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Experimental demonstration of a 'rate–size' trade-off governing body size optimization

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ABSTRACT

Questions: Can the decline in ectotherm body size with increasing temperature be explained using a simple body size optimization model? Does the pattern conform to the rate–size trade-off wherein organisms trade asymptotic size for mass-specific resource demand in order to maintain maximal resource uptake rates?

Organism: The predatory protist, *Actinosphaerium* sp., feeding on *Paramecium bursaria*.

Methods: I measured biovolume production rate (an index of mass-specific resource demand) and cell size across three environmental temperatures. I controlled prey supply by mixing and standardizing culture media across treatments and replicates.

Results: Biovolume production rates increased and cell size decreased with increasing temperature. The slope between these two variables on logarithimic axes (–0.91) was almost exactly as predicted by the optimization model (–0.94), lending quantitative support for the existence of a rate–size trade-off that governs the plasticity of size in this species and leads to the temperature–size rule.

Keywords: *Actinosphaerium*, body size evolution, life-history evolution, *Paramecium bursaria*, supply–demand model, temperature–size rule.

INTRODUCTION

Body size influences a wide array of ecological, physiological, and evolutionary processes, and its evolution is of fundamental importance (Peters, 1983; Calder, 1996; Brown *et al.*, 2004; Bonner, 2006; Brose, 2010). Although considerable research has addressed the evolution of and plasticity in body size, a broad and general explanation for why a particular size comes to characterize the adults of a species is still lacking. For this reason, many long-standing patterns of variation in body size, including Bergmann's, Cope's, the island, and the temperature–size rules remain unexplained (Jablonski, 1997; Blackburn *et al.*, 1999; Arendt, 2011).

The temperature–size rule describes a decrease in body size with increasing temperature (a negative reaction norm of size on temperature) (Atkinson, 1994; Berrigan and Charnov, 1994; Sibly and Atkinson, 1994; Atkinson *et al.*, 2003; Kingsolver and Huey, 2008). The pattern applies to ectotherms and is

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widespread but not universal (Atkinson, 1994, 1995), and explanations for the temperature–size rule have to date met with limited success. For example, the MASROS (maintain aerobic scope and regulate oxygen supply) hypothesis suggests that ectotherms grow to a smaller size because this facilitates the maintenance of aerobic scope when oxygen concentrations decline with temperature (Atkinson *et al.*, 2006; Forster *et al.*, in press). This hypothesis, however, fails to explain reversals of the temperature–size rule and does not apply to terrestrial organisms or very small ectotherms such as protists or prokaryotes. Using the von Bertalanffy growth model, others have suggested that the temperature–size rule arises due to differential sensitivities of the 'enabling' and 'constraint' processes that generate growth (von Bertalanffy, 1960; Perrin, 1995; Kozlowski *et al.*, 2004). Their model, however, requires that growth efficiencies decline with temperature, which a meta-analysis did not support (Angilletta and Dunham, 2003). Similarly, recent work expanding on the ontogenetic growth model of Hou *et al.* (2008) suggests that differential temperature sensitivities of growth and development account for the temperature–size rule (Zuo *et al.*, 2012). This model provides a potentially powerful framework for understanding ontogeny, yet it appears to apply only to determinate growers, as it hypothesizes that the temperature–size rule occurs when less growth occurs by the time of sexual maturity at warmer temperatures. Furthermore, Zuo and colleagues' (2012) model offers no explanation as to why growth and development would not have similar temperature sensitivities, and it casts the final size as simply a consequence of biochemical kinetics that is unrelated to fitness. And finally, although fairly successful in demonstrating that observed adult sizes may maximize fitness (Roff, 1986; Stearns, 1992; Charnov, 1993), classic life-history theory is unable to explain the temperature–size rule because temperature has not been connected logically to the costs and benefits of further growth.

THE SUPPLY–DEMAND MODEL

The supply–demand model is intended to be a general model of body size optimization, but here I develop it specifically to help understand the temperature–size rule. The supply–demand model proposes that the optimal body size is that which matches the bodily demand for resources with the average expected level of per capita resource supply from the environment. This matching of supply and demand should be fitness maximizing: a body that requires less than the expected amount of available resource is competitively inferior due to unnecessary limits on its biomass production, and a body that requires more resource than the environment can supply is at a disadvantage because it is taking in less energy than its allocation patterns require. A body that was too large would thus experience nutritional stress (or mortality), or be forced to allocate available production in suboptimal ways (all to maintenance, for example). In short, the hypothesis suggests that an organism should continue to grow until it uses as much energy as it can reliably use on average, and no more.

In the supply–demand model, a demand (D) curve shows the relationship between the size of an organism as it grows, m, and the amount of resource it requires to grow, function, maintain itself, and eventually, reproduce (Fig. 1). The D curve is the ontogenetic allometry of metabolic rate and is a straight line with a positive slope because it is a power law function plotted in log space. A supply (S) curve stipulates the available per capita resource supply rate from the environment. Although the S curve may take on a variety of shapes, here I draw it as horizontal because experimental conditions will impose this shape (other shapes are possible and perhaps likely). The key hypothesis of the supply–demand model is that the optimal asymptotic (final) body size, m_∞, occurs where the S and D curves intersect.

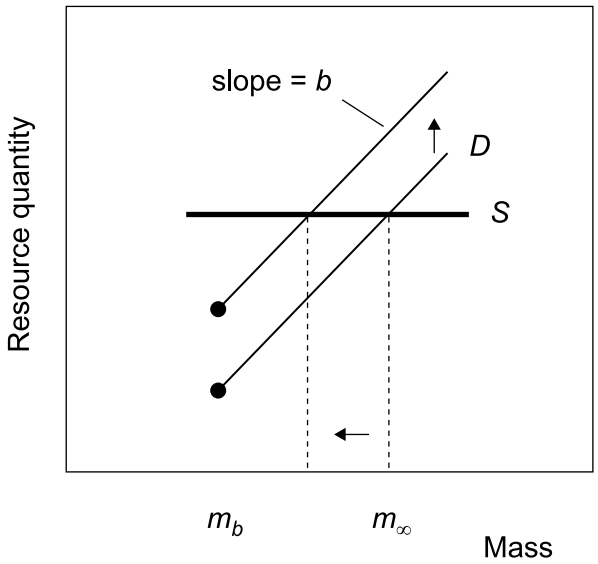

Fig. 1. Graphical explanation of how the supply–demand model predicts a decrease in body size with an increase in temperature (temperature–size rule). Increasing temperature raises the demand (D) curve and shifts the intersection of the supply (S) and demand curves to the left, following equation (1).

I suggest this intersection point is fitness maximizing because it is where the organism can access, and therefore allocate, the greatest amount of resource towards essential components of fitness such as biomass production and somatic maintenance, whatever the balance of those competing ends may be. Note that the supply–demand model does not depend upon a particular model of ontogenetic growth.

With S held constant, variation in D is predicted to induce a change in m_∞. Because the height of the D curve reflects the mass-specific rate of resource use, a fixed S imposes a 'rate–size' trade-off whenever the height of D changes (Fig. 1). The rate–size trade-off enables organisms to use the optimal amount of resource by adjusting their eventual body size in response to an environmental influence on metabolic rate. The optimal asymptotic body size occurs when $S = D$, so we may write, $S = D = b_0 m_\infty^{\,b}$, where b_0 is the pre-factor for the body-size scaling of resource demand and b is the scaling exponent. We can then solve for m_∞, yielding a trade-off expression: $m_\infty = (S/b_0)^{1/b}$. Taking the log of both sides gives

$$\log(m_\infty) = \frac{1}{b}\log(S) - \frac{1}{b}\log(b_0). \quad (1)$$

Equation (1) shows that m_∞ is linearly related to supply and mass-specific demand on log scales. When S is held constant, m_∞ is predicted to relate negatively to b_0 with a slope of $-1/b$, and when b_0 is held constant, it is predicted to relate positively to S with a slope of $1/b$. For ectotherms, temperature has an exponential effect on b_0 (Gillooly *et al.*, 2001), and equation (1) suggests that the temperature–size rule arises via a rate–size trade-off because increases in temperature raise b_0 and lower m_∞ given a constant S. A robust test of this hypothesis would involve measuring temperature-induced changes in physiological demand and body size and determining if the association between these variables had a slope of $-1/b$.

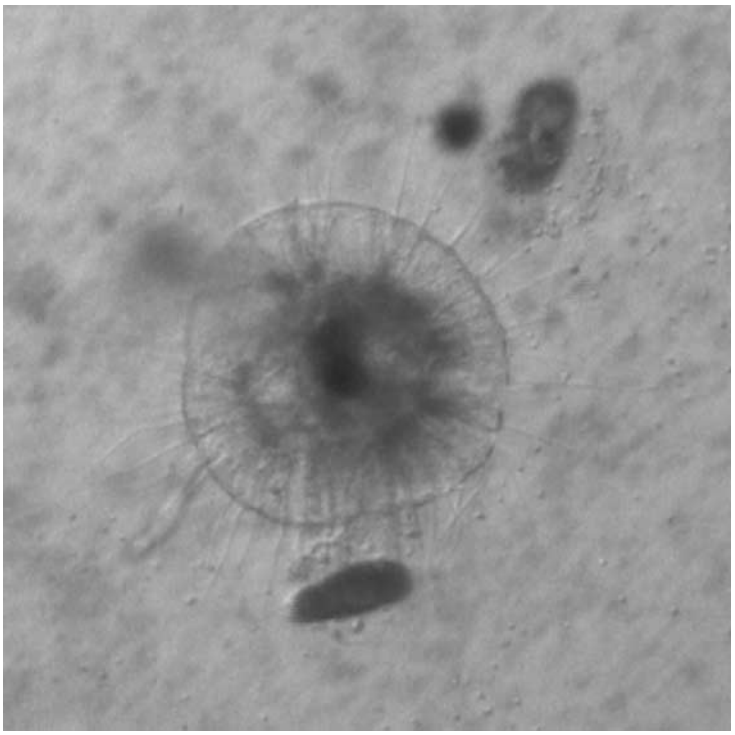

Fig. 2. An *Actinosphaerium* with a recently captured prey, *Paramecium bursaria. Actinosphaerium* capture its prey with long sticky spikes, which are then withdrawn into the cell, pulling the paramecium into a single vacuole within the cell (two vacuoles are visible within the cell).

I conducted a robust test of the supply–demand model using the protist *Actinosphaerium* sp. feeding on the protist *Paramecium bursaria* (hereafter just *Paramecium*; Fig. 2). I measured the effect of temperature on both biovolume production rates and cell volume, controlled the *S* curve, and used the supply–demand model to predict the change in cell volume of *Actinosphaerium*. The observed pattern matched the prediction quite closely, suggesting that body size is optimized at the point where supply meets demand for this species. This study raises the possibility that a simple energetic optimization principle may be sufficient to understand the temperature–size rule, and potentially, other patterns of body size variation.

METHODS AND MATERIALS

Actinosphaerium and *Paramecium* were isolated from cultures obtained from Carolina Biological Supply (CBS, Burlington, NC, USA). *Actinosphaerium* was washed free of other protists and maintained in the laboratory with *Paramecium* as food. Culture medium was made from a 1:20 dilution of CBS protozoa medium inoculated with the microorganisms present in the original *Paramecium* culture, which contained an unknown array of bacteria and small flagellates. *Paramecium* and *Actinosphaerium* cultures were maintained in jars and flat dishes in the light (1000 lux) at 25°C.

The experimental microcosms were 50 mm diameter plastic Petri dishes with lids, filled with 5 mL of media. Microcosms were incubated in Percival incubators with 24 h light at three treatment temperatures: 20°C, 25°C, and 28°C. Irradiance at the surface of the media (below the clear lid) was ~2000 lux. In total there were 16 replicate microcosms, five each at 25°C and 28°C and six at 20°C. The replicates were staggered in time to allow for more replicates because it would not have been possible to manage all 16 replicates on the same day. The total duration of the experiment was 2 weeks.

Microcosms were inoculated with a standardized density of *Paramecium*, about 40 cells per millilitre (indicated by acquiring ~25 cells in three separate draws of 0.2 mL from the culture). Five millilitres of this culture were put into each microcosm, and 1–2 medium-sized *Actinosphaerium* cells with 1–2 food vacuoles (each containing one *Paramecium*) visible inside (Fig. 2) were transferred to each microcosm. Each day, including the initiation day, the *Actinosphaerium* cells were counted and photographed. The size of each *Actinosphaerium* cell was determined by measuring cell diameter in two directions and converting to volume using either the formula for a sphere or prolate spheroid depending on which shape better approximated each cell. Dividing cells also were measured, and if sufficient separation had occurred, each daughter cell was measured and summed to give a total cell volume of the parent cell.

To impose a horizontal *S* curve, I normalized *Paramecium* levels across treatments, cultures, and days. I did this by removing 3 of the 5 mL from each culture each day (taking care not to remove the *Actinosphaerium*), mixing these extracts in one container, and augmenting the *Paramecium* levels in this mixture with a concentrated culture of naïve *Paramecium* prey grown on the same media until three separate 0.2 mL draws from the culture produced a total count of ~25 *Paramecium* individuals, which matched the starting conditions of the microcosms. Then 3 mL of this normalized culture were added back to each microcosm.

The *Actinosphaerium* multiplied after inoculation. Although all cells were photographed and measured as the population grew (a total of 1019 cells were measured across the three treatments), only the later days when five or more cells were present were included in measurements of production and the analysis of cell size. The early period of growth was excluded from the analysis because the number of *Actinosphaerium* cells has a strong impact on foraging rates. Five cells was deemed a density with sufficient within-replicate subsamples to produce a meaningful replicate-level average and a low enough density to avoid large density effects, as pilot experiments showed little suppression of vacuole formation at this density (one cell per millilitre). A minimum of four sampling days with densities above five cells was measured for each replicate, with five or six days used in some replicates. On weekends, measurements were not taken but resource normalization across replicates was conducted once per weekend.

Each day during the sampling period, all *Actinosphaerium* cells were measured, and then the population was culled to five individuals. The culling procedure was standardized in the following manner. Each cell was found using a clockwise screening from the six o'clock position of the dish. The first cell encountered was cell number 1, then cell number 2, and so on. Then, if there were six cells, cell 2 was removed. If there were seven cells, cells 2 and 5 were removed. If there were eight cells, cells 2, 5, and 7 were removed. If there were more than eight cells, cells 2, 5, 7, and all cells 9 and above were removed. The volumes of all retained cells were noted, and these five cells were allowed to grow over the course of the next day, when the procedure was repeated. Biomass production was taken as the total cell

volume on the subsequent day less the cell volume of the five retained cells. This value was expressed per mass by dividing by the total volume of the five retained cells.

I averaged size and mass-specific biomass production rates for each replicate across sampling days and evaluated the relationship between these observations using logged data and reduced major axis regression (RMA). The best estimate of the metabolic scaling slope available for protists is $b = 1.06$ (95% CIs: 0.91 to 1.28) for active cells (DeLong *et al.*, 2010), taken from a large compilation of measurements and also analysed using RMA regression. Confidence intervals for both the observed and predicted slopes were made using a bootstrapping procedure with 1000 samples.

RESULTS

Resource levels were maintained independent of treatment and replicate (Fig. 3A), indicating that the standardization methods successfully controlled the *S* curve with respect to temperature. Temperature therefore influenced mass-specific physiological rates (demand) without influencing supply.

Overall, production rates increased and cell size decreased with temperature (Fig. 4). Six individual production estimates were negative, mostly from cold microcosms that had very small production rates that were more affected by measurement error than at the warmer temperatures, and these were excluded from the analysis (11% of 53 production measurements). The slope of the relationship between mass-specific demand and cell size was −0.91 (95% CIs: −1.2 to −0.69; $R^2 = 0.71$), which is very close to and statistically indistinguishable from the predicted slope of −0.94 (95% CIs: −1.1 to −0.78).

The minimum recorded cell volume was similar among treatments (Fig. 3B), suggesting that some daughter cells were generated at similar sizes across temperatures. This corresponds with the increasing tendency for plasmotomy in the colder *Actinosphaerium*

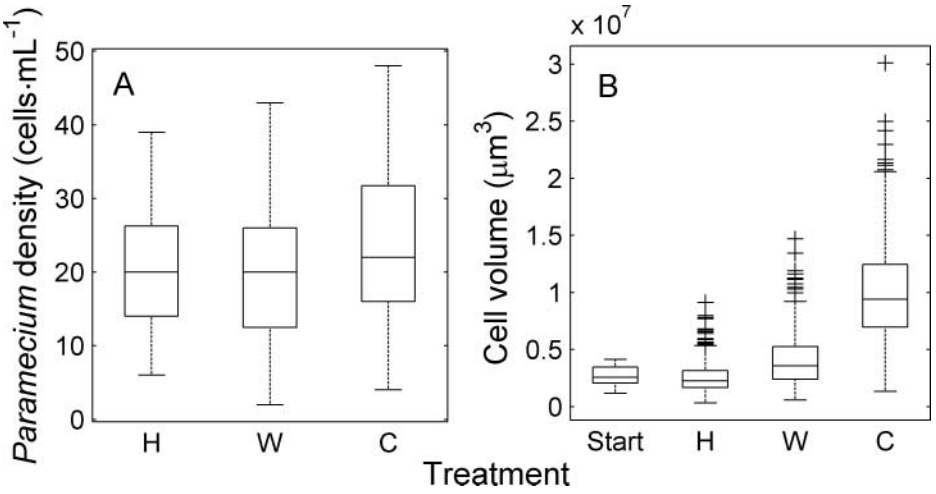

Fig. 3. Association between temperature and (A) prey density and (B) cell size. (A) Prey density was similar across treatments, because it was controlled by remixing cultures among microcosms, eliminating any dependence of resource level on temperature and enforcing a horizontal *S* curve. (B) The 1019 cell volumes measured during the course of the experiment, indicating similarity of starting conditions (start) with the broadening of cell size with cooling temperature.

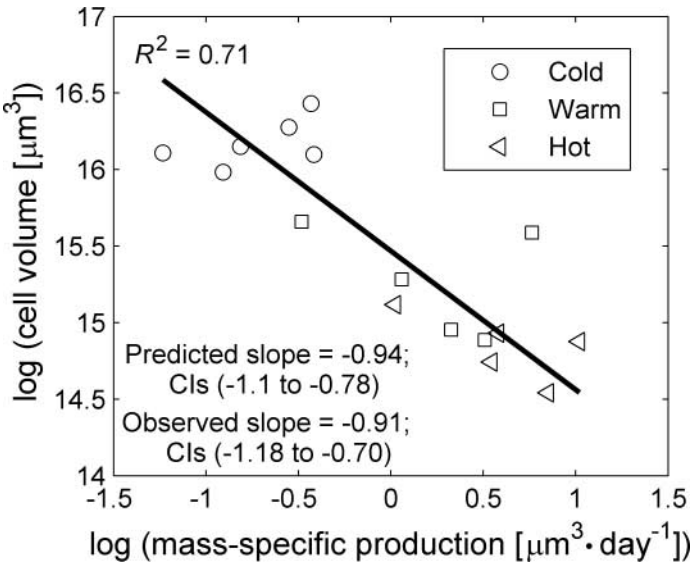

Fig. 4. Rate–size trade-off in *Actinosphaerium* induced by experimental manipulation of temperature. The observed slope is very close to the predicted slope, indicating support for the supply–demand model.

cells, where large cells frequently divided into three rather than two daughter cells. Plasmotomy was never observed in the 28°C treatment.

DISCUSSION

Because it correlates with so many ecological, physiological, and evolutionary processes, adult body size is often viewed as a fundamental biological trait. The supply–demand model proposes a simple hypothesis about size: organisms should grow to the point where they use as much resource as can be expected to be available. The supply–demand model quantitatively predicts the magnitude of size change given changes in the *S* and *D* curves. In this study, I predicted the slope of the relationship between the log of mass-specific demand and the log of asymptotic size using the supply–demand model and known information about the scaling of metabolic rate with size. This prediction was made without reference to the size data itself, and the observed slope was very close to the prediction, indicating strong support for the supply–demand model. However, I did not measure fitness directly, and therefore cannot conclude that this trade-off is fitness maximizing. Nonetheless, it is hard to imagine why such a precise compensating mechanism such as the rate–size trade-off would arise if it did not enable organisms to adjust their phenotypes to an optimal level.

The supply–demand model also clarifies the importance of controlling for resource supply when evaluating the effect of temperature on size, since without this control, warming treatments would also influence the productivity of the prey species and create an inadvertent supply effect as well, confounding the results. By controlling resource supply levels, which strongly influence foraging rates (DeLong and Vasseur, 2012) as well as cell size (Jiang and Morin, 2005; Forster *et al.*, 2012) in protists, I was able to ensure that the results were the

consequence of a kinetic effect on mass-specific demand and not of a supply effect. The supply–demand model also suggests that failure to control resource supply would produce a temperature–size rule of a smaller magnitude or no such rule at all. It is noteworthy that protists have typically shown small declines in size with temperature (~2.5% per degree centigrade) (Atkinson *et al.*, 2003), generally in laboratory studies where resource supplies were allowed to change with temperature. In the current study with a controlled supply, *Actinosphaerium* mean cell size declined ~15% per degree centigrade, suggesting that the independent effect of temperature on cell size may be more pronounced than previously thought.

One question that the supply–demand model leaves unanswered is how an optimal size is achieved. The supply–demand model only suggests that a thermal reaction norm should evolve to have a particular slope, but it does not explain how that adjustment comes about. The mechanism of attaining an optimal size may be one area in which competing models may come together, as models that focus on proximate underlying mechanisms (Kozlowski *et al.*, 2004; Zuo *et al.*, 2012) may help to illustrate how the supply–demand or other optimum may be achieved.

Although it appears that *Actinosphaerium* adjusts its size to precisely compensate for a temperature-induced change in its bodily demand for resources, existing models do not suggest how a growing organism determines the expected level of supply. Some work has shown that early life signals of environmental resource levels correlate with adult traits, including body size (Kuzawa *et al.*, 2010). For *Actinosphaerium*, similar signals such as the rate of encountering prey early in life may play a role in setting the organism on a path to an optimal size. Resources handed down from the parent cell also may be a factor, as daughter cells frequently are produced still digesting the *Paramecium* in vacuoles formed by the parent cell. It would be interesting to examine whether at-division food stores are linked to asymptotic body size.

That the supply–demand model has survived a quantitative test raises the possibility that a simple, general process could explain why body sizes are what they are. Nothing about the supply–demand model is specific to *Actinosphaerium*; for any species and any body size evolution question, one could ask how a factor influences the S and D curves. Then it is possible to produce a qualitative and quantitative prediction about how body size should evolve. Thus, the supply–demand model may be able to shed light on a wide range of observations, including Cope's, Bergmann's, and the island rules, as well as their absences and reversals.

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