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The bioenergetic cost of building a metazoan

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All life forms depend on the conversion of energy into biomass used in growth and reproduction. For unicellular heterotrophs, the energetic cost associated with building a cell scales slightly sublinearly with cell weight. However, observations on multiple *Daphnia* species and numerous other metazoans suggest that although a similar size-specific scaling is retained in multicellular heterotrophs, there is a quantum leap in the energy required to build a replacement soma, presumably owing to the added investment in nonproductive features such as cell adhesion, support tissue, and intercellular communication and transport. Thus, any context-dependent ecological advantages that accompany the evolution of multicellularity come at a high baseline bioenergetic cost. At the phylogenetic level, for both unicellular and multicellular eukaryotes, the energetic expense per unit biomass produced declines with increasing adult size of a species, but there is a countergradient scaling within the developmental trajectories of individual metazoan species, with the cost of biomass production increasing with size. Translation of the results into the universal currency of adenosine triphosphate (ATP) hydrolyses provides insight into the demands on the electron-transport/ATP-synthase machinery per organism and on the minimum doubling times for biomass production imposed by the costs of duplicating the energy-producing infrastructure.

energetics | ATP synthase | multicellularity | respiration | scaling relationship

Significance

Organismal success ultimately depends on the ability to efficiently transform environmental materials into biomass essential to growth and survival. The energetic cost per unit biomass produced is more than ten-fold higher in multicellular organisms than in unicellular species with comparable size. Thus, the additional support structures and functions that endow metazoans represent a significant barrier to the evolution of multicellularity unless they are offset by ecological advantages that come with such a life style. Consideration of the features of biology's energy-making machine, ATP synthase, provides insight into the investments made into this key molecule and yields estimates of the upper bound to rates of organismal doubling times.

As all aspects of growth, reproduction, and survival depend on the conversion of external carbon sources into biomass production and operational activities, bioenergetic efficiency plays a key role in the evolutionary potential and sustainability of species. A century ago, Lotka (1) emphasized this point in postulating that maximization of energy flow through biomass production is the central target of natural selection, and Van Valen (2, 3) made similar arguments nearly half a century later. Some have gone so far as to argue that maximization of the efficiency of energy utilization is one of the few "laws" in the life sciences (4, 5). The basic idea is that, in a competitive environment, natural selection should favor genotypes that maximize the efficiency and/or rate of conversion of environmental energy into biomass. Drawing on the same logic, Brown et al. (6–8) argue that fitness is equivalent to the rate of conversion of energy into offspring.

Nonetheless, although bioenergetic principles play a central role in the fields of physiology, biochemistry, and biophysics (9–11), and were highly influential in the early development of ecosystem science (12–14), these fields remain largely disconnected from mainstream evolutionary theory. Progress in reconnecting this loop is now becoming possible with the emergence of an understanding of how the costs of building and maintaining cells and their constituent parts can be computed in general ways that transcend species boundaries (15–17). However, substantial conceptual difficulties remain in incorporating bioenergetic principles into a conventional evolutionary genetics framework based on genotypic differences in offspring production rates (18).

As a critical first step in moving the field of evolutionary cell biology forward in ways that might make this connection, it is necessary to establish a basic understanding of the bioenergetic costs of building biomass in organisms from different phylogenetic backgrounds. Among other things, such information yields insight into the ease of adding or modifying various phenotypic elaborations based on the fractional cost to the total energy budget (18). The same approach can be used to estimate the gains from trait loss when the elaboration is no longer paying for itself in terms of energetic advantages.

Previously, we summarized and extended analytical methods for estimating the costs of constructing and maintaining cells of unicellular species (15, 16, 19). However, difficulties arise in applying these approaches to multicellular organisms, as they minimally require information on age/size-specific rates of respiration and biomass production. To this end, to provide insight into the costs of building multicellular organisms (aerobic metazoans in particular), this study draws on extensive analyses of the

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growth, reproductive, and respiration rates of 23 species of cladocerans (mostly in the genus *Daphnia*) ranging in size by two orders of magnitude, as well as from extrapolations of published data on several other species. Comparison of bioenergetic costs as a function of organism size illustrates that although there are some scaling similarities between unicellular and multicellular eukaryotes, there is a quantum leap in the cost of building metazoan biomass. Combining these results with prior studies on unicellular species yields insights into the performance challenges for biology's energy production machine, ATP synthase.

Results

Cladocerans. To gain an understanding of the ways in which rates of biomass production scale with organism size in a phylogenetically and ecologically widely distributed group, detailed life-table analyses were performed on single clones from 23 species of planktonic cladocerans, grown at 20 °C on a defined medium containing phytoplankton at food-saturating conditions. From a knowledge of average daily increments in individual lengths throughout life, numbers of eggs carried per adult instar, instar durations, and instar-specific weights of molts (shed exoskeletons), eggs, and individuals (with eggs removed), total rates of biomass production (dry weight) were computed on a daily basis. With parallel information available for active respiration rates (*SI Appendix*, Fig. 1), the amount of oxygen consumed at each growth interval was also determined.

Several generalizations can be drawn from the resultant data. First, within each species, the scaling of the respiratory cost of producing biomass with individual biomass can be adequately expressed as an allometric "power-law" function with form $y = ax^b$, obtained by least-squares regression of the log-transformed data. Results for six representative species are shown in Fig. 1, and displays for the remaining 17 species are given in *SI Appendix*, Fig. 2 (with all statistics given in Dataset S1). These regressions exclude data for apparent periods of senescence when there are substantial declines in total biomass production at very late ages, and for a few small species, data are also excluded for the first one to three days of life when there was high mortality (presumably due to sticking to the surface film of culture vessels). Within all species, the metabolic cost of producing biomass scales positively with individual biomass, i.e., with increasing age (Fig. 1). Although there is scatter in the data, the scaling exponent (b) does not vary significantly with size at maturity (B_{mat}, dry weight, exclusive of eggs) across species (*SI Appendix*, Fig. 3), averaging 0.352 (SE = 0.038), and increasing to 0.410 (0.032) if four apparent low-outliers (for which there is no apparent explanation) are removed.

Second, the normalization constant (a), which is an estimate of the metabolic cost at 1-μg dry weight, scales negatively among species with increasing size at maturity, with $\log_{10}(a) = -0.272(0.146) - 0.466(0.103)\log_{10}(B_{mat})$; $r^2 = 0.495$; $P = 0.00018$; SEs in parentheses (Fig. 2). That is, the respiratory cost per unit biomass production in the smallest species is substantially

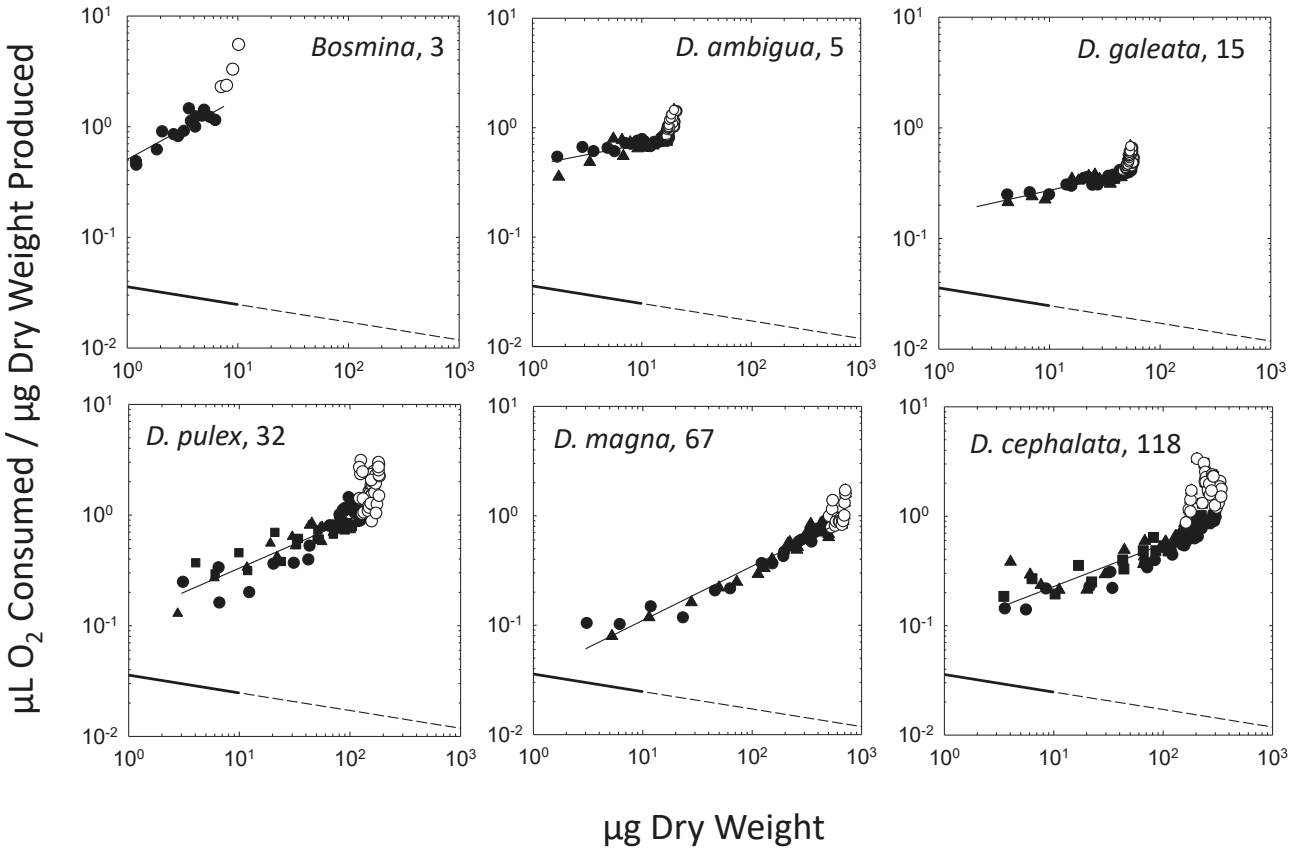

Fig. 1. Profiles for the response of the metabolic cost of biomass production to increasing size during the growth trajectories of isolates of 6 species of filter-feeding cladocerans, distributed over the full range of body sizes for such species (results for 17 other species are given in *SI Appendix*, Fig. 1). Circles, squares, and triangles refer to results from different replicate experiments. Open symbols denote presumptive stages of senescence that are not used in the least-squares regressions. *Inset* numbers are the sizes at first appearance of eggs in the brood chamber, in μg dry weight, i.e., size at first reproduction. *Lower* lines denote results from multiple species of unicellular ciliates, $y = 0.108x^{-0.16}$ (19); solid lines cover the size range for such species, and dashed lines are linear extensions beyond the range of ciliate sizes.

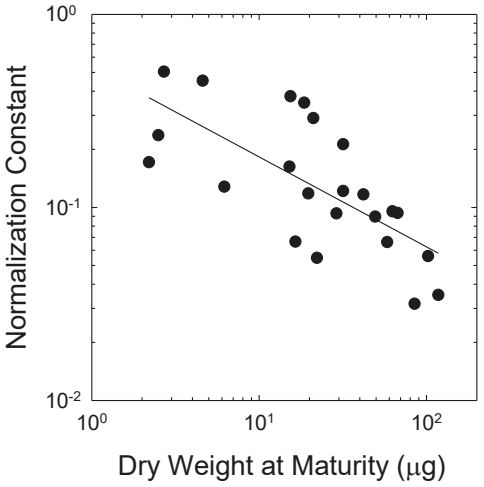

Fig. 2. Relationship of the fitted normalization constants (elevations of the log–log regression) for the within-species allometric relationships in Fig. 1 (and *SI Appendix*, Fig. 1) with dry weight at maturity for 23 species of cladocerans.

higher (up to 10×) than that for the larger species in the range of overlap in size. Third, the costs of biomass production in cladocerans are uniformly higher, up to 60×, than previous estimates for a diverse set of ciliated protozoans, even in the range of overlapping sizes (Fig. 1).

Finally, whereas the preceding analyses are concerned with the efficiency of biomass production at specific time points in the ontogenetic trajectory, it is also of interest to evaluate the total (cumulative) costs of growing a soma to maturity. This can be computed as the sum of instar-specific respiration rates weighted by instar durations from time of birth to the time of first offspring production (defined here by the time at which the first clutch is released). The interspecific data adhere closely to the allometric relationship,

$$C_{\mathrm{mat}} = 2.38 B_{\mathrm{mat}}^{0.736}, \quad [1]$$

where C_{mat} is defined as the cumulative μL of O_2 consumed from birth to the time of first reproduction, and B_{mat} is the dry weight at the size carrying the first clutch (in μg) (Fig. 3; $r^2 = 0.954$, $P < 10^{-15}$, SEs of intercept and slope on the $\log_{10}$ scale are 0.048 and 0.034). This cost scaling is nearly parallel to that obtained for ciliates in prior work (19) but elevated by an average factor of ∼30×.

Other Unicellular Heterotrophs. As ciliates were used as a comparative benchmark in the preceding analyses, it is desirable to evaluate whether other unicellular lineages have similarly low costs of biomass production. Although the requisite data are scant for most phylogenetic groups, sufficient observations on both respiration and growth rates for free-living amoeboids enable an extension of analyses to this group. Respiration rates for ciliates (19) and amoebae (μL O_2/cell/day, normalized to 20 °C using $Q_{10} = 2.5$ correction), scale as

$$M = 0.089 B^{0.62}, \quad [2a]$$

$$M = 0.034 B^{0.76}, \quad [2b]$$

respectively, where B denotes mean cell dry weight in μg (*SI Appendix*, Fig. 4). Maximum cell-division rates (days^{-1}, again scaled to 20 °C,) equivalent to the inverse of doubling times) scale as

$$G = 1.18 B^{-0.22}, \quad [3a]$$

$$G = 0.52 B^{-0.21} \quad [3b]$$

(19). Thus, relative to ciliates, which move by use of flagella, amoebae that rely on crawling motility have ∼50% reductions in both respiration and cell-division rates. As a consequence, the costs per cell division (M/G) in the two groups are quite similar,

$$C = 0.075 B^{0.84}, \quad [4a]$$

$$C = 0.065 B^{0.97}, \quad [4b]$$

respectively (Fig. 3). With B denoting reported mean cell sizes, under the assumption of approximately linear growth, cell sizes at birth and maturity must $\simeq 2B/3$ and $4B/3$, implying a growth in cell size of $\sim 2B/3$ per division. Thus, for unicellular eukaryotes, the rate of biomass production (μg dry weight/day) can then be estimated as $2BG/3$, and the cost per unit biomass produced (μL O_2/μg dry weight) as $3C/(2B)$.

Other Metazoans. Determining the generality of the *Daphnia* results for other multicellular species requires joint information on interval-specific respiration and growth rates under food-saturating and temperature-controlled conditions, from birth to maturity. Unfortunately, despite the substantial attention given to respiration rates and growth potential of metazoans, the vast majority of studies provide information only for narrow size ranges or for mixed size classes, or only for one of the two measures, and many studies simply report respiration rates per unit biomass under the (generally incorrect) assumption of isometric scaling.

Nonetheless, limited data for other crustaceans and other metazoan phyla are generally compatible with those for cladocerans (Fig. 3). In all cases, there is a 10 to 100× inflation of the cumulative respiratory cost to the time of first reproduction

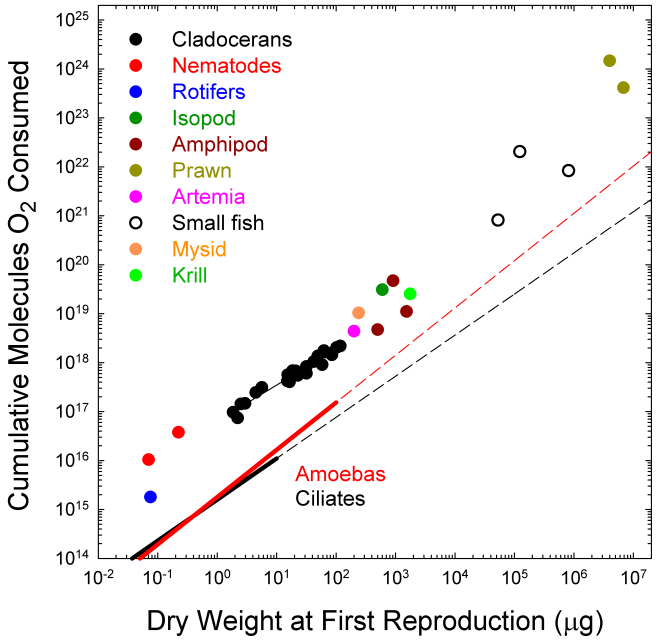

Fig. 3. Relationship between the cumulative consumption of O_2 from birth to age at first reproduction and the size at maturity. Black points and associated regression line denote results for cladoceran species. *Lower* lines denote results from multiple species of unicellular ciliates (19) and amoeboid species (Dataset S2); solid lines cover the size ranges for such species, and dashed lines are linear extensions beyond such ranges shown only for comparative purposes. Sources of data for other metazoans are given in Dataset S3.

relative to the situation in unicellular species. The analyses are least reliable for rotifers and nematodes, for which the temporal surveys are rather coarse-grained, but these are also consistent with the conclusion that even the smallest metazoans have elevated costs of biomass production relative to protists of similar sizes. The data also suggest that for organisms maturing at sizes beyond 10^4 μg dry weight (fish and prawn), the total oxygen consumption to age at maturity scales at an accelerating rate with size at maturity, i.e., there appears to be an increasing cost of biomass production (cumulative to the age at first reproduction) with increasing size.

Discussion

Over the past century, enormous effort has been invested in compiling and interpreting patterns of scaling of respiration rates with organism size (see refs. 21–24 for a small sample of recent reviews). However, remarkably little attention has been given to how such rates relate to the ultimate manifestation of metabolism in the form of biomass production essential to fitness. The focus here is on the energetic cost of growth and reproduction and how this varies with body size and phylogenetic groupings of heterotrophic organisms. The methods utilized provide a conceptually simple and informative approach to estimating the total cost associated with biomass production, in that the respiration measures reflect the full suite of normal organismal activities requiring ATP (e.g., search for resources, somatic maintenance, and biosynthesis) and the production measures make minimal assumptions about assimilation efficiencies of resources of unknown quality.

Prior work in the field of ecological energetics associated with metazoans has focused on various indices such as assimilation efficiency (e.g., ref. 25), i.e., the fraction of ingested (or assimilated) energy that is converted to biomass, but such ratios do not yield immediate insight into the total energetic investment in growth and reproduction and often make assumptions about conversion efficiencies. Although attempts have been made to identify resource allocation rules in homeothermic vertebrates (e.g., refs. 26 and 27), evaluation of the total costs associated with the construction of biomass has been secondary. In his review, Wieser (28) outlined some basic approaches relating to classical methods of microbial physiology (29, 30) that are similar in spirit to those deployed herein, and with limited data suggested that the cost of biomass production may be similar in both microbes and metazoans. The results outlined above suggest otherwise.

Relative to the situation in protists, there is a quantum leap in the bioenergetic cost of producing biomass even in the simplest metazoans (Fig. 3). Presumably, these elevated costs are at least in part a consequence of the added investment in nonproductive organismal infrastructure and activities, e.g., cell adhesion and support tissue, nervous system maintenance, and intercellular communication and transport. In addition, even in nongrowing individuals, multicellular species can invest heavily in recurrent somatic-cell turnover, imposing replacement costs not incurred by unicellular species (31). As the cost of DNA constitutes a relatively small fraction of the total cost of building a cell (18), and ciliates invest more intensively in DNA (amplified in macronuclei), differences in genome size cannot explain the substantial excess costs of biomass production in metazoans.

In appropriate ecological contexts, numerous advantages of multicellularity can be imagined, e.g, an ability to consume smaller unicellular species en masse, avoidance of gape-limited predators, and an ability to move across terrestrial environments. However, the evolutionary privilege of acquiring such adaptations appears to come at a steep cost. That being said, the current study is restricted to aerobically respiring, heterothermic metazoans, and to achieve a fuller understanding of the energetic costs of multicellularity, future extensions to plants, red algae, fungi, multicellular prokaryotes, and anaerobic eukaryotes are desirable.

It remains to be determined which diversions of resources contribute most to the declining growth efficiency as multicellular individuals expand in size (Fig. 1). However, the fact that the cost per unit biomass production increases with size by a factor of 2 to 10 within the lifespan of an individual implies that increased size imposes structural changes that impede the efficiency of further biomass production in growing individuals. The scaling exponent associated with this behavior does not have an obvious relationship with species-specific sizes at maturity, at least among cladoceran species, for which there are considerable data.

On the other hand, the fact that the normalization constant for this relationship increases with decreasing size at maturity across cladoceran species implies that the overall infrastructure and maintenance costs are substantially elevated in smaller species, in ways that are inconsistent with within-species ontogeny (Fig. 2). That is, smaller species are not simply scaled-down versions of larger species. As the energetic content of biomass varies by no more than ∼10% across all forms of cellular life (32–37), and the biosynthetic pathways for producing nucleic acids, proteins, and lipids are strongly conserved across eukaryotes, the reduced cost of biomass production by larger species must be a consequence of reduced energetic investment in nonproductive structures and/or activities, e.g., maintenance, environmental sensing, and motility. One potential explanation for the lower growth efficiency in small multicellular species is the increased surface area: volume ratio at the organismal level, which will reduce the relative amount of internal biomass allocated to resource assimilation. However, this same argument would seem to apply to size differences within species, and yet at that level, the opposite pattern is observed—costs increase with increasing size (Fig. 1). An alternative possibility is that smaller species have smaller cells, and hence more internal surface area per unit biomass, leading to increased investment in expensive membrane lipids that do not directly contribute to biomass production (18, 38).

Although the evolutionary mechanisms responsible for this shift in the direction of the scaling of bioenergetic costs with size at the developmental vs. phylogenetic level remains unclear, it is notable that the negative phylogenetic scaling of cost per unit biomass production extends to unicellular eukaryotes (Fig. 1). In both cases, larger-bodied species have reduced bioenergetic costs of producing biomass but require longer times to reach maturity.

Conversion to ATP Equivalents. As oxygen consumption is directly related to ATP production by aerobic respiration, the preceding results can be used to approximate the ATP demands associated with total biomass production (18, 19). The utility of such an accounting is that 1) ATP is the universal energy currency used by cells across the Tree of Life, with other energy carriers such as nicotinamide adenine dinucleotide being directly translatable to ATP equivalents; 2) the costs (in terms of ATP hydrolyses) of synthesizing essentially all cellular building blocks (e.g., nucleotides, amino acids, and lipids) and the additional costs of assembling them into higher-order structures and cellular components can be computed from known biochemical pathways, providing a basis for computing the costs of individual parts; 3) the operational

costs of cellular functions can only be quantified in terms of ATP-generated processes (rather than, for example, use of particular chemical elements); and 4) the energetic content of carbon skeletons abstracted from food sources can be expressed in ATP equivalents (18).

The following analyses rely on the assumptions that the majority of oxygen consumption in the study organisms is a reflection of aerobic respiration and that the majority of total ATP regeneration is based on the former. Some metazoans rely substantially on anaerobic metabolism (39, 40), but such organisms inhabit permanently or transiently anaerobic environments, and anaerobic eukaryotes have average rates of biomass production that are 5× lower than those included in this study (41). Some cancer cells are known to engage in the Warburg effect whereby energy production is heavily subsidized by aerobic glycolysis, resulting in the production of lactate as a waste product. Although this is not thought to be a common feature of nonproliferating cells and does not necessarily inhibit the operation of oxidative metabolism, it has been observed in cultures of nonmalignant mammalian cells (42). Some flatworms and cnidarians may engage in anaerobic mitochondrial metabolism (producing succinate and short-chain fatty acids as waste products), although direct evidence for this remains to be developed (43). All organisms reported on in the current study were raised in well-oxygenated environments, and as there are no reports on significant production of the by-products that would result from the above-mentioned processes, there is no reason to expect major deviations from standard aerobic respiration as the dominant mode of ATP production. However, as the baseline data reported here are in units of oxygen consumption, the following numbers can be readily modified in the light of new physiological observations. To put things on a solid footing, future empirical research should address whether aerobically growing unicellular eukaryotes and/or invertebrates invest significantly in anaerobic mitochondrial metabolism and/or glycolysis as modes for ATP production, and if so, how this compares with the amount of ATP resulting from aerobic respiration.

For microbes that can be grown on a simple (one compound) substrate serving as the sole source of carbon and energy (e.g., glucose), the rate of uptake of the limiting resource molecule can be directly converted into ATP equivalents, providing a relatively simple means for estimating the total costs of cell construction and maintenance (15, 29, 30). Although this approach cannot be easily applied to organisms consuming complex diets consisting of biological materials of unknown composition, rates of oxygen consumption provide indirect insight into the energy content of the assimilated food supply. Based on diverse observations on the physiology of aerobic respiration, the number of ATP molecules produced per oxygen atom consumed (the so-called P:O ratio) is typically in the range of 2.0 to 3.0 for metazoans, with an average value near 2.5 (44, 45). This is true for crustaceans (e.g., refs. 46–48), mollusks (49–51), and likely for all other invertebrates based on the known structural and functional features of the electron-transport chain and ATP synthase (18). Noting that there are 2.69×10^{16} molecules of O_2 per μL O_2, then after converting the respiration rate M to numbers of O_2 molecules, $5M/G$, where $1/G$ is the doubling time, provides a first-order approximation of the number of ATP molecules produced over time interval $1/G$, i.e., per generation for a unicellular organism.

One can then go a step further to estimate the total energetic content of food assimilated by noting that $5M$ only represents the fraction of the energy composition of assimilated food that is directly converted to ATP (θ), the remainder being used as carbon skeletons in biomass production (which does not result in respiration, but nonetheless harbors potential energy that can be converted into ATP). From analyses of the biosynthetic pathways of amino acids, nucleotides, and lipids, $\theta = 0.21$, 0.17, and 0.27 for these monomeric building blocks (ref. 18, Chapter 17). Given that nucleotides typically comprise <10% of total biomass (18) and that there are numerous other small additional ATP-consuming costs associated with biomass assembly, e.g., polymerization, protein folding, cargo delivery, messenger RNA and protein replacement, etc. (15, 16), $5M$ represents approximately half of the energy assimilated by a metazoan. Thus, $10M/G$ approximates the organism-wide rate of energy consumption in units of ATP hydrolysis equivalents, providing a basis for comparison across different species (19). For ciliate species within the range of cell sizes for which there are more direct estimates of ATP equivalents derived from growth on defined media (e.g., glucose as the only carbon and energy source; 15), this indirect approach yields estimates between 0.5× and 1.1× those from direct estimates (19), further justifying the application of this approximation.

Drawing from the results above, the total cost of building and maintaining a ciliate cell growing at maximum rate at 20 °C is

$$C_{\mathrm{ATP}} = (1.58 \times 10^{16}) B_{\mathrm{mat}}^{0.84}, \qquad \textbf{[5a]}$$

where the size at cell-division, B_{mat}, is in units of μg dry weight. This derivation assumes that the size of a dividing cell, B_{mat}, is equal to 1.33× the cell average size typically recorded in the literature. Likewise, for amoeboids,

$$C_{\mathrm{ATP}} = (1.37 \times 10^{16}) B_{\mathrm{mat}}^{0.97}. \qquad \textbf{[5b]}$$

Whereas there is a clean delineation between generations in a unicellular species reproducing by binary fission, for a metazoan engaging in multiple reproductive events per lifespan, the average generation time will be dictated by environmental influences on age-specific mortality schedules. The most objective approach for cladocerans is to consider the age at first reproduction as the minimum generation time, in which case Eq. **1** leads to

$$C_{\mathrm{ATP}} = (64.0 \times 10^{16}) B_{\mathrm{mat}}^{0.74} \qquad \textbf{[5c]}$$

as the estimated total ATP budget from birth to first progeny release. Thus, for the size range in which cladocerans and protists overlap, the former incur ∼30 to 50× higher costs in producing new individuals. The magnitudes of these disparities are conservatively low because the birth-to-maturity cost estimates do not include the additional metabolic cost of the egg that produced the juvenile. Shifting the benchmark to a later age (beyond maturity) will further increase the total lifetime energy expenditure. From Fig. 3, it is apparent that these same conclusions apply to all other metazoans examined.

Demands on ATP Synthase. Noting that 1 mol ATP ≃ 507 g and retaining the assumption that half of C_{ATP} is reflected in direct ATP turnovers, according to Eq. **5c**, a small metazoan of 1 μg dry weight, will expend the equivalent of ∼270× its own weight in recycling adenosine diphosphate (ADP) to ATP en route to first reproduction, with ratios of 81, 24, and 8 for organisms of size 10^2, 10^4, and 10^6 μg dry weight, and possibly ∼10× higher for the two larger size classes (Fig. 3).

Such calculations provide further insight into organismal requirements for the machinery involved in ATP production. During aerobic respiration, oxygen is consumed via electron-transport chains housed in inner-mitochondrial membranes,

which ultimately export protons that then return via the proton-motive force. In this process, mechanical force generated by the latter is converted to chemical energy (ATP) by rotational motion involving the cell's energy turbine, ATP synthase. Given that 5× the rate of consumption of O_2 molecules provides an estimate of the rate of ATP production, the number of ATP-synthase complexes necessary for such production can be estimated if the turnover number (ADP → ATP) of the complex is known.

Several in vitro studies have shown that turnover numbers can be 200 to 400 s^{-1} in complexes embedded in liposomes (e.g., refs. 52–55), but under optimal physiological conditions, the maximum rate may be no greater than 100 (56–61). Applying the latter estimate to the allometric relationships for respiration rates in ciliates, amoebae, and *Daphnia,* the inferred numbers of ATP synthases (in billions) per organism are $1.39B^{0.62}$, $0.50B^{0.76}$, and $5.45B^{0.86}$, where B is in units of μg dry weight. If the turnover number for ATP synthase is 200 s^{-1}, these numbers would be 2× too high, but unless there is a dramatic increase in the turnover number in metazoans, there must be a 5 to 17× increase in numbers of this complex in *Daphnia* relative to that in protists of equivalent size (in the range of 2 to 50 μg dry weight). Further empirical work will be required to ground-truth these estimates with direct observations, although the results for unicellular species are in rough accord with estimates from whole-cell proteomic data (18).

To account for the respiration rates of *Daphnia* of sizes 1, 10, and 100 μg dry weight, ∼5.4, 23, and 95 billion ATP-synthase complexes are required per individual, respectively. It is less clear how such complexes are distributed over cells and among mitochondria. However, if one assumes the average number of mitochondria per mammalian cell to be 400 (62), then proteomic analyses (reviewed in ref. 18) suggest 2,000 to 7,000 complexes per mitochondrion in various cell types. An *Arabidopsis* mitochondrion (in heterotrophic cells) contains ∼6,400 complexes (61). Assuming that an average mitochondrion in *Daphnia* contains 5,000 complexes, then dividing the numbers just cited by 5,000 provides an estimate of the number of mitochondria per individual.

The Speed Limit on Cell-Division Times. Estimation of the energetic investments that organisms make in the construction of the electron-transport chain and ATP synthase provides insight into the minimum time required to build biomass, as cell duplication minimally requires duplication of the machinery deployed in power production. In metazoans, an ATP synthase complex consists of ∼5,380 amino acids, which with an average cost of 30 ATPs/amino acid synthesized and 4 more ATPs per residue required for polymer construction (18) implies a construction cost of 1.83×10^5 ATPs per complex. Estimation of the additional cost of the remaining four complexes of the electron-transport chain requires information on their in vivo stoichiometries, which relative to ATP synthase, scale as 1.1 : 1.3 : 3.0 : 6.7 : 1.0 in mammals (63); a similar stoichiometry exists in heterotrophic *Arabidopsis* cells, except for an apparent sixfold reduction in abundance of Complex IV (61). Using the known molecular weight for the complexes in bovine, there are an additional 8,698 amino acids associated with the electron-transport chain (ETC) per ATP synthase. As there are additional small contributions from coenzyme Q, cytochrome c, and other small membrane proteins, these results indicate a total investment in the ETC/ATP synthase population of ∼3× that for ATP synthase alone.

Considering the numbers of ATP synthases inferred above for *Daphnia* of sizes 1, 10, and 100 μg dry weight, the costs of the ETC/ATP-synthase machinery are 3.0×10^{15}, 2.2×10^{16}, and 1.6×10^{17} ATP-hydrolysis equivalents, respectively. Relative to the cost of constructing an entire individual, C_{ATP} (Eq. **5c**), these are equivalent to investments of 0.5, 0.6, and 0.8%, respectively. Such estimates assume that the complexes have a negligible chance of degrading (and needing replacement) during the generation time of an organism (31), which if not correct would lead to downwardly biased cost estimates (18).

Although these investments in the infrastructure for power generation may seem relatively small, the essentiality of such complexes imposes a biological limit on cell-division potential. Assuming an upper limit of ATP production of 100/s per ATP synthase complex, the above results imply that ∼1.3 h of continuous operation of a complex is necessary to generate enough energy to synthesize another eukaryotic ETC/ATP synthase stoichiometric assemblage. However, ATP synthesis must also subsidize the production of all other proteins in the cell. For unicellular species, the total number of proteins per cell can be estimated from the allometric scaling relationship in figure 7.4 of Lynch (18). Assuming that ∼100 total proteins constitute an ETC/ATP synthase complex, there are 90 additional proteins synthesized per complex in the smallest ciliates with known minimum doubling times of 1.7 h (20), which can be contrasted with the expectation of $1.3 \times 1.9 = 2.5$ h based on bioenergetic extrapolation. For amoeboid species, there are 390 additional proteins synthesized per complex in the smallest species with minimum doubling times of 2.8 h (20), which contrasts with the expectation of $1.3 \times 4.9 = 6.4$ h.

The numbers for *Daphnia* are less secure in that cell volumes necessary for ascertaining protein numbers are uncertain and that 9 of the 20 amino acids cannot be synthesized in metazoans, and must be acquired directly from food sources. If an average cell volume of 1,500 μm^3 is taken as a first-order approximation (based on observations in nematodes and mammals), then each ETC/ATP synthase complex must produce another 2.7× equivalent amount of cellular protein, leading to a predicted minimum doubling time of $1.3 \times 3.7 = 4.8$ h. This calculation, which assumes that costs of acquiring the 9 "essential" amino acids by food protein breakdown are approximately the same as those synthesized, can be contrasted with a minimum doubling time of 8.3 h observed for the smallest cladocerans (20).

For all three of these cases, the observed minimum doubling times are ∼0.4 to 0.7× the theoretical expectations, but the two are brought into close accord if it is assumed that ∼50% of assimilated reduced carbon compounds are allocated directly to carbon skeletons (rather than respired). As noted above, any additional yield of ATP from glycolysis and/or from anaerobic mitochondrial metabolism has been ignored here, but this is unlikely to constitute much more than 10% of energy production in the organisms noted above. Thus, these results suggest that the peculiar energy-producing machinery endowed upon all organisms via inheritance from the last universal common ancestor imposes a hard constraint on the maximum rate at which biomass can be produced.

Methods

This paper reports on substantial datasets for 17 species of *Daphnia* and one of *Simocephalus*, all grown in the laboratory at 20 °C on a food consisting of 100,000 cells *Scenedesmus dimorphus* and 20,000 cells of *Chlamydomonas reinhardtii* per mL in a synthetic medium, which as described in refs. 64–66, leads to maximum levels of growth and reproduction in all species. Cohorts of individuals were raised together in 3.5 L of medium, replaced on a daily basis.

Initial cohort sizes ranged between 100 and 150 individuals, which were then gradually culled to ensure that total grazing activities did not reduce the standing levels of algae by more than 10% per day. Each day, 30 random individuals were measured for body length (from the tip of the head to the base of the tail spine) and numbers of eggs/embryos carried, with this sample size only diminishing as natural mortality occurred in aging individuals. Total numbers of offspring released per day were also recorded, and the overall cohort size was enumerated so as to yield survival data. As described in the preceding references, dry weights of several dozens of individuals of various sizes (with eggs removed) were determined to enable a conversion of lengths into mass. Dry weights of several dozens of eggs were determined, as were lengths of adult instar durations, to enable estimation of investments in reproduction. With this information, along with estimates of molt weights (*SI Appendix*), it was possible to estimate per-individual daily investments in growth, reproduction, and molting (in units of dry weight), as outlined in further detail in the prior references. All of these studies were pursued using two to three replicate experiments, with the results reported herein being averages over all replicates. These analyses were supplemented with computations made possible with parallel data for four other cladoceran species reported in the literature: *Bosmina longirostris* (67); *Ceriodaphnia lacustris* (68); *Ceriodaphnia quadrangula* (66); and *Ceriodaphnia reticulata* (68).

Data on size-specific respiration rates for several species were obtained from ref. 58, as well as from the literature for several other species. For species without such data, extrapolations on respiration rates were made by using data from closely related species or from the average allometric regressions over all species. As noted in *SI Appendix*, the among-species differences in these relationships are small enough to have minimal influence on the results reported herein. Similarly, data on molt weights had to be extrapolated for various species.

Data, Materials, and Software Availability. All study data are included in the article and/or supporting information.

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