i. Thermodynamics
    ii. Chemical kinetics
    iii. Physiological harshness and environmental stress
    iv. Cell size
    v. Levels of biological organization, including host-endosymbiont mutualisms, consortia, biofilms, multicellular prokaryotes, and multi-domain superorganism complexes.
3. The metabolism and chemical kinetics of the higher levels of biological organization emerge from the complex interaction of the energetics of the individuals and their biochemical reactions.

4. Identifying shifts in metabolic scaling across major transitions in ecological and evolutionary organization can elucidate some of the most fundamental features of bioenergetics that shaped the early evolution of life and shape the ecology of microorganisms today.

Introduction

Microscopic organisms are of macroscopic importance. Microorganisms are everywhere. They make up a majority of the biomass on Earth. Prokaryotes alone have an estimated abundance of 4-5 x 10^{30} cells, a global carbon mass 60-100% that of plants, and global nitrogen and phosphorous masses about 10-fold more than plants (Whitman et al. 1998). The metabolic activities of microorganisms have crucial roles in local and global biogeochemical cycles. Our food industry, biotechnology, medicine, agriculture, and health rely on the biological activities of microbes.

The majority of explicit research in ecological theory has been conducted on macro- organisms. In several respects, however, microbial organisms harbor the greatest biological diversity – in biochemistry, in phylogeny, in habitat, in metabolic lifestyle, in resource use, and in range in body size. Thus the greatest challenges and most promising advances for ecological theory arguably lie in its applications and extensions to understanding the ecology of bacteria, archaea, and microbial eukaryotes.

Microbes exhibit an astounding range of values along multiple dimensions of diversity, and this documented variety continues to increase as we look more carefully at the microbial world (e.g., Brock et al. 2011). The size of their cells spans sixteen orders of magnitude (a factor of ten quadrillion or 10,000,000,000,000,000), from the tinniest

bacteria weighing ~10^{-15} g to the largest protists weighing ~1 g (Table 2). Collectively these tiny organisms harness a huge diversity of metabolic pathways, substrates, and lifestyles that use dozens of different elements as energy sources. They maintain physiological activity across the widest range in temperatures, from -40 to 122 °C, pressures, salinity, and pH, and inhabit nearly every location in the Earth's crust where free energy is available, rocks up to kilometers deep underground and microscopic liquid veins kilometers deep in Antarctic glacial ice (Morita 1980; P. Price & Sowers 2004; Rothschild & Mancinelli 2001). Microorganisms organize themselves across multiple levels of organization – growing as single reclusive cells, multi-species social consortia, multicellular organisms, and members of multi-domain superorganisms. This biodiversity is hardly surprising given that prokaryotes and unicellular eukaryotes occupy at least two-thirds of the tree of life – at least 50 different phyla. The evolutionary and phylogenetic diversity of microbes resulting from the billions of years of evolution of their lineages has allowed them to generate and conserve novel metabolic niches and occupy every corner of the Earth's crust explored by scientists.

Given the importance of microbial metabolic processes and their remarkable biological diversity, some of the most significant applications of ecological theory are in identifying the major ecological dimensions governing the metabolism of microbes and determining how metabolism scales across extremes along these dimensions. Because of their high abundances and biological rates, microbes offer a useful model system for metabolic ecology. Sufficient data can be generated in short periods of times from field and lab studies. Their high rates of mutation and horizontal gene transfer mean that evolutionary and ecological perspectives must be integrated. And big-picture ecological,

biogeographic, and evolutionary experiments can be conducted that would never be possible in higher organisms.

So, in order to develop a metabolic theory of ecology that addresses the geographically heterogeneous distribution of phylogenetic and metabolic diversity on Earth we must study microbes. Their integration into metabolic theory is necessary in order to unify biological theory across levels of organization. The question is: what are the major dimensions of the metabolic ecology of prokaryotes and unicellular eukaryotes? In other words, what sets of variables must be considered in order to understand the role of energy in the interactions between organisms and their environments? I shall employ a scaling perspective to explore the five fundamental dimensions that characterize a metabolic theory of ecology of microorganisms:

1) Thermodynamics
2) Chemical kinetics
3) Physiological harshness and environmental stress
4) Cell size
5) Levels of biological organization, including host-endosymbiont mutualisms, consortia, biofilms, multicellular prokaryotes, and multi-domain superorganism complexes.

Each dimension influences the metabolic rate of microorganisms and thus the interaction between microbes and their environments. Explicit consideration and application of these dimensions in the development of metabolic theory has great potential. It provides a basis for extending metabolic theory to explain patterns in biodiversity, such as diversity gradients and community assembly rules. After presenting an abbreviated history of the

metabolic ecology of microbes, I will delve into the foundations of the energetics of individual cells that must be considered in order to develop a quantitative metabolic theory of microbial ecology. Then I will discuss the first four intrinsic dimensions as they affect individual cells. And I will end by considering the fifth dimension (levels of organization) and its interaction with the other dimensions.

Brief history of metabolic ecology of microbes

Microbial ecologists have long studied the energetics and metabolic ecology of microbes. They have investigated the temperature dependence of microbial growth and respiration employing the Arrhenius equation (e.g., Ingraham 1958a; Price & Sowers 2004; Johnson & Lewin 1946; Davey 1989; Button 1985; Goldman & Carpenter 1974). They have investigated how substrate and growth conditions affect growth rate and efficiency (e.g., Droop 1973; Panikov 1995; Button 1978). Protists biologists showed early interest in the effects of body size on biological rates (e.g., Fenchel 1974; Fenchel & Finlay 1983); prokaryote biologists have showed less interest. Often performed in laboratory experiments and bioreactors, much of the research has been motivated (explicitly or implicitly) by applications to medical, industrial, food, and environmental technology. Historically, much of microbial ecology has advanced relatively independently of theoretical developments in macro-organism and ecosystem ecology - the exception being ecologists studying phytoplankton, who have extensively studied the effects of cell size and resource stoichiometry on growth rate and community structure (Fenchel 1974; Droop 1973; Litchman et al. 2007; Yoshiyama & Klausmeier 2008; Sheldon et al. 1972; Litchman Chapter 14).

Relatively speaking, a formal metabolic theory of ecology (MTE) for microbes is in its infancy. A few MTE papers have made important initial steps. The integration of the effects of body size with kinetic effects of temperature on metabolic rate into one equation was a particularly important step in the development of metabolic ecology (Gillooly et al. 2001; Brown et al. 2004) and in the metabolic ecology of microbes (López-Urrutia et al. 2006). Subsequently, microbial ecologists have sought to integrate the core MTE equation (Brown and Sibly Chapter 3) with the effects of resource availability and stoichiometry (López-Urrutia & Morán 2007; Sinsabaugh et al. 2010; Sinsabaugh et al. 2009; Sinsabaugh & Follstad Shah 2011; Sinsabaugh et al. 2011; Sinsabaugh & Shah 2010). These scaling and metabolic perspectives, together with exciting advances in prokaryote and eukaryote cell physiology, may provide the necessary stimulus to begin to develop an integrated, unified, and quantitative understanding of physiological and metabolic ecology spanning the three domains of life.

Physiological foundations

All organisms require energy and materials to build and maintain their complex structures far from thermodynamic equilibrium. Enzymes have evolved to harness energy from a variety of sources: sunlight, organic carbon, and energy-yielding (exergonic) geochemical substrates. Carbon is one of the essential elements used in building physiological infrastructure. It can be obtained from organic sources or carbon dioxide. So the most basic classification of trophic lifestyles is according to the energy and carbon sources utilized by an organism (Table 1). All of the major trophic groups of life are used

by the Archaea and Bacteria, the two domains making up the prokaryotes, whereas the Eukarya cannot perform lithotrophy without the assistance of prokaryote symbionts.

Table 1. The major metabolic lifestyles of life.

Energy source	Carbon source	Terminology
Light	Carbon dioxide	Photoautotroph
Light	Organic compounds	Photoheterotroph
Inorganic chemicals	Carbon dioxide	Lithoautotroph
Inorganic chemicals	Organic compounds	Lithoheterotroph
Organic carbon	Organic compounds	Organoheterotroph

Organoheterotrophs and photoautotrophs are often referred to as heterotrophs and phototrophs, for short. Mixotrophs use a mix of different energy and/or carbon sources. Lithotrophs and organotrophs together are called chemotrophs; the prefix *chemo* encompasses both *litho-* and *organo*.

In order for an individual organism to maintain cellular integrity and function, the power supply (energy per unit time) available to an organism, R_{org}, must be sufficient to fuel the whole-organism minimum metabolic rate, I_{min}, required to repair macromolecular damage (Price & Sowers 2004). R_{org} must be even greater in order to supply the power used to support basic metabolic functions and activities, known as the maintenance metabolic rate I_{maint} (more or less comparable to inactive metabolic rates called "standard metabolic rate" or "basal metabolic rate" in macroorganisms). Even more power is required for a cell to actively create new biological material, grow, and reproduce, known

as the active or growth metabolic rate I_{grow}. Thus, $I_{grow} > I_{maint} > I_{min}$[1]. In order for an environment to be habitable for life, over a reasonable period of time R_{org} must be greater than or equal to I_{grow}: $R_{org} \geq I_{grow}$ (Hoehler 2004; Hoehler 2007a; Hoehler et al. 2007; Shock & Holland 2007). The closer R_{org} is to I_{grow}, I_{maint}, and I_{min}, the more extreme and relatively unsuitable the environment (Fig. 10). Thus the difference or ratio of R_{org} to I_{grow}, I_{maint}, and I_{min} determines an environment's habitability. An environment may be extreme because R_{org} is low or because I must be high in order for an organism to survive, maintain biological activity, and grow. Because of the challenges of studying microbes in the field, there is still fragmentary knowledge of the different metabolic rates and associated growth and survival rates of microorganisms *in situ.*

How is the metabolic rate of an individual defined and quantified? There are no intrinsically superior definitions of metabolic rate. An organism obtains power from exergonic chemical reactions or phototrophy. This supply side of an individual's metabolism could be considered its metabolic rate. This rate may be the most general and theoretically useful rate, at least in microorganisms, and so this definition is used in this chapter. Some of the supplied power may then be coupled to ATP synthesis. So the total power used for whole-organism rate of ATP synthesis could also be considered the metabolic rate. The cell's ATP molecules are used to power endogenic reactions, so the total power produced through ATP hydrolysis could be considered the metabolic rate. The rate of the membrane electron transport chain may be used to provide a more general

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[1] The ratios $I_{grow} : I_{maint} : I_{min}$ have been estimated to be on the order of 10^6:10^3:1 in bacteria communities *in situ* (Price & Sowers 2004); $I_{grow} : I_{min}$ seems to more typically have maximum values of 1-2 orders of magnitude when bacteria species isolates are measured and in protist species (DeLong et al. 2010).

and encompassing measure than ATP synthesis since the electron transport chain powers both ATP synthesis and other activities, such as bacteria flagella and secondary active transport. However, some exergonic reactions may in fact power ATP synthesis without the use of an electron transport chain (substrate-level phosphorylation) or power anabolic reactions without the use of ATP hydrolysis, for example, by using the biosynthetic pathways associated with glycolysis. Therefore, the rate of ATP synthesis, ATP hydrolysis, or the electron transport chain reaction may not always provide the most useful measure of metabolic rate and may not always give an accurate measure of the total power expended by an organism.

In fact, in microorganisms the population growth rate μ (number of divisions per unit time, known in microbiology as specific growth rate) or biomass production rate P (biomass produced per unit time by a cell or population of cells) may provide a meaningful proxy of metabolic rate. These rates have been widely used by microbiologists (e.g., Fenchel 1974; Panikov 1995; Dawson 1974; Ratkowsky et al. 2005). The value of μ reflects supply-side metabolic power and the energetic efficiency H by which this energy is used to power the organism's biological and reproductive activities:

$$\mu = H \times (I/M), \qquad (1)$$

where M is cell mass. H is in dimensions of mass per unit energy (e.g., gJ^{-1}) and can be thought of as the amount of energy required for an organism to produce a unit mass of biomass. Metabolic rate is partitioned between energy use for growth, p_{grow}, and energy

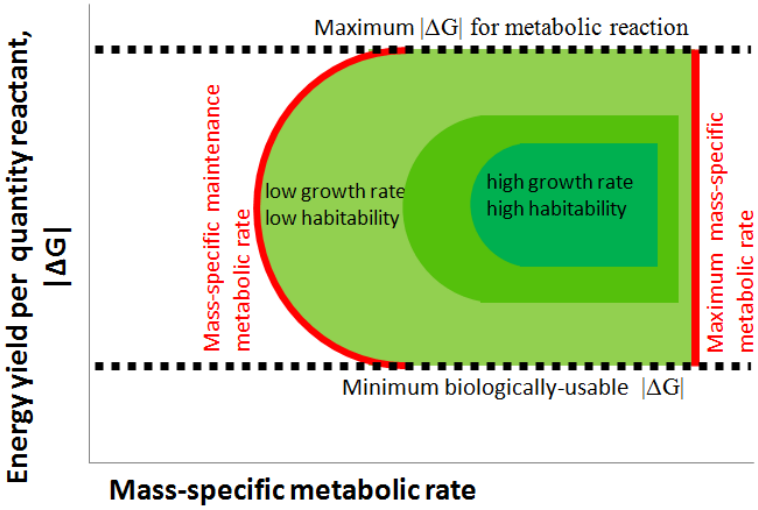

Figure 10. Conceptual diagram illustrating the effects of energetics as mediated by thermodynamics, kinetics, and environmental stress on the productivity of an organism or ecosystem and on the habitability of its environment. For simplicity, the figure is presented for an organism or ecosystem with one single energy source. Life can only produce biomass in the green area. A living thing must be able to obtain energy from its environment at a rate greater than its biomass-specific maintenance metabolic rate (I_{maint}/M , left-side red curved line) in order to produce biomass. The metabolic design of the living thing, physicochemical conditions, and resource availability impose an upper boundary on its metabolic rate (right-side red line). In order for a reaction to provide biologically usable free energy, the reaction must have an energy yield |ΔG| equal to or greater than the minimum |ΔG| (lower red line) and less than the maximum |ΔG|. Metabolic reactions close to max |ΔG| induce greater oxidative cellular damage and reactions close to the minimum |ΔG| require more complex and expensive metabolic machinery, thereby increasing maintenance energy requirements (a_{maint} and I_{maint}/M) and decreasing the amount of energy allocated to growth. Therefore, a living thing's mass-specific growth rate and biomass production tend to increase as its power and reaction's |ΔG| value approach the middle right region of the plot.

use for maintenance activities I_{maint}, giving $I = p_{grow} + I_{maint}$ and $a_{maint} = I_{maint}/I$, where a_{maint} is the proportion of whole-organism metabolic rate that is allocated to maintenance. The partitioning of energy between maintenance processes and growth affects H and μ, as shown by the following commonly used mass-balance equations (e.g., Pirt 1965; Panikov 1995):

$$\mu = Y\left(\frac{I - I_{maint}}{M}\right) \text{ and} \quad (2)$$

$$\mu = Y(1 - a_{maint})(I/M), \quad (3)$$

where Y is the growth efficiency or growth yield—the efficiency by which growth-allocated energy p_{grow} is converted into new biomass (since $\frac{I - I_{maint}}{M} = \frac{p_{grow}}{M}$). The relationship between H and Y is

$$H = Y(1 - a_{maint}). \quad (4)$$

These mass-balance equations show that a decreased mass-specific metabolic rate or increased allocation to maintenance metabolism leads to a linear decrease in population growth rate and division rate. H and other comparable measures of metabolic efficiency, such as growth yield and ATP yield, are fundamental quantities of great interest to microbiologists and ecologists (e.g., Dawson 1974; Panikov 1995; Maier et al. 2009; Pirt 1965; Russell & Cook 1995; Chapin et al. 2002)[2].

Quantitative outline of the dimensions of metabolism

[2] They are also important parameters relevant to maximizing the efficiency of industrial processes that depend on microbial metabolism. This quantitative framework can be generalized to modeling any substrate use, not just energy use. The growth yield, also referred to in microbiology as the cell yield, biomass yield, or growth efficiency, is generalized to the amount of biomass produced per unit amount of substrate consumed.

Whole-organism metabolic rate is a function of ΔG, the energy yielded per unit quantity of reactant molecule by each type of exergonic reaction supplying energy to the organism[3], the rate of each reaction *r* (number of product molecules produced per unit time), and the number of different reactions, *n*:

$$I = \sum_i^n |r_i \Delta G_i| \,, \qquad (5)$$

where brackets denote the absolute value of $r_i \Delta G_i$ (since energy-yielding reactions have by definition negative values of ΔG). This equation can be combined with equation 3, giving

$$\mu = Y(1 - a_{maint})(\sum_i^n |r_i \Delta G_i|)/M, \qquad (6)$$

These are core equations quantifying the dependence of metabolic and growth rate on an organism's biochemistry, energy partitioning, and efficiency. The major dimensions of the energetic ecology of microbes underlie the variables in these equations: (*i*) *n* and ΔG constitute the thermodynamic dimension; (ii) *r* is the dimension of chemical kinetics; (*iii*) the dimension of physiological harshness reflects the niches of species and has important effects on a_{maint}, and can also influence the other variables; (*iv*) cell mass unavoidably constrains *r* and μ, and also may have a positive effect in prokaryotes on *n* and ΔG; (*v)* the level of biological organization can influence all variables in these equations.

Equation 5 can be rewritten as

$$I = n|\langle r \Delta G \rangle|, \qquad (7)$$

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[3] ΔG is the Gibbs free energy. It is the difference between the potential energy of reactants and products. A negative ΔG means that the total free energy (potential energy) of the products of the reaction is lower than the total free energy of the reactants. Reactions with more negative ΔG values yield more energy per mol of reactants.

where $|\langle r\Delta G\rangle|$ is the average reaction metabolic power, highlighting the linear dependence of metabolic rate on the number of energy-yielding reactions and the average metabolic power of energy-supplying reactions.

Dimension 1: Thermodynamics

The enormous metabolic diversity of microbes is striking. Organotrophic microbes can consume a multitude of organic carbon compounds too recalcitrant or toxic for animals. Phototrophic prokaryotes have several different kinds of pigments for harvesting light energy. They can harness wavelengths from 385 nm to over 800 nm, which affects the ΔG of the photoreactions; and, new pigments and types of photoreactive centers are still being discovered (Fuhrman et al. 2008). Lithotrophic prokaryotes can derive energy from hundreds to thousands of geochemical reactions, using a variety of minerals and elements functioning as electron acceptors and donors (e.g., Shock et al. 2010).[4]

The first step towards understanding biological activities and their dependence on the environment is to elucidate an organism's possible available number of metabolic pathways for obtaining energy (n) and how much energy is yielded by each metabolic pathway, ΔG. This is a vibrant area of research in the fields of geomicrobiology and systems biology (e.g., Amend & Shock 2001a; Hall et al. 2008; Inskeep et al. 2005; Shock et al. 2010; Spear et al. 2005; Price et al. 2004; Raymond & Segre 2006). The study is necessary in order to predict whether or not a microbe can persist in a particular

[4] Some of the most important electron donors are hydrogen, ammonium, nitrate, hydrogen sulfide, and iron and some of the most commonly used electron acceptors are oxygen, carbon dioxide, nitrite, sulfur, magnetite, hematite, and carbon monoxide.

environment, essential to microbial biogeography, and to predict energy and biogeochemical fluxes in particular environments, essential to ecosystem ecology.

ΔG varies widely between reactions and environments (Fig. 11). It is dependent on the energy in the bonds of the substrate molecules, the thermodynamic activity of the reactants and products (which depend on the concentrations and activity coefficients of the reactants and products, pH, and the ionic strength of the aqueous environment), temperature, and pressure according to principles of thermodynamics and theoretical geochemistry. A naïve prediction would be that temperature has a linear effect on the overall Gibbs free energy change ΔG based on the thermodynamic equation $\Delta G = \Delta G^0 + RTln(Q)$, where ΔG^0 is the standard Gibbs free energy change reflecting the reaction's thermodynamic properties, *R* is the gas constant, *T* designates temperature, and *Q* denotes the activity product, which is calculated from the concentrations and thermodynamic

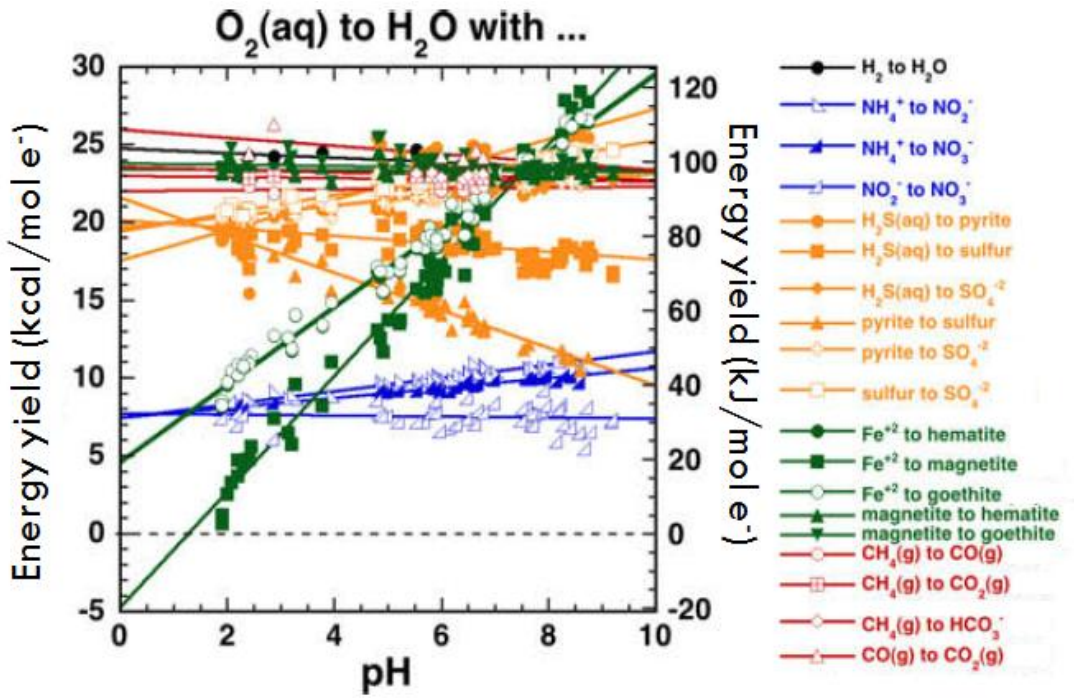

Figure 11. The energy yield of potential metabolic reactions varies greatly between reactions and with pH according to principles of thermodynamics. Plotted here are geochemical reactions with O_2 as the electron acceptor and H_2, NH_4^+, NO_2^-, H_2S, S, pyrite, Fe^{+2}, magnetite, CH_4, and CO as the electron donor (as listed on the right-hand side). The energy yields are for hot springs in Yellowstone National Park, USA, and were determined based on geochemical data and thermodynamic calculations. These reactions potentially provide sources of metabolic energy to chemotrophic microorganisms in these hot springs. Many prokaryotes are known to utilize these pathways for catabolism. Modified from Shock et al. (2010).

activities of the reactants (Amend & Shock 2001a). In reality, temperature's effect on ΔG is complex because temperature influences the concentrations and activities of dissolved reactants and products, in addition to its direct effect on the thermodynamic favorability of the reaction (Hammes 2007; Amend & Shock 2001). However, because the logarithmic term diminishes the effects of variation in *Q*, ΔG may often tend to vary approximately linearly or very little with temperature over biochemically-relevant temperature ranges. Over such ranges, ΔG is more strongly dependent on pH and is well

approximated by a linear function of pH (Fig. 11; Amend & Shock 2001a; Shock et al. 2010).

An exergonic reaction must have a sufficiently large enough energy-yield in order for an organism to be able to exploit it (Thauer et al. 1977; Schink 1997). There are also constraints on the maximum Gibbs free energy change that can be harnessed by organisms. Reactions with high absolute values of Gibbs free energy change are more likely to cause the cell oxidative damage and there are biophysicochemical limits to the ability of enzymes to catalyze high energy-yielding reactions (Hoehler 2007). There appear to be at least two orders of magnitude variation in the ΔG values of life's exergonic metabolic reactions (Hoehler 2007).

What are the constraints on exergonic metabolic diversity, the number *n* of energy-yielding metabolic reactions used by an organism? *n* depends on the diversity of enzymes an organism has that are involved in exergonic reactions. In prokaryotes, number of kinds of enzymes and metabolic reactions scales positively with genome size, which in turn scales with cell size; in eukaryotes, number of kinds of enzymes and metabolic reactions is weakly, if at all, dependent on *M* (DeLong et al. 2010; Molina & Van Nimwegen 2008). *n* reflects the number of available metabolic reactions having negative values of ΔG and so depends on thermodynamic conditions as discussed above. Also, the chemical diversity of an organism's environment ultimately imposes an upper boundary on *n*. Thus in chemotrophs *n* may be a positive function of the chemical diversity of its ecosystem.

Dimension 2: Chemical kinetics

Systems biology, an important area of cell, molecular, and microbiology, is determining how the conditions affecting reaction kinetics, the effectiveness of the enzymes catalyzing the reactions, and the network properties of an organism's biochemical network influence the metabolic and growth rate of cells (Westerhoff & Palsson 2004; Price et al. 2004). The metabolism of an organism operates in heterogeneous and non-mixed spaces, such as along the surfaces of membranes and in the fractal-like volume of the cytosol. Metabolism is composed of a complex network of reactions, and cells respond dynamically to changes in substrate availability and temperature. Therefore often the assumptions underlying the application of basic physical chemistry and biochemistry to organism metabolism are not upheld (e.g., Savageau 1995; Berry 2002). It is essential to determine which assumptions are robust and which are violated governing the biological kinetics of an organism. Despite the complexity of the cell, basic physicochemical models have been found to provide useful models for the kinetics of organism metabolism.

Temperature. The rate of a simple uncatalyzed reaction, *r*, scales with temperature according to the Arrhenius equation as $r \propto e^{-E/kT}$ (otherwise known as the Boltzmann

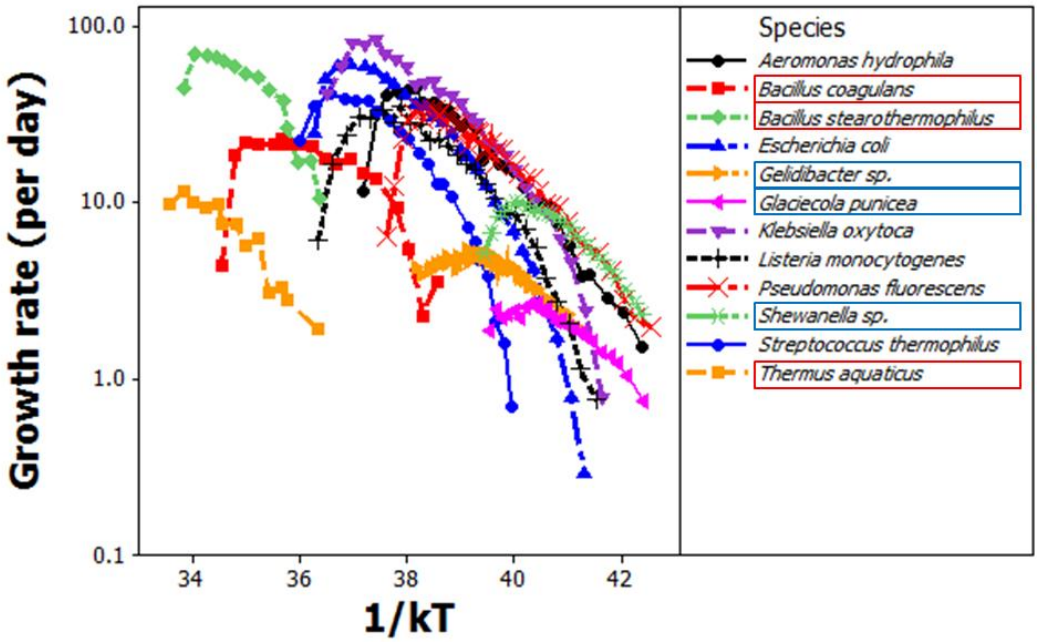

Figure 12. Arrhenius plot of the temperature dependence of the growth rate of different species of bacteria. The optimum temperature for growth varies from 15°C or less in cold-adapted species (psychrophiles; species names boxed in blue) to 65°C or more in hot-adapted species (thermophiles; species names boxed in red). Data are from Mohr and Krawiec (2005) and the work of Ratkowsky (2005) and graduate students at University of Tasmania.

factor), where *E* is the activation energy, *k* is Boltzmann's constant, and *T* is temperature in kelvins (Atkins & De Paula 2009). In theory and in practice, reaction rate has a more complicated temperature dependence (Johnson et al. 1974). However, this temperature dependence can often be approximated by the Arrhenius equation because variation in the other effects of temperature are comparatively small over biologically-relevant temperature ranges (e.g., -40°C to 130°C). For a complex enzyme-catalyzed reaction, reaction rate scales over some limited temperature range approximately according to the Arrhenius function with the activation energy of the rate-limiting step (Stegelmann et al.

2009). However, as temperature increases, the reaction rate will increasingly deviate from following the Arrhenius equation, because temperature will increasingly have a negative influence on enzymes and catalysis by increasing the probability that the enzymes are in their denatured states as opposed to their native and active states (Ratkowsky et al. 2005). At some threshold temperature, the rate of reaction begins to decrease, usually quite steeply.

Metabolic rate and growth rate exhibit comparable temperature dependences; however, in organisms temperature also has important effects on the functioning of physiological infrastructure, such as bilipid membranes supporting electron transport chain reactions or the compartmentalization of reactants. Thus, the temperature response curves for physiological rates depend on the properties of the enzymes and of the bilipid membranes. These temperature response curves vary greatly between organisms and reflect the evolutionary optimization of enzyme and membrane function for a particular temperature range (Fig. 12).

Biologists have long used the Arrhenius model to quantify the temperature dependence of biological rates over the increasing phases of temperature response curves, in particular of respiration, production, and population growth rates, and division times (Price & Sowers 2004; Ingraham 1958b; Johnson & Lewin 1946; Davey 1989; Button 1985). They have also modeled the entire temperature response curve using empirically-derived models (Ratkowsky et al. 1982; Ratkowsky et al. 1983; Rosso et al. 1995). Recently, in order to advance understanding of the temperature dependence of growth and respiration rate in microbes, Ratkowksy (2005) developed quantitative theory to incorporate the effects of temperature on the stability of enzymes, thereby modeling the

entire temperature response curve. The effects of temperature on the fluidity and integrity of the cell membrane, given its importance for energy transduction and transport, must also be incorporated into biophysical models. In many microbial habitats, temperature will vary enough that consideration of the shape of the temperature response curve beyond the Arrhenius regime will be necessary in order to accurately model microbial responses to temperature.

Although the kinetic effects of temperature are important, ultimately, understanding of the temperature dependence of the power of a reaction and of an organism's metabolism is sought. The power produced by a reaction is equal to$|r\Delta G|$, so it is a more complex function of temperature that reflects both kinetic and thermodynamic dimensions.[5] Researchers have sought to rigorously combine these effects into one model of respiration (LaRowe & Helgeson 2007; Jin & Bethke 2003; Jin & Bethke 2007). Although much work remains, these models are laying the grounds for developing a foundation for a quantitative theoretical biogeochemistry and metabolic theory of microbes that integrates thermodynamics and kinetics.

Substrate concentration. Resource availability may account for several orders of magnitude variation in the metabolic rate and growth rate of a cell (Price & Sowers 2004; Glazier 2009). As the availability of energy and essential materials increases, an organism can increase its use of those resources, thereby increasing its metabolic, growth, and reproductive rates. Initially, metabolic rate tends to increase linearly with resource availability but eventually metabolic rate will saturate at a maximum possible rate.

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[5] This temperature dependence can often be approximated by the Arrhenius equation because variation in ΔG with temperature is comparatively small.

Kinetic theory is used to model the dependence of the speed of a reaction, *r*, on the substrate concentration, [S], found at the location of the biochemical reaction (Panikov 1995; Button 1985; Button 1998). Biologists often make the assumption that the metabolic and growth rate of a cell is proportional to the total rate of relevant metabolic reactions. Michaelis-Menten kinetics, which have been derived for modeling biochemical reactions involving enzymes, have been widely applied in microbial ecology to model the effects of resource availability on growth rate (often called the Monod equation in this case) and photosynthesis rate (Liu 2007; Litchman Chapter 14):

$$r = \frac{v_{max}[S]}{K_M + [S]}, \qquad (8)$$

where v_{max} is the maximum possible reaction rate and $K_M$is the half-saturation constant. The assumptions underlying its derivation for a simple biochemical system may often not be strictly upheld when applied to an organism (Savageau 1995; Liu 2007) or community (Sinsabaugh & Shah 2010). However, it is often a good predictive model that provides an approximation of the kinetics of the complex metabolic network of organisms.

The substrate concentration of the bulk fluid surrounding a cell and in an ecosystem, $[S_o]$, is not necessarily equal to the substrate concentration at the site of an organism's biochemical reactions, [S], that are involved in Michaelis-Menten kinetics. Ecologists are interested in the dependence of biological rates on $[S_o]$ because is easier to empirically measure than [S] and reflects the general availability of a substrate to different organisms in an ecosystem. However, $[S_o]$ is not necessarily equal to [S] when the enzymes of the biochemical reaction are immobilized by being attached to a solid surface, such as the enzymes located in the membranes of cells. In this case, substrate must diffuse from the bulk pool to the site of biochemical reactions at a flux rate *F*

according to Fick's law, $F = k_s([S_0] - [S])$, where k_s is a parameter related to the physical conditions near the reaction site. Thus, F,$[S]$, and cell uptake rate I are codependent on each other: the flux rate depends on the concentration gradient, the gradient depends on $[S]$, and $[S]$ depends on the equilibrium between the cells' uptake/reaction rate and the flux rate from the bulk fluid to the cell surface [6]. The interaction of these variables ultimately determines the form of the functional dependence of r on $[S_o]$. Numerous physically-derived models have been successfully developed in microbiology and chemical engineering to model these dynamics under various conditions (e.g., Williamson & McCarty 1976a; Bosma et al. 1996; Bailey & Ollis 1986; Patterson 1992; Siegrist & Gujer 1985).

Dimension 3: Physiological harshness and environmental stress

Physiological harshness of the environment greatly influences the metabolic rates of cells. Physiologically harsh environments are prevalent. One organism's mild environment is another organism's extreme environment. A tiny microenvironment of a cubic millimeter will be a macro-environment to thousands of microorganisms. An environment that may seem benign and homogeneous to us, such as the soil in a forest, may in fact harbor environments stressful to the physiologies of organisms. Therefore, in

[6] There are two limit cases for the dependence of the reaction rate on environment substrate concentration. The reaction-limited regime occurs when the Damköhler number $Da = \frac{v_{max}}{k_s[S_0]} \rightarrow 0$, giving $r = \frac{v_{max}[S_0]}{K_M + [S_0]}$ (assuming equation 8). In the mass-transport or diffusion-limited regime, $Da \rightarrow \infty$ and $r = k_s[S_0]$. Systems that are both reaction and diffusion-limited exhibit intermediate functional dependences on $[S_0]$ and $[S]$ (Bailey & Ollis 1986).

order to understand the distribution, abundance, and activity of microorganisms and ecosystems, the influence of physiological harshness must be considered.

Physiological stress arises because of the existence of inescapable tradeoffs in an organism's biochemical and physiological attributes. For example, for biophysical reasons enzymes cannot perform well as catalysts at both extremely cold and extremely hot temperatures. Thus an organism will evolve to be best adapted to a particular temperature range. Temperature and chemical harshness are probably the most important kinds of physiological harshness affecting microbial metabolism. However, in many environments high levels of UV radiation and extremely low or high pressures can also have important impacts on physiological harshness. In general, physiological harshness reduces an organism's growth rate by: (*i*) forcing the organism to allocate more energy to maintaining its physiological functions, resulting in reduced allocation of energy to growth and reduced energetic efficiency *H* as previously discussed; and/or (*ii*) negatively influencing the rate and energy yields of an organism's exergonic reactions. Here I illustrate the application of these general principles by discussing the specificities for chemical harshness. There are numerous different chemically extreme environments—salinity, desiccation, pH, concentrations of heavy metals are some of the important chemical conditions that can stress the physiologies of microbes.

There are two different evolutionary and physiological strategies that chemical extremophiles may adopt in order to withstand such harshness (Rothschild & Mancinelli 2001). First, they can maintain homeostasis by keeping the external environment out. Such a strategy may require serious investment of energy in order to pump chemicals against concentration gradients or in materials in order to build the necessary structures to

prevent chemicals from diffusing down a chemical gradient into the cell. Second, they can allow their cells to have the same chemistry as the outside but alter their biochemistry and physiology or enhance repair mechanisms in order for their cell interiors to withstand the chemical extreme. The first strategy necessitates an increase in energy allocated to maintenance processes, leading to an increase in a_{maint} and consequently a decrease in growth rate and biomass production. The second strategy may also require an increase in a_{maint} . Also, importantly, the altered chemical environment of the cell and macromolecules can influence the ΔG and rates of reactions. Analogous considerations can be made for the other kinds of physiological harshness.

pH is one chemical condition that is of great importance in scaling the biological rates of unicellular organisms[7]. It varies greatly across the habitable areas of the planet, from 0 to 11 or more (Rothschild & Mancinelli 2001), and has significant effects on the geographic distribution of microbial diversity (Fierer & Jackson 2006). In addition to the previously mentioned thermodynamic effects of pH on the energy-yield of reactions, changing the pH that a cell is adapted to is physiologically stressful to it, causing a decrease in growth rates (Fig. 13). Cells tend to favor the homeostasis strategy, keeping cytosol pH relatively independent of environmental pH; however, cytosol pH still varies from 6 in acidophiles to 9 in alkoliphiles (Kroll 1990; Ingledew 1990). And, their cell surfaces must still deal with pH extremes, so they still have to alter the biochemistry of some of biomolecules on cell surfaces. For an individual strain, population growth rates

[7] Environmental pH affects metabolism by: (*i*) influencing a cell's transmembrane pH gradient, which contributes to the proton motive force that powers ATP synthase; (*ii*) increasing the energy expended to maintain non-extreme pH inside the cell; (*iii*) affecting the structure and functioning of a cell's enzymes; (*iii)* influencing the Gibbs free energy changes of reactions.

are a unimodal function of pH (Fig. 13). Since microorganisms often do not inhabit their optimal pH, understanding the functional form between biological rates and pH is an important step towards developing metabolic scaling theory for microbes.[8]

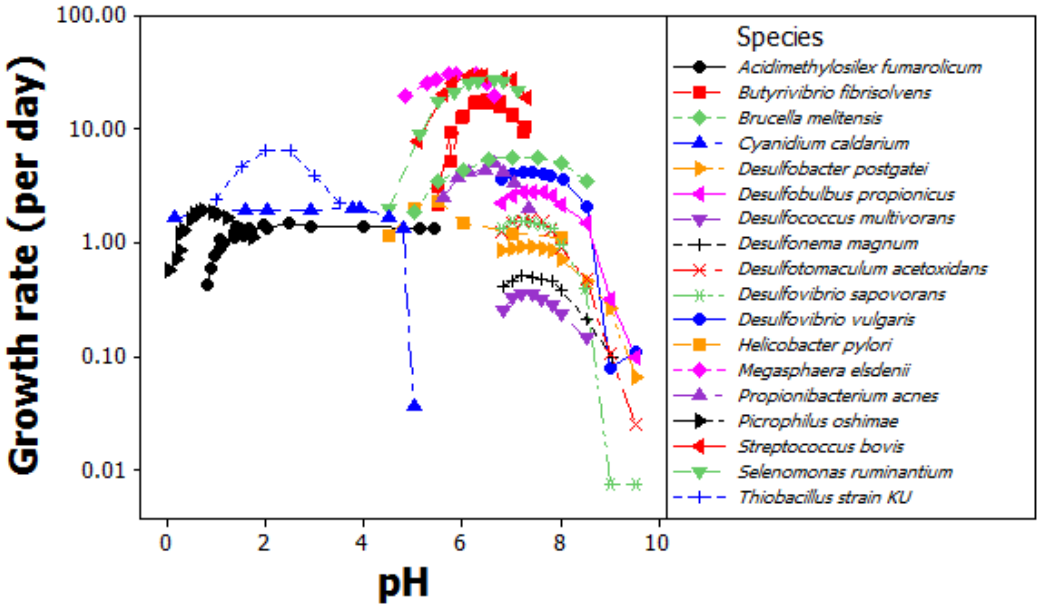

Figure 13. The unimodal dependence of microorganism population (specific) growth rate on pH in laboratory grown species isolates. Most species can only grow at ± 1.5 pH from their optimum pH, but species have adapted to be able to grow at pH values ranging from 0 to more than 9. Graphical inspection suggests that species optimal pH growth rates may also be a unimodal function of pH. (Data from Rosso et al. 1995; Schleper et al. 1995; O'Flaherty et al. 1998; Hallberg & Lindstrom 1994; Kangatharalingam & Amy 1994; Pol et al. 2007; Doemel & T. D. Brock 1977).

Dimension 4: Cell size

[8] Microbiologists have used phenomenological models that successfully modeled the combined effects of pH and temperature on growth rate (e.g., Tienungoon et al. 2000; Rosso et al. 1995). An enzyme kinetic model has been developed that models the effects of pH on enzyme stability and the consequential effect on enzyme kinetics (Antoniou et al. 1990; Bailey & Ollis 1986), but more work needs to be done to develop mechanistically-based quantitative models that incorporate the effects of pH on the proton-motive force and to develop an integrative model of the dependence of metabolic rate and growth rate on pH.

Cell size must have an important effect on metabolic rate because it affects the total available volume and surface area available for biochemical reactions and the distances necessary for the transport of materials. Prokaryote cells vary from 10^{-15} to 10^{-4} grams, protists cell vary from 10^{-13} to 1 gram, and yeast by at least 3 orders of magnitude, from the typical yeast cell size of 10^{-11} grams to 10^{-8} grams[9] (Table 2). The study of the scaling of organismal traits with organism size is known as allometry. Given the variation in cell size in microorganisms, microbial allometry is a promising area of study.

Prokaryotes. In prokaryotes much of ATP synthesis occurs in the cell membrane by oxidative phosphorylation and photophosphorylation. Therefore, the naïve expectation is that metabolic rate should be proportional to the surface area *A* of the cell membrane. If cell shape and surface roughness are not changing consistently with size, the external surface area scales with volume *V* as $A \propto V^{2/3}$and so the prediction is $I \propto V^{2/3}$ and $I \propto M^{2/3}$ (assuming $V \propto M^{1}$). For decades, however, many biologists thought that like other organisms in prokaryotes metabolic rate scales as a three-quarter power function of cell mass and volume—that is, as Kleiber's Law: $I \propto M^{3/4} \propto V^{3/4}$ (Brown et al. 2004). This conclusion was based on a few bacteria species that were grouped together with protists for statistical analysis (e.g., Gillooly et al. 2001; Fenchel & Finlay 1983; Peters 1986; Hemmingsen 1960). Increases in data availability and more resolved statistical

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[9] Prokaryotic cells range in size from the tiniest mycoplasma bacteria with reduced genomes weighing about 10^{-15} g (Himmelreich et al. 1996), to the giant spherical sulfur bacterium *Thiomargarita namibiensi*, which can weigh 10^{-4} g (Schulz et al. 1999). Unicellular protists span fourteen orders of magnitude in cell mass, from around 10^{-13} g in the green algae *Ostreococcus tauri* (Courties et al. 1994) to 1 g in the largest Foraminifera, Acanthophora, and Radiolaria protists. Yeast span over several orders of magnitude variation in cell mass; typical yeast cells are 3-4 μm in diameter and the largest reported yeast cells are 40 μm in diameter in the species *Blastomyces dermatitidis* (Walker et al. 2002).

analyses have generated debate. Makarieva et al. (2008; 2005) emphasized that the mean mass-specific metabolic rate of a group of organisms, such as heterotrophic bacteria or phototrophic unicellular protists, does not vary very much between groups of organisms. Yet as suggested by their analyses and demonstrated by analyses by Delong et al. (2010), whole-organism metabolic rate increases superlinearly in organoheterotrophic bacteria: $I \propto M^{\beta}$, where $\beta > 1$ (Fig. 14). This exceptional finding means that larger bacteria in fact have higher metabolic rates per unit body mass than smaller bacteria; and so if the

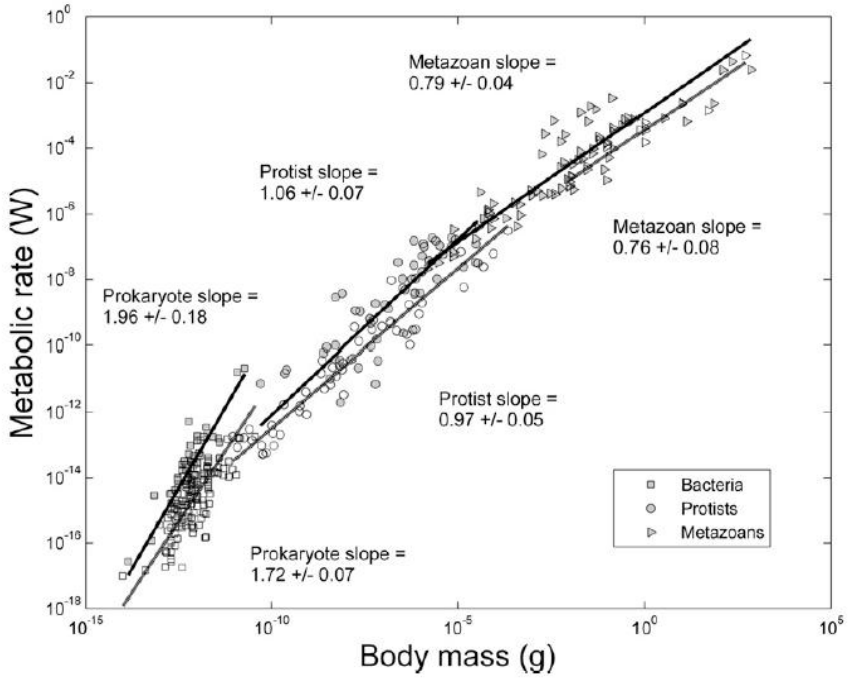

Figure 14. Relationship between whole-organism metabolic rate and body mass for organoheterotrophic bacteria, heterotrophic protists, and animals. Fits are RMA slopes +/- SE. Filled symbols with solid line fits are for active metabolic rates and unfilled symbols with grey lines are for inactive, starving metabolic rates (from DeLong et al. 2010).

allocation of energy to growth is invariant with size, then population growth rate is expected to scale positively with cell mass as $\beta - 1$, and generation time to scale negatively as $1 - \beta$.

Delong et al. (2010) hypothesize that the superlinear scaling is made possible by a concomitant increase in an individual's number of genes, which in prokaryotes scales with cell size. In prokaryotes, cells with larger genomes have metabolic networks composed of a larger number of reactions and enzymes. This increased network size and complexity may be able to confer greater metabolic power in the following non-mutually exclusive ways: by increasing energy yields, $|\Delta G|$; by increasing reaction rates r through autocatalytic feedback pathways in reaction networks and through better designed enzyme catalysts; or by increasing the number of substrates and reactions used as energy sources. This can explain why the metabolic scaling exponent is greater than two-thirds, but work is necessary in order to explicitly show how such network changes lead to superlinear scaling. The hypothesis proposed by DeLong et al. (2010) may apply to lithotrophic bacteria and to archaea; however, empirical scaling relations in these organisms have not been reported.

It is less obvious how this theory applies to phototrophs, since they have one source of energy. Once the effectiveness of the photosynthetic reactions and their density on the cell surface is maximized, the total photosynthetic rate will necessarily be limited by the surface area exposed to solar radiation, which scales sublinearly (Niklas 1994). Indeed, current analyses suggest the scaling of metabolic and associated biological rates in phototrophic prokaryotes is sublinear (Nielsen 2006) or only slightly superlinear (Makarieva et al. 2008).

Unicellular eukaryotes. In unicellular eukaryotes, applying the same logic used to build an *a priori* expectation in prokaryotes, the expectation is that metabolic rate scales with the total surface area of the mitochondria inner membranes, A_{MT}. All else being equal, $A_{MT} \propto V^1$ because cells can increase the number of mitochondria linearly with cell volume, thereby leading to $I \propto M^1$ (Okie 2011 *dissertation*). On the other hand, a slow rate of uptake and transport of oxygen and organic compounds from the environment and through the cell to the mitochondria could limit the total rate of activity of the mitochondria. If surface area limits the uptake of resources, then all else being equal the expectation is $B \propto M^{2/3}$ (however, see Okie 2011 *dissertation* and Patterson 1992 for reasons why deviations from two-thirds may be common). If the distribution of resources within the cell is the limiting factor, network scaling theory suggests $I \propto M^{3/4}$ (Banavar et al. 2010; West et al. 1999).

Historically, most biologists thought protists followed quarter-power biological scaling relations such as $I \propto M^{3/4}$. Few studies have investigated metabolic scaling in yeast and mold cells, despite their ecological, industrial, agricultural, gastronomical, and medical importance. Larger and higher quality data sets have led to a re-evaluation of scaling relations in unicellular protists. In heterotrophic protists, $I \propto M^1$ (DeLong et al.; Makarieva et al 2008). In phototrophic unicellular protists, biological scaling appear to follow quarter-powers, with $I \propto M^{3/4}$ (e.g., Niklas & Enquist 2001; Johnson et al. 2009; Nielsen 2006), but the subject is still open to some debate. For example, Makarieva et al. (2008) found linear scaling of metabolic rate in eukaryotic microalgae. Phototrophic protists, however, likely cannot sustain linear scaling as size increases because as in phototrophic prokaryotes their surface areas govern their ability to harness solar energy.

And packaging chloroplasts at higher densities and further within the cells leads to increased shading by surrounding chloroplasts and cytoplasm—the "package effect" (Niklas 1994).

In sum, central to elucidating understanding allometric scaling is identifying the fundamental constraints on metabolic rate for a given size. Because these constraints may change with body size and organismal design, the metabolic scaling exponent may also shift with changes in size and major evolutionary transitions. Determining empirically and theoretically at what sizes and in what groups of organisms these scaling shifts occur is an important avenue for future research.

Dimension 5: Levels of biological organization

Cells in nature rarely live in isolation, and a cell's interaction with other cells profoundly alters major dimensions of its metabolism. On ecological timescales, microbes group together in tightly knit populations and communities, which I call a major ecological transition in level of organization. Increased cooperation and decreased conflict between individuals in a population or community causes levels of organization to become more permanent and integrated. Eventually this can lead to the evolution of the integration of groups of individuals into a new higher-level unit of natural selection, a process called a major evolutionary transition (Maynard Smith & Szathmary 1995; Michod 2000)**.** Thus, ecological and evolutionary dynamics have organized life into different levels of organization (Fig. 15A).

The history of life is characterized by dramatic increases in the complexity and size of living things as a result of ecological and evolutionary transitions (Table 2; also

see Payne et al. 2009). Major ecological transitions influencing microbial metabolism include the formation of microbial consortia of syntrophic species (microbial mats, biofilms, and microbial aggregates)[10], colonies, endosymbionts living within the cells of other single-celled and multicellular organisms, and unicellular microbes living in close association with multicellular organisms (Table 2, Fig. 15A). Although these complexes develop on ecological timescales, many of the species co-evolve and consequently their metabolisms are the manifestation of eco-evolutionary processes. In major evolutionary transitions, prokaryotes evolved into eukaryotic cells via endosymbiosis, unicellular prokaryotes and eukaryotes into multicellular organisms via cooperation between related cells, and unitary multicellular organisms into obligatory social organisms living in eusocial colonies called "superorganisms" (Queller & Strassmann 2009; Maynard-Smith & Szathmary 1995; Michod 2000; Szathmary & Smith 1995).

These ecological and evolutionary transitions have significant effects on the metabolism of microbial cells, microbial communities, plants, animals, and ecosystems. Interactions between cells in the collection can also influence the cell's allocation of

[10] Microbes form complex networks of mutualistic and competitive interactions with inter-agent flows of metabolites and toxins (Costerton et al. 1995). These communities are called microbial consortia. Their species are interdependent and many of the interactions are synergistic and syntrophic, allowing metabolic processes not possible to one individual cell. Many of the species in the consortia are so dependent on their neighbors that biologists have not yet found ways to cultivate them in isolation in the laboratory. Consortia that grow on solid surfaces such as rocks, desert soils, the bottom of lakes, teeth, and human lungs are biofilms and microbial mats. Biofilms are microscopic with typical thicknesses around 30-500 μm, can have geometrically complex structures, and are pervasive (Ghannoum & O'Toole 2004); microbial mats are similar but are macroscopic formations that can grow up to several centimeters in height (Staley & Reysenbach 2002). Thus the range in size of these surface-growing microbial consortia varies by at least three orders of magnitude.

energy and materials to growth, maintenance, and infrastructure[11]. By altering the diffusion and active transport of resources and waste products, these collections influence the flux and concentration of substrates available to cells. By inducing syntrophy and the metabolic specialization of cells, these groups influence the kinds of substrates and associated Gibbs free energies available to a cell.

For example, although the species found in mature biofilms and mats may be found as plankton in the surrounding water, the consortia cells have fundamentally different traits and behaviors from their free-living counterparts. The mats and biofilms are composed of layers of metabolically-distinct species and characterized by pronounced physical and chemical heterogeneity, specialized niches, and complex spatial organization. The transport and transfer of nutrients and gases are generally rate controlling in biofilms and mats (Teske & Stahl 2002; Petroff et al. 2010), and channels in the consortia may form that function as primitive circulatory systems with wate

[11] Prokaryote cells can communicate with each other by releasing chemical signals. Prokaryotes utilize quorum sensing, regulating the gene expression of the population in response to changes in cell population density (Miller & Bassler 2001). Bacteria use quorum sensing to regulate a variety of physiological activities, including virulence, competence, conjugation, antibiotic production, motility, sporulation, and biofilm formation, thereby leading to the coordination of the behavior of the entire community and bestowing quorum sensing communities qualities of higher organisms, sociality, and multicellularity (Dekas et al. 2009; Nadell et al. 2009; Strassmann & Queller 2010; Queller & Strassmann 2009; Branda & Kolter 2004; Miller & Bassler 2001).

Table 2. The biological units created by the major ecological and evolutionary transitions of life and their ranges of reported sizes and their number of levels of ecological and evolutionary organization.

Unit	Volume (μm^3)*		Number of levels of organization (cell = 1)	References
	Min	Max		
Prokaryote cell	10^{-3}	10^{8}	1	(Schulz & Jørgensen 2001)
Prokaryote colony	10^{1}**	10^{11}	2	(Beardall et al. 2009)
Multicellular prokaryote	10^{1}	10^{3}	2	(Keim et al. 2007)
Biofilm or microbial mat	(10^{1})***	(10^{5})[f]	2	(Staley & Reysenbach 2002; Ghannoum & O'Toole 2004)
Aggregate consortium	10^{1}	10^{4}	2	(Alperin & Hoehler 2009; Orcutt & Meile 2008)
Prokaryote endosymbionts and prokaryote host****	$5x10^{1}$	$4x10^{3}$	2	(Von Dohlen et al. 2001)
Unicellular eukaryote (without additional endosymbionts)	10^{-1}	10^{9}	2	(Courties et al. 1994; Beardall et al. 2009)
Unicellular eukaryote host & prokaryote endosymbionts	10^{0}	10^{6}	3	(Guillou et al. 1999; Curds 1975; Heckmann et al. 1983)
Colony of eukaryote cells	--------	10^{16}	3	(Beardall et al. 2009)
Unicellular eukaryote host & eukaryote endosymbiont	10^{2}	10^{13}	3	(Beardall et al. 2009; Tamura et al. 2005)
Multicellular eukaryote (may include microbial symbionts)	10^{5}	$5x10^{21}$	3-4	(Stemberger & Gilbert 1985; Payne et al. 2009)
Eusocial colony of multicellular eukaryotes & its microbial symbionts	10^{12}	$>10^{15}$	5	(Hou et al. 2010)

*1 μm^3 of biomass $\approx 10^{-12}$ g of biomass
**As calculated for a colony of 10 small *E. coli* cells
***minimum thickness of biofilms in µm
****has rarely been observed
[f]maximum thickness of mats in µm

flowing through channels, augmenting the exchange of gases and resources between the consortia and environment (Davey & O'toole 2000). Consequently, the thickness of biofilms and mats has been shown to affect the consortia's rate of metabolism and production (Williamson & McCarty 1976). There also are many prokaryote consortia that grow in spherical aggregates in which cells in the spherical core carry out different metabolic pathways than the cells forming an exterior shell of the aggregate (e.g., Dekas et al. 2009). [12] Modeling suggests that the metabolic rates and reaction energy-yields of cells decrease significantly with increasing aggregate size, leading to sublinear scaling of aggregate metabolism (Orcutt & Meile 2008; Alperin & Hoehler 2009).

A diversity of prokaryotes, protists, yeasts, and molds also form organized colonies of varying sizes[13] and studies have found that their biological rates scale with colony size (Nielsen 2006; Beardall et al. 2009), as is also found in eusocial animal colonies (Hou et al. 2010; Gillooly et al. 2010). In addition to the well-recognized primitive multicellular organisms found in eukaryotes, there are many colonies in prokaryotes that are considered by many biologists to be multicellular organisms (Shapiro 1998).[14] Like the endosymbionts that evolved into mitochondria, there are also

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[12] The size of these aggregates have been observed to vary by at least four orders of magnitude variation in size, from 0.5 μm^3 to 8200 μm^3, and have been observed to be composed of from 60 to ~100,000 cells[12].

[13] For example, cyanobacteria colonies of *Trichodesmium* spp. can attain volumes of 10^{11} μm^3 and protist colonies of the Chlorophycean *Hydrodicyton* can attain volumes of 10^{16} μm^3 (Beardall et al. 2009b)

[14] Examples include the magnetotactic prokaryotes made up of 15-45 coordinated cells and varying over two orders of magnitude in volume, from 6 μm^3 to 1020 μm^3 (calculated based on diameters reported in Keim et al. 2007), heterocyst-forming cyanobacteria such as *Anabaena* spp. and *Nostoc* spp. (Flores & Herrero 2009; Beardall et al. 2009b), *Proteus* spp., myxobacteria (Shapiro 1998), and Myxococcus xanthus (Queller & Strassmann 2009). These species have some of the hallmarks of multicellularity: cell-cell

abundant and diverse prokaryotes, protists, and yeasts that live within the cells of protists, plants, fungi, and animals (Douglas 2010; Lee et al. 1985). Many protists, bacteria, and yeast also live in the interspaces between cells of multicellular hosts and on the skin and within the guts of animals, forming multi-domain communities. Many of these interactions are mutualistic, for example, with the microbe fixing carbon and respiring oxygen (e.g., Kerney et al. 2011), providing bioluminescence, fixing nitrogen, or digesting recalcitrant organic carbons, and the host providing the microbe with habitat, protection, and resources.[15] These communities have coevolved such that the multicellular organism cannot properly function without its microbial symbionts. Thus, some of these communities could even be considered organisms or superorganisms, and the metabolic ecology of higher-level animals is in fact the metabolic ecology of a multi-domain complex involving both the plant or animal and its symbiotic microbes. In sum, the metabolism of the complex formed by an eco-evolutionary transition is more than simply the sum of its parts. An important avenue of research will be to understand how biological rates of cells and their complexes scale with the complexes' size and number of levels of organization.

In Fig. 15B, I present a unified theory largely based on DeLong et al. (2010) to explain the relationship between metabolic scaling and the major transitions in

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adhesion, complex intercellular communication and coordination, cell differentiation, and lack of cell autonomy.

[15] Animal gut microbes have essential roles in the metabolisms of the animal hosts. For example, understanding obesity in humans requires understanding the ecology of the microbes living within the guts of humans (Ley et al. 2006). Bacteria form endosymbiotic relationships with leguminous plants that is coordinated by chemical signaling between microbe and host plant (Jones et al. 2007) and such inter-domain signaling also occurs between many animal hosts and their gut microbes (Hughes & Sperandio 2008).

organization. At the core of the theory is identifying the shifting constraints on metabolic scaling at different sizes and levels of organization. Scaling within each eco-evolutionary group of organisms is bounded by the linear scaling with body mass of the total membrane surface area on which membrane-bound metabolic processes are localized, leading to a potential for linear metabolic scaling at smaller sizes as observed in heterotrophic protists (DeLong et al. 2010), animals (Zeuthen 1953), and plants (Mori et al. 2010; Enquist et al. 2007; prokaryotes being the exception to this generalization, as discussed in the cell size section). However, as size increases geometric constraints on exchange surfaces and transport distances limit the supply of substrates to energy-yielding or ATP synthesizing sites on the membranes, thereby imposing sublinear scaling (Banavar et al. 2010; West et al. 1999). Each transition incorporated innovations in metabolic design that allowed newly-integrated organisms or complexes to initially escape the sublinear scaling constraint by increasing the uptake and distribution of resources to the sites of catabolism, thereby allowing for greater metabolic rate, growth rate, and hence, all else being equal, greater fitness (see Brown & Sibly 2006). Also, each added level of organization requires additional allocation of materials and energy towards building and maintaining more complex metabolic infrastructures. So with each increase in level of organization, the energetic efficiency of biomass production, *H*, declines.

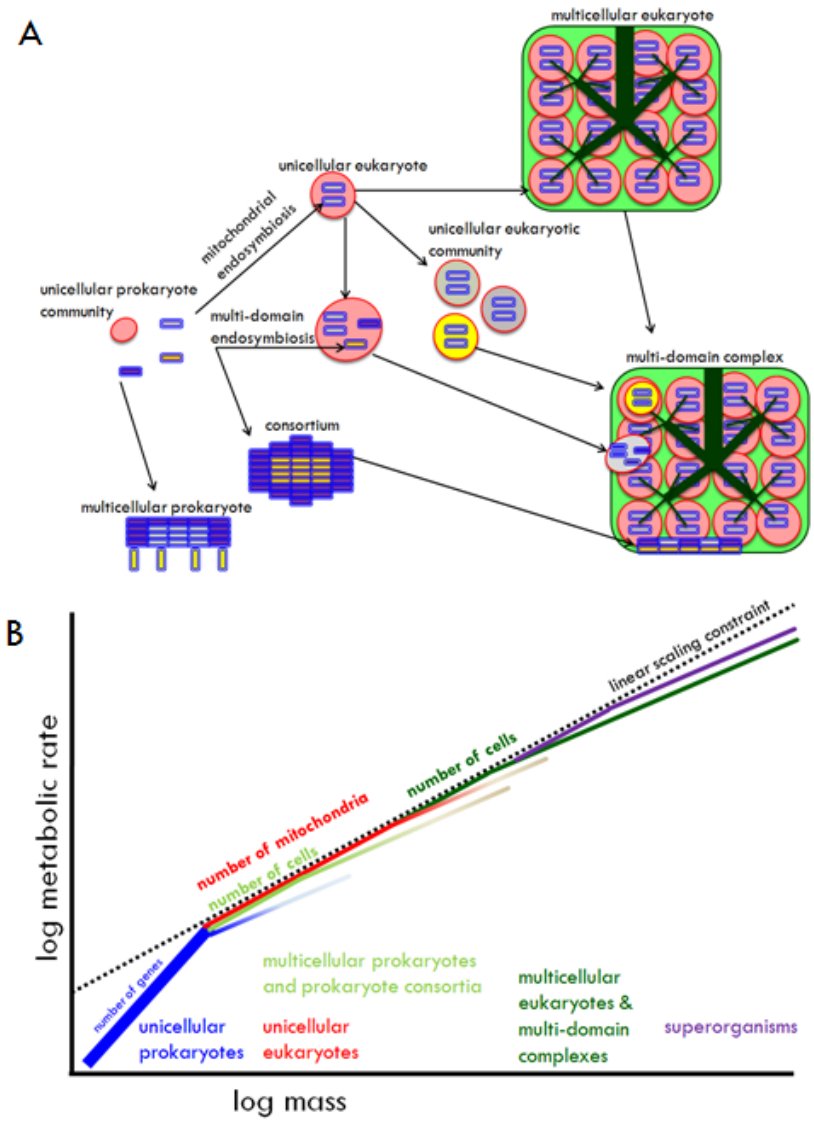

Figure 15. The metabolic ecology and scaling of prokaryotes and unicellular eukaryotes inhabiting different levels of biological organization. (A) Arrows show how higher levels of organization are formed from lower levels by ecological and evolutionary processes. The larger multicellular complexes require transportation networks for exchanging resources and wastes with the environment and distributing them within the complex, as denoted by the dark green networks. (B) The theorized relationship between metabolic scaling and major evolutionary and ecological transitions. Each transition allows organisms to avoid sublinear scaling constraints at the smaller sizes. However, as size increases surface area and distribution constraints eventually impose sublinear scaling (faded lines), at which point individuals tend to be outcompeted by the individuals of the same size but at the higher level of organization. Superlinear scaling in unicellular prokaryotes (solid blue line) reflects the increase in number of genes and metabolic

enzymes with cell size. This eventually gives way to a new constraint (fading blue line) imposing sublinear scaling as a result of respiratory complexes and proton pumps being localized in the cell surface. Protists overcome this constraint by incorporating respiratory complexes on internal surfaces through the endosymbiosis of mitochondria. Larger protists can accommodate more of these organelles, resulting in metabolic rate scaling linearly with cell mass (solid red line), until a new geometric constraint of surface area or transport distance limits rate of resource supply to the mitochondria, imposing sublinear scaling (fading red line). Since the smallest multicellular organisms are composed of relatively few cells and minimal vascular or skeletal systems, the scaling should initially be near linear as observed empirically in both animals and plants (Zeuthen 1953; Enquist et al. 2007; Mori et al. 2010). As body size increases, transport distances within organisms and exchanges of resources across surface areas increasingly come into play, leading to sublinear scaling (green lines). Similarly, small eusocial colonies, consortia, and multi-domain complexes may be able to increase their resource acquisition rate linearly with size, but the resource acquisition rate in larger complexes must be constrained by the transport of resources from the environment to and within complexes, imposing sublinear scaling in larger complexes (green and purple lines).

Community metabolism and the interplay of dimensions

An ecosystem's metabolism is a distributed network of metabolic reactions—a meta-metabolome (Vallino 2010; Raes & Bork 2008). The temperature dependence of a microbial community's metabolic rate, I_{TOT}, is the sum of the temperature dependences of the energy fluxes of all the concerned metabolic reactions or individuals in the community (e.g., see Panikov 1995), giving

$$I_{TOT} = \sum_k^N I_k = \sum_i^{n_{TOT}} |r_i \Delta G_i| \;\; \longrightarrow \;\; I_{TOT} = N\langle I \rangle = n_{TOT}\langle |r\Delta G| \rangle, \quad (9)$$

where N is the number of individuals, I_k is an individual's metabolic rate, n_{TOT} is the total number of reactions in the community, and brackets denote average quantities. If the community is composed of individuals all having the same temperature response curves, then the functional form of the community temperature dependence is identical to the individual temperature response curve. If the individuals have different response curves,

then the community temperature response will depend on the distribution of individual response curves along the temperature axis. Microbial species can grow along an extraordinary range of temperatures, so consideration of the distribution of individual temperature response curves is important. Most ecosystems and microenvironments experience temperature fluctuations, both over short daily timescales and seasonal timescales.[16] Ecosystems with temporal temperature variation are likely to be composed of species with different temperature response curves because competition causes species to spread out along the temperature niche axis. Immigration of microbes from locations with different temperatures will also lead to variation in the thermal niches of species within the community. Therefore, most microbial communities are probably made up of a variety of temperature response curves. The challenge will be to determine how this variation affects community-level temperature dependence.[17]

The higher community metabolic rates found at higher temperatures require greater amounts of resources. If these resources are unavailable, then the community level biological rate will be lower than expected based on temperature alone. Thus resource limitation and stoichiometric imbalances at high temperatures can reduce the observed temperature dependence of the community. Examples include the limited

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[16] For example, the top layer of desert soils may experience diurnal temperature fluctuations in the summertime of up to 40°C.

[17] Remarkably, despite these complexities, there are striking concordances in the responses of organism respiration and terrestrial ecosystem-level metabolic rates to temperature through the Arrhenius phases of temperature response curves (e.g., J. F. Gillooly et al. 2001; AP Allen et al. 2005). Such similarities suggest that confounding effects of the variation in properties of enzymes within these ecosystems are outweighed by universal thermodynamic and kinetic effects of temperature on whole-ecosystem metabolic reactions. Given the dominant contribution of aerobic respiratory and oxidative photosynthetic reactions to the energy budgets in terrestrial biomes, the observed concordance probably results from the activation energies of these reactions, which are the metabolic reactions used by most species in metabolic scaling data sets.

availability of phosphorus or nitrogen in aquatic and marine planktonic communities (see Chapters 4, 10, and 14). Temperature also influences the physical properties of the environment, which may in turn affect the metabolism of the cells. Thus, temperature may also have indirect effects on microbial communities that complicate the temperature dependence of the community. Probably one of the most important effects relevant to terrestrial microbes is the increased evaporation of water at higher temperatures. Under water-limited conditions microbes must decrease their metabolic rates in order to survive, thereby leading to a reduced temperature dependence of the community (Rothschild & Mancinelli 2001).

Concluding remarks

The Earth microorganisms harbor amazing metabolic diversity. Five major dimensions must be invoked to develop metabolic theories of the ecology of microbes. These dimensions involve the physicochemical attributes of life and its environment and the uniquely organic features of natural selection, competition, and cooperation that have organized life into hierarchical levels of organization. Exciting opportunities are available for contributing to the development of a unifying understanding of ecology that integrates across all three domains of life.

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CHAPTER 5: Conclusion

My dissertation has shown that Kleiber's Law does not apply universally across life. However, universal principles of thermodynamics, kinetics, and evolution may explain metabolic scaling across the major evolutionary transitions. Essential to understanding the metabolism and scaling of organisms is identifying the appropriate constraints governing the uptake, distribution, and transformation of resources. My research identifies some of the essential adaptations and innovations that allow organisms to shift these constraints on ecological and evolutionary timescales—fractal surfaces, geometric dissimilitude, internalization of surface processes, and major evolutionary and ecological transitions. The surfaces of organisms, the thermodynamics and kinetics attributes of metabolic networks, and the transportation networks within organisms are key metabolic features of organisms that depend on organism size, temperature, pH, substrate concentrations, and interspecific interactions according to fundamental principles of physics, chemistry, and evolutionary ecology.