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LETTER

Size-abundance rules? Evolution changes scaling relationships between size, metabolism and demography

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Abstract

Body size often strongly covaries with demography across species. Metabolism has long been invoked as the driver of these patterns, but tests of causal links between size, metabolism and demography within a species are exceedingly rare. We used 400 generations of artificial selection to evolve a 2427% size difference in the microalga *Dunaliella tertiolecta*. We repeatedly measured size, energy fluxes and demography across the evolved lineages. Then, we used standard metabolic theory to generate predictions of how size and demography should covary based on the scaling of energy fluxes that we measured. The size dependency of energy remained relatively consistent in time, but metabolic theory failed to predict demographic rates, which varied unpredictably in strength and even sign across generations. Classic theory holds that size affects demography via metabolism – our results suggest that both metabolism and size act separately to drive demography and that among-species patterns may not predict within-species processes.

Keywords

Allometry, artificial selection, Centre for Geometric Biology, demographic parameters, evolutionary size-shift, experimental evolution, metabolic ecology, metabolic energy, scaling.

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INTRODUCTION

For decades, ecologists have investigated the relationships between the body size of a species and its demographic rates (Fenchel 1974; Peters 1983; Calder 1984). Most life-history traits covary strongly with size. For example, smaller species tend to have populations with higher productivities and maximum growth rates (Ernest *et al.* 2003; Savage *et al.* 2004; Marba *et al.* 2007). Perhaps the best-known ecological pattern with regard to body size is Damuth's rule, whereby populations of smaller individuals achieve higher densities than populations of larger individuals. This size–density covariance among species is strikingly consistent across a wide range of species (Damuth 1981; Blackburn *et al.* 1990; Marquet *et al.* 1990; Andersen & Beyer 2006; Perkins *et al.* 2019), but the mechanisms driving these relationships are still debated.

A widely accepted explanation for why demographic parameters correlate with body size among species is based on how size affects energy use. Metabolic rate does not increase in proportion to body size, particularly among species. Rather it tends to show a nonlinear relationship described by the function $MR \propto M^{\beta}$, where MR is the metabolic rate, M is the mass and β is the scaling exponent, which is usually <1 for metazoans [but see DeLong *et al.* (2010) for deviations across the natural kingdom]. Because variation in metabolic rates among species is strongly determined by size, it has been repeatedly argued throughout the history of ecology that this size–metabolism relationship is what drives much of the covariance between body size and demography among species (Peters 1983; Reiss 1991; de Roos & Persson 2003). More recently, many of those classic theories have been unified into a broader 'Metabolic Theory of Ecology' (or simply 'metabolic theory') framework that has the merit of proposing testable mathematical equations of how each demographic rate should scale with organismal size (reviewed in Brown *et al.* 2004; and in Isaac *et al.* 2012). For example, metabolic theory holds that the maximum intrinsic rate of population increase (r) should scale with metabolism at $(M^{\beta}/M^{1} =)\ M^{\beta-1}$. Assuming metabolism dictates resource requirements, the population density at carrying capacity (K_{pop}) of a resource-limited population should scale at $(M^{0}/M^{\beta} =)\ M^{-\beta}$. From these assumptions, it follows that the total biomass at carrying capacity (K_{bio}) should scale at $(M \times K_{pop} = M \times M^{-\beta} =)\ M^{1-\beta}$. Similarly, the maximum production of individuals (or rate of new individuals per unit time; r_{pop}) should scale at $(r \times K_{pop} = M^{\beta-1} \times M^{-\beta} =)\ M^{-1}$.

Metabolic theory also predicts that some demographic parameters should be size invariant. The maximum productivity (or the total biomass production per unit time; r_{bio}) should scale at $(r \times K_{bio} = M^{\beta-1} \times M^{1-\beta} =)\ M^{0}$ and, hence, should be independent of size. Similarly, the total energy use per unit area (E_{tot}) of a population should scale at $(E \times K_{pop} = M^{\beta} \times M^{-\beta} =)\ M^{0}$, which is also known as the 'Energy Equivalence rule' (Damuth 1981). Metabolic theory is a very active field of research and has been continuously refined over the years (reviewed in Sibly *et al.* 2012), with developments including elemental stoichiometry and nutrient fluxes (Allen & Gillooly 2009), animal space use (Jetz *et al.* 2004), species competition (Berlow *et al.* 2009) and the self-thinning rule (Jonsson 2017). Nevertheless, the fundamental relationships describe above have all been retained.

This metabolic explanation for size–demography patterns has strong support. Some studies show predictions based on interspecific metabolic scaling with remarkably high congruency with demographic processes, including phytoplankton, trees, fish, mammals and birds (Calder 1983; Peterson *et al.* 1984; Lindstedt *et al.* 1986; Reiss 1988; Silva *et al.* 1997; Andersen & Beyer 2006; Hatton *et al.* 2015; Perkins *et al.* 2019). Yet, other groups show systematic deviations from

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such expectations (Russo *et al.* 2003; Muller-Landau *et al.* 2006; Russo *et al.* 2007; McGill 2008; Hayward *et al.* 2009; Reuman *et al.* 2009). The congruence between metabolic scaling and the scaling of demographic rates among species is not definitive evidence that metabolism (and its covariance with size) drives demographic rates (e.g. Hayward *et al.* 2010). Importantly, whether the same size–metabolism–demography covariance occurs within species also remains unclear (Kozlowski & Weiner 1997; Glazier 2005).

A problem with interspecific comparisons of body size and demography is that size covaries with so many other traits simultaneously. This collective size dependency of most life-history traits complicates the identification of causal links between size, metabolism and demography. For example, metabolic theory holds that body size is a key factor regulating the rate at which organisms use energy, which in turn influences the carrying capacity and intrinsic rate of population increase (Brown *et al.* 2004). This seems reasonable, but it is also possible that demographic parameters are more affected by other size-related traits, such as growth rate, generation time, mortality rate or resource acquisition. As Tilman *et al.* (2004) point out, it is competitive ability, dispersal and predator defences that directly determine the fitness of a species, not metabolism or body size. Similarly, Glazier *et al.* (2011) argue that ecological factors can shape the scaling of metabolism and size, rather than the other way around. Finally, standard theory assumes that species of very different sizes all access resources in the same way, but this assumption is unlikely to hold in nature (Loeuille & Loreau 2006; Hayward *et al.* 2009). Thus, it remains unclear as to whether among-species patterns reflect within-species processes when it comes to size and demography.

It is also unclear whether interspecific scaling patterns have any relevance for the scaling of intraspecific demographic processes. Heusner (1991) and Kozlowski & Weiner (1997) show that size-scaling exponents of among-species relationships do not necessarily indicate any functional relationships within a species, but can originate simply from artificial combinations of subset allometries. Moreover, reviews of metabolic theory have noted that covariances between metabolism, size and demography often break down when specific taxonomic groups are examined (Hayward *et al.* 2010; Isaac *et al.* 2012). This is partly because it is difficult to obtain precise size-scaling exponents with less than two orders of magnitude in the range of body sizes (Hayward *et al.* 2010). On the other hand, metabolic theory has been strikingly successful at predicting intraspecific patterns in some instances. For example, self-thinning of crickets (Jonsson 2017) and lifespan of fish (Munch & Salinas 2009) both adhere to predictions based on metabolic scaling. Similarly, Bernhardt *et al.* (2018) recently found that metabolic theory predicted the effects of temperature on the carrying capacity of a phytoplankton species.

Establishing whether intraspecific covariation between size and metabolism causally determines demography has taken on new urgency because of global anthropogenic change. Within many species, body sizes are declining worldwide because of global temperature increases and exploitation. Standard theory, based on an interspecific metabolic scaling exponent (β) of 0.75, would predict that such declines within species should lead to higher intrinsic rates of population increase but lower biomass carrying capacities (Table 1). Changes in both demographic parameters would have massive consequences (both positive and negative) for biological systems worldwide, with implications for carbon cycles, species persistence and harvesting.

Table 1 Observed size-scaling exponents of each demographic parameter compared to theoretical predictions calculated from general theory using body size (M) and metabolic energy use (β). See Tables S1 and S2 for equivalent comparisons based on photosynthetic energy and total daily energy use respectively. Underlined in red are observed scaling exponents whose credible intervals do not overlap predictions

Parameter (units)	General theory	Generation	Predicted scaling exponent	Observed scaling exponent
Max. intrinsic growth rate (r; 1/time)	$M^{\beta-1}$	200	0.17	0.18
		300	−0.11	0.13
		400	−0.07	0.38
Biomass (K_{bio}; biovolume)	$M^{1-\beta}$	200	−0.17	0.7
		300	0.11	−0.28
		400	0.07	0.35
Density (K_{pop}; cell density)	$M^{-\beta}$	200	−1.17	−0.47
		300	−0.89	−0.88
		400	−0.93	−0.59
Biomass product. (r_{bio}; biovol./time)	M^{0}	200	0	0.83
		300	0	−0.13
		400	0	0.41
Cell product. (r_{pop}; cell/time)	M^{-1}	200	−1	−0.17
		300	−1	−1.13
		400	−1	−0.59
Total energy use (E_{tot}; energy)	M^{0}	200	0	0.71
		300	0	0
		400	0	0.34

Total congruence: 6 parameters (4 in gen 300).

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What type of changes are to be expected is unclear because we lack studies that examine the causal links between size, metabolism and demography within species.

The dearth of intraspecific studies is driven partly by practical issues. It is difficult to generate substantial intraspecific variation in body size without confounding ontogeny, diet or temperature, all of which can confound the role of size on demographic rates (Hayward *et al*. 2010). Here, we overcome this limitation by taking a common ancestor and evolving a 20-fold range of body sizes and repeatedly estimating key metabolic and demographic parameters in a common environment. This approach allows us to examine the causal links between size, metabolism and demography directly.

We applied 400 generations of artificial selection (*c*. 2.5 years) to generate differences of 2427% in the cell volume of the green microalga *Dunaliella tertiolecta* (Chlorophyta). We used metabolic theory to generate *a priori* energy-based predictions for each demographic parameter of the population: maximum intrinsic rate of population increase (r), maximum biomass productivity (r_{bio}), maximum production of new individuals (r_{pop}), together with carrying capacity in terms of total biovolume (K_{bio}) and population density (K_{pop}) and total energy use of the population (E_{tot}) at carrying capacity. To examine how evolutionarily labile these relationships are, we estimated size scaling of all of our energy and demographic parameters after 200, 300 and 400 generations of artificial selection.

MATERIAL AND METHODS

Experiment overview and rationale

Our goal was to isolate the effects of body size as much as possible to examine how size drives demography and whether metabolic scaling predicts these effects. Thus, we use an artificial selection approach to generate evolutionary trajectories that by definition are artificial. Such rapid evolution is unlikely to occur naturally. The artificiality of this approach is both a strength and limitation. Its strength is that it allows to focus on size to test mechanistic links between size, metabolism and demography, as well as explore the consequences of rapid, human-induced evolution in size. Its limitation is that it does not increase our understanding of how size and demography are related across species that take millennia to evolve to different sizes, with major concurrent changes in other life-history traits. Importantly however, in our study there were at least two generations of natural selection between each round of artificial selection. This means that our evolved lineages are not shaped by artificial selection alone, rather they are the products of both artificial and natural selection. Hence, only those phenotypes that have evolved to maximise production while also accommodating changes in size will proliferate. As such, we believe this experiment allows to explore the consequences of size evolution but does not unrealistically insulate the lineages from other selection pressures.

Study species and culturing conditions

The cosmopolitan, fast-growing green microalga *Dunaliella tertiolecta* (Butcher) was originally sourced from the Australian National Algae Culture Collection (ANACC; strain code CS-14). Cultures were reared in standard autoclaved F/2 medium without silica (Guillard 1975) prepared with 0.45 μm filtered seawater and kept in a temperature-controlled room at 21 ± 0.5 °C. Light intensity was set at 150 μM m^{-2} s^{-1} with a 14–10 h day–night cycle, using low-heat 50 W LED flood lights (Power-lite™, Nedlands Group, Bedfordale, Australia). Samples were not axenic, but bacterial loads in the cultures were kept to low levels by centrifuging and resuspending cells in autoclaved medium twice a week and by handling samples using sterile materials under a laminar-flow cabinet (Gelman Sciences Australia, CF23S, NATA certified).

Artificial selection

We used artificial selection to evolve cells of *D. tertiolecta* to smaller or larger volumes (Malerba *et al*. 2018c). Briefly, the artificial selection method relied on larger cells sinking and forming a pellet at the bottom of test tubes at lower centrifugal forces compared to smaller cells, which instead will remain in solution (i.e. differential centrifugation). On 25 April 2016, 72 cultures were inoculated from a single mother culture into aseptic 75 cm^2 plastic cell culture flasks (Corning®, Canted Neck, Nonpyrogenic). Since then, lineages have been kept independent and selected twice a week, each Monday and Thursday: 30 lineages were large-selected, 30 small-selected and 12 were the control. Control cultures experienced identical conditions (including centrifugation) without being size selected. Growth curves and metabolic rates were measured for all lineages after around 200, 300 and 400 generations of artificial selection across 2 years, reaching an overall size difference of 2427% among the smallest and the largest mean cell volume. Before collecting any data from the lineages, phytoplankton cells were sampled from 10 randomly selected lineages for each size treatment and were grown in nutrient-replete common garden under neutral selection (i.e. with no centrifugation) for three generations (7 days) to remove any environmental effects and non-genetic phenotypic differences.

Cell size

The mean cell area was monitored monthly for each lineage at the end of the neutral selection period. We used optical light microscopy after staining cells with 2% Lugol's iodine. All measurements were taken using software ImageJ and Fiji (version 2.0; Schindelin *et al*. 2012). Cell biovolume was calculated assuming a prolate spheroid shape, as recommended for this species by Sun & Liu (2003).

Energy rates

Oxygen dynamics were measured using six 24-channel PreSens Sensor Dish Readers (SDR; AS-1 Scientific Wellington, New Zealand) using the methods described in Malerba *et al*. (2018c). Briefly, at each generation all lineages were standardized to the same blank-corrected optical density and were loaded into 5 mL sealed vials, being careful to remove all air bubbles inside. Sodium bicarbonate (2 mM) was added to the media to ensure that photosynthesis and oxygen evolution were not limited by

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carbon availability. At least three vials per SDR plate were used as blanks and were filled with filtered supernatant. Oxygen dynamics were recorded in the dark (i.e. metabolism) and at saturating light (~ 300 μmol quanta m^{-2} s^{-1}; i.e. net-photosynthesis), each replicated at least four times. Rates of metabolism (oxygen consumption) and net-photosynthesis (oxygen production) were calculated from linear regression slopes of O_2 concentration over time and converted into units of J min^{-1}, using the conversion factor of 512×10^{-3} J $(\mu mol\ O_2)^{-1}$ from Williams & Laurens (2010). Rates were standardised by population density to obtain per-cell energy use in units of J min^{-1} $cell^{-1}$. Finally, the daily net energy production (J day^{-1} $cell^{-1}$) was calculated by adding the photosynthetic rate during the day and removing energy used at night (assuming a 14–10 h day–night photocycle).

Demographic rates

Using body mass is typically more appropriate than using body volume when calculating allometric relationships, because biologically inert components of a cell (e.g. vacules) can bias estimates of the size-scaling exponent. However, we showed for this species that cell volume and cell carbon (C) mass are linearly proportional (i.e. they are isometrically related; Malerba *et al.* 2018a), indicating that using cell C mass or cell volume would produce equivalent allometric scaling. Hence, throughout this study we used cell volume as the proxy for cell size, because it can be measured for individual cells more accurately and precisely than mass.

Following neutral selection, growth curves were collected after resuspending all samples into standard fresh F/2 medium. Samples were loaded into 96-well plates (Corning® polystyrene, flat bottom, with lid, sterile, non-treated, Sigma-Aldrich) after standardising to the same blank-corrected optical density (750 nm), which we showed to be a reasonable proxy for total biovolume in a culture (Malerba *et al.* 2018b; Malerba *et al.* 2018c). Each lineage was loaded into three 250 μL wells on different well plates after randomising the position within the plate. All samples were grown at light saturated conditions (> 200 μM m^{-2} s^{-1}) at 14–10 h day–night cycle and blank-corrected optical density (750 nm) was monitored daily (at the same time into the photoperiod) using a plate reader SPECTROstar® Nano (BMG labtech, Offenburg, Germany), until cultures reached a stable final biomass. This indirect way of measuring biomass allowed for far more frequent (non-destructive) monitoring compared to direct methods (e.g. flow cytometer). A pilot study showed that evaporation in the wells was low (~ 1% per day) and was, therefore, ignored in the analysis.

Demographic parameters were estimated using the same model-fitting techniques detailed in Malerba *et al.* (2018b). Briefly, the analysis of growth curves consisted of three parts. First, linear calibration curves measured for each of the three sets of experiments were used to convert units of blank-corrected optical density into units of total biovolume (μm^3 μL^{-1}; R^2 = 0.81–0.92). Second, we fitted five different growth models to accommodate different qualitative dynamics in the time series of total biovolume over time for all lineages collected at the three generations – each replicated three times (total of 297 time series) – and we used Akaike information criteria (AIC; Burnham & Anderson 2002) to determine which model showed the best goodness of fit. The five growth models were: Michaelis–Menten (2 parameters), Gompertz (3), logistic (3), 4-parameter logistic (4) and a Gompertz including mortality (4; for details on model structures, see figs S3 and S4 in Malerba *et al.* 2018b). Third, we used the best-fitting model favoured by AIC to estimate five demographic parameters for each lineage: total biovolume (K_{bio}; units μm^3 μL^{-1}), total cell density (K_{pop}; cells μL^{-1}) and total daily energy use (E_{tot}; J day^{-1} μL^{-1}) characterized the carrying capacity of the culture, whereas the maximum intrinsic rate of population increase (r ; day^{-1}) quantified the maximum proportional growth of the culture and finally the maximum production of biomass (r_{bio}; μm^3 μL^{-1} day^{-1}) and of new individuals (r_{pop}; cells μL^{-1} day^{-1}) defined the absolute yield of the culture. The parameters of each lineage were calculated using the maximum observed slope (i.e. r, r_{bio}, r_{pop}) or the final density (i.e. K_{bio}, K_{pop}, E_{tot}) of the best-fitting growth model. Cell density (cells μL^{-1}) was inferred from total biovolume by using calibration curves between per-cell optical density and mean cell volume (R^2 = 0.72) presented in fig. S3 of Malerba *et al.* (2018a). Although estimating population densities from calibration curves is prone to measurement error, we verified that including this source of uncertainty in the analyses did not change any of the conclusions (data not shown). Finally, total energy use (E_{tot}) was calculated by multiplying population density at carrying capacity (K_{pop}) by per-cell respiration rate.

Predicting demographic rates from energy rates

In ecology, rates of energy use (i.e. metabolism), and how these scale with size and demography have received much attention. Nevertheless, ecological models linking population demography to energy rates can be applied not only to metabolism, but also to other types of energy, including photosynthetic rates or daily energy budget (de Roos & Persson 2003). Thus, for all demographic rates considered in this study (i.e. K_{bio}, K_{pop}, E_{tot}, r, r_{bio}, r_{pop}) we generated three sets of predictions using either respiration rates, net-photosynthetic rates or daily net energy production (energy budget integrated over the light : dark photoperiod).

While Reduced Major Axis (RMA) techniques are often used to calculate size-scaling exponents (e.g. Niklas 1994; DeLong *et al.* 2010), we instead used ordinary least-squares linear models, as recommended by Kilmer & Rodriguez (2017) when the uncertainty in the X-axis (cell volume) is lower than that on the Y-axis (demographic and energy rates), which was indeed our case. Importantly, we verified that all conclusions remained unchanged when using RMA techniques instead (data not shown). All analyses were carried out in R (R Core Team 2016) using packages nlme (Pinheiro *et al.* 2016), lme4 (Bates *et al.* 2015) and plyr (Wickham 2011) for model fitting and ggplot2 (Wickham 2009) and cowplot (Wilke 2016) for plotting.

RESULTS

Cell size evolution

The difference in cell sizes evolved to be around 20-fold between small and large lineages after 300 generations of

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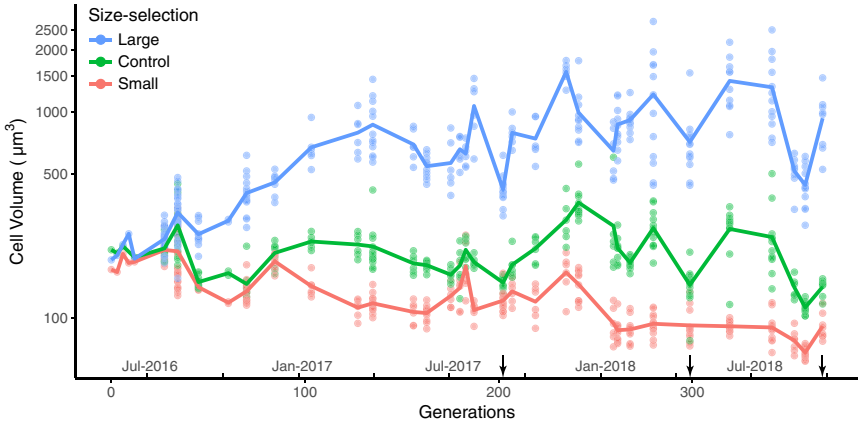


Figure 1 Effects of more than 2 years of artificial selection on the mean cell volume of the green microalga *Dunaliella tertiolecta*. Each point represents the mean of an independently size-selected culture. Lines indicate running means in cell size among lineages of each size-selection treatment. Arrows indicate when samples were collected and analysed for demographic rates and energy use.

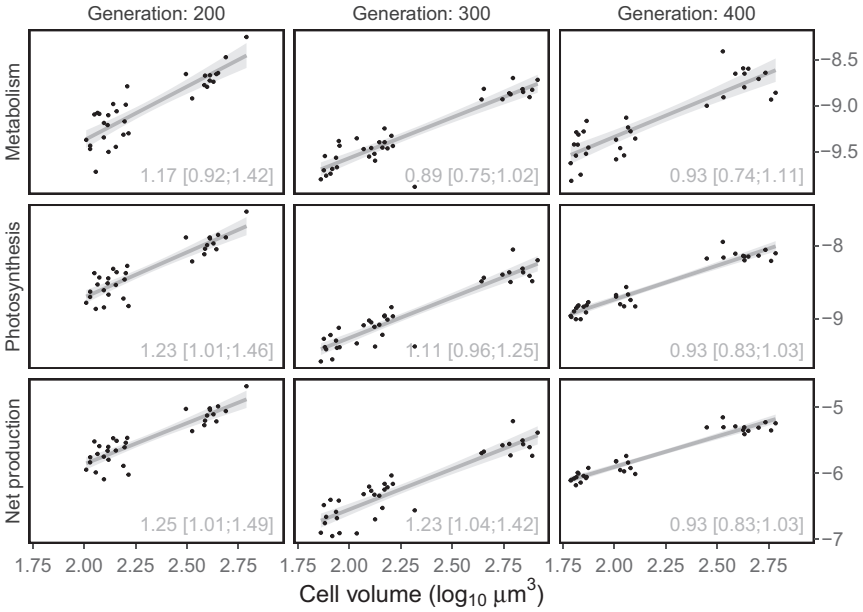


Figure 2 Observed allometric size-scaling for all ($\log_{10}$ transformed) per-cell energy rates measured after 200, 300 and 400 generations. Each dot represents the average state ($N = 3$) of an independently size-selected lineage. Lines (± 95% confidence intervals) indicate the fit of ordinary least-square linear models (see Fig. 4a for R^2 coefficients). Bottom right of each panel reports the mean and 95% confidence interval for each linear size-scaling exponent. Energy rates were recorded in the dark (i.e. metabolism; J cell^{-1} min^{-1}) and at saturating light (i.e. net-photosynthesis; J cell^{-1} min^{-1}). The daily net energy production was calculated by adding the energy produced during the day and removing energy used at night (assuming a 14–10 h day–night photocycle; J cell^{-1} day^{-1}). Scaling exponents for metabolic rates (i.e. respiration rates) were used to generate predictions on species demographic parameters (dashed lines in Fig. 3).

artificial selection. Beyond this point, size evolved very little despite maintaining the same selection regime, although occasionally still showing rapid evolutionary changes (Fig. 1).

Scaling of energy fluxes

Energy fluxes were relatively constant across 200 generations of size evolution, although they did show some variation (i.e. rates were 30–50% higher after 200 and 400 generations, compared to 300 generations; Fig. 2). Respiration rates scaled with size with an exponent of around 1 throughout the study and while it fluctuated, it was never statistically different from 1. Photosynthetic rates had consistently higher scaling exponents than respiration rates for generations 200 and 300 but were the same after 400 generations – again, it was not possible to statistically distinguish it from 1 throughout the experiment. The total daily net production of energy tended to scale with an exponent > 1 and was statistically > 1 for generations 200 and 300, but not after 400 generations. After 400 generations, the scaling exponent of all three energy parameters was the same: 0.93.

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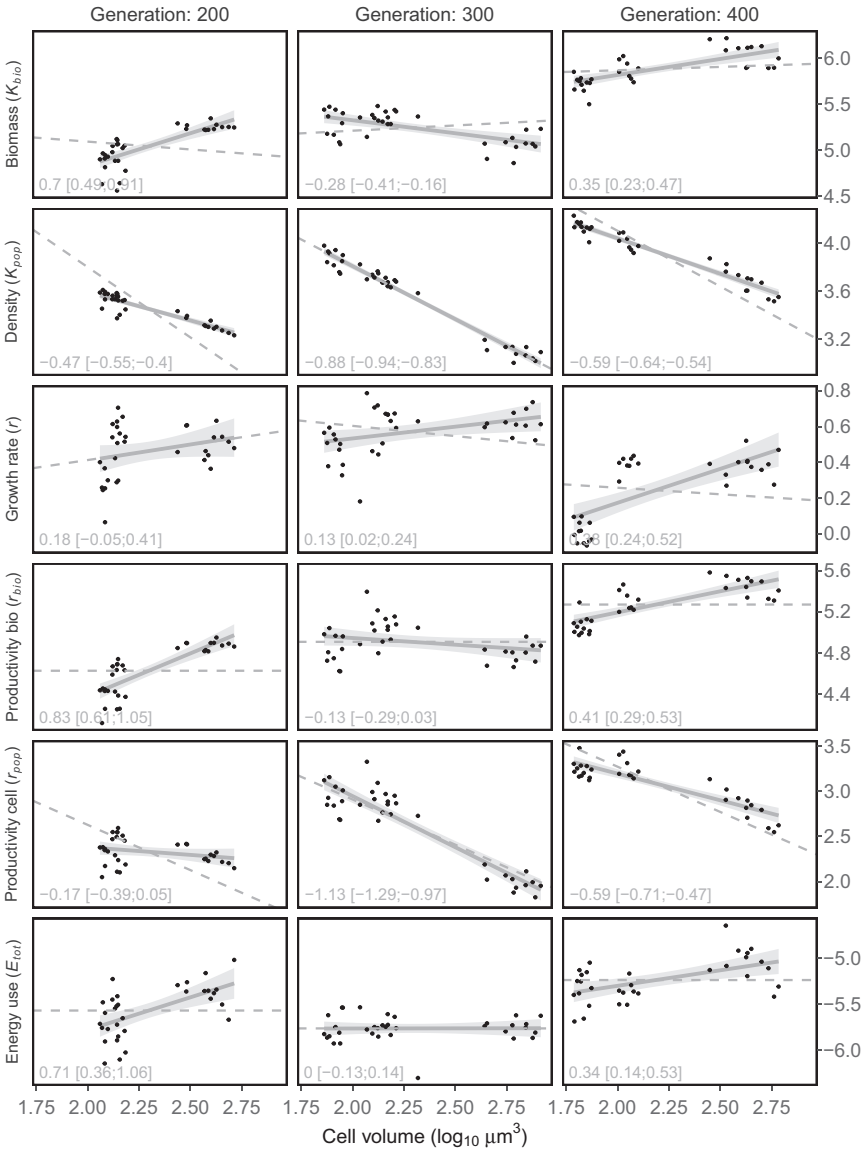


Figure 3 Observed and predicted allometric size-scaling relationships for all ($\log_{10}$ transformed) demographic parameters measured after 200, 300 and 400 generations of artificial size selection. Each dot represents the average state ($N = 3$) of an independently size-selected culture. Continuous lines (± 95% confidence intervals) show the fit of the ordinary least-square linear model to observations (see Fig. 4b for R^2 coefficients). Dashed lines show the predicted allometric relationship calculated based on respiration rates: the slopes were taken from Fig. 2 and equations in Table 1. To best allow comparison, the intercepts of the predicted lines were calculated to go through the centroid of the observed data. Bottom of each panel reports the mean and 95% confidence interval for each observed slope coefficient in the linear models.

Scaling of demographic rates

Demographic rates scaled with size but the strength and direction of this relationship fluctuated across 200 generations of size evolution (Fig. 3). Some demographic rates showed relatively consistent patterns – for example, the maximum intrinsic rate of population increase (r) scaled positively with size at around 0.13–0.38 from 200 to 400 generations. On the other hand, the carrying capacity of biomass (K_{bio}) went from a scaling relationship that was significantly positive to significantly negative and returned to positive across the 200 generations of evolution.

Predicted scaling relationships

We applied standard metabolic theory to generate energy-based expectations of how body size should scale with various demographic parameters at 200, 300 and 400 generations (Table 1). At every generation measured, at least some parameters matched theoretical predictions but none matched consistently. For example, the maximum intrinsic rate of population increase (r) initially matched predictions of a size scaling of 0.18, but later observations diverged substantially from predictions, both in direction and magnitude. Similarly, after 300 generations, the population density at carrying

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capacity (K_{pop}), the maximum productivity of new individuals (r_{pop}), the maximum biomass productivity (r_{bio}) and total energy use of the populations (E_{tot}) all matched theoretical predictions, but this congruence disappeared after 400 generations of size evolution.

Using the size scaling of photosynthesis (Table S1) or net daily energy production (Table S2) was equally (un)successful in predicting the scaling of demographic rates as using metabolic scaling (Table 1), but the parameters that were predicted successfully were different. Using the scaling of either photosynthesis or the rate of net energy production successfully predicted the scaling of maximum intrinsic rate of population increase (r) for generations 200 and 300, as well as the biomass at carrying capacity (K_{bio}) after 300 generations.

After 400 generations of size evolution, only one of the demographic scaling relationships was successfully predicted by energy, although only marginally within the realm of the predicted value (0.07 vs. 0.35). This is despite having more statistical power from accessing a larger size range after 400 generations relative to 200.

The observed demographic scaling exponents were the same or higher than the predicted exponent in 15 of 18 cases. In other words, the demographic benefits of increased size [e.g. higher growth rate (r) and biomass at carrying capacity (K_{bio})] were greater than expected and the demographic costs of increased size [e.g. lower population density at carrying capacity (K_{pop})] were less than expected.

Energy fluxes and demography are size dependent

Size consistently explained more than 75% of the variation in metabolic rate (i.e. respiration), photosynthesis and net daily energy production (Fig. 4). Instead, the capacity of size to explain variation in demographic rates varied greatly among parameters and also across generations. For example, size explained 80–95% of the variation in population density at carrying capacity (K_{pop}), but explained only 30–60% of the biomass at carrying capacity (K_{bio}). Interestingly, body size explained a substantial proportion (on average ~ 40% (and up to 80%) of the variability of demographic parameters that theory predicts should be unrelated to size, such as productivity (r_{bio}, r_{pop}) and total energy usage (E_{tot}; Fig. 4).

DISCUSSION

Using artificial selection, we showed that size, energy and demography did not scale within a species as predicted by metabolic theory (Isaac *et al.* 2012). Size has long been recognised to determine the rate at which organisms assimilate and use energy. Therefore, it is reasonable to expect size to drive population dynamics indirectly, and indeed interspecific patterns support this expectation. Yet, our study showed that these classic 'rules' regarding how body size and metabolism should covary with demography did not apply within a species. Instead, size covaried with demography in mostly unpredictable ways, changing strength and even sign across generations. Hence, how size influences demography in our model system is not fixed, but appears that these scaling relationships are evolutionarily labile.

Classic metabolic theory had limited success in predicting how size scales with demography. Even when a parameter showed the expected allometric scaling in one generation, that relationship changed in unexpected ways in other generations. Deviations from the size-scaling exponents of ecological traits among species have been often documented for both terrestrial (Russo *et al.* 2003; Russo *et al.* 2007; McGill 2008) and aquatic (Hayward *et al.* 2009; Reuman *et al.* 2009) ecosystems. Predictions from metabolic theory rely on simplifying assumptions that minimise the role of species-specific differences in ecology. For example, for carrying capacity to scale with size at an exponent that is inverse to that of its energy use, it requires assuming specific parameters to be size-invariant and the unbiased partitioning of resources among organisms of different size classes (Damuth 1991; Hayward *et al.* 2010). When demographic patterns among species differ, then species-specific differences in their ecologies are often invoked. Yet, we found similar deviations from expected patterns across evolved lineages even in the same species, suggesting

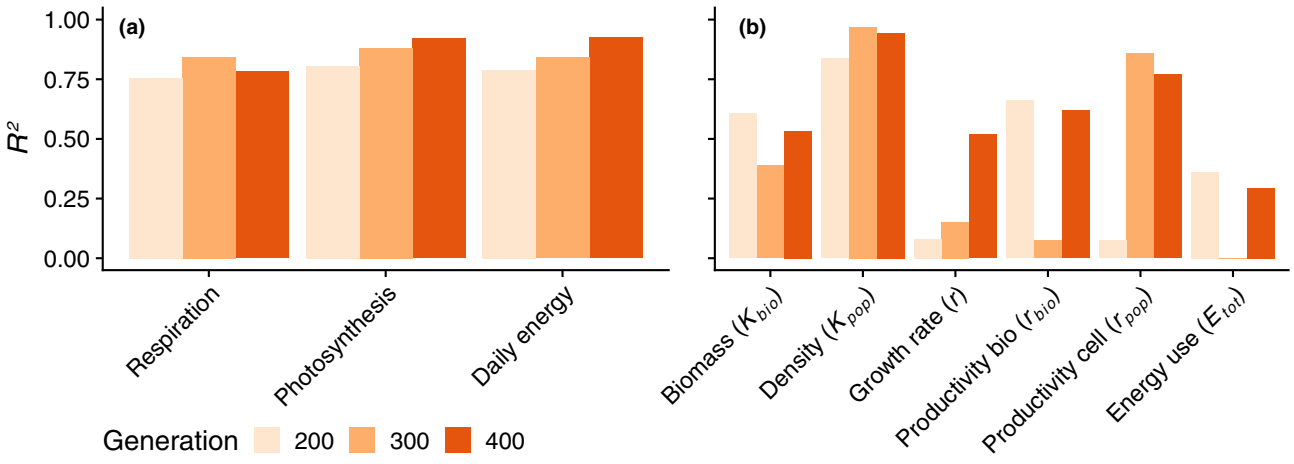


Figure 4 Coefficients of determination (R^2) of the linear models for the allometric size-scaling relationships of (a) energy rates and (b) demographic parameters. See Figs 2 and 3 for data and model fits of energy rates and demographic parameters respectively.

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that evolution can very quickly alter the relationship between size and demography independently of metabolism.

We suspect that coevolution between size and other important traits were responsible for the deviations from theory we observed. We found size-scaling slopes for carrying capacity (K_{pop} and K_{bio}), maximum intrinsic growth (r) and maximum productivity (r_{pop}, r_{bio}) that were generally higher than predictions based on energy use. This systematic deviance from expectations is consistent with large-selected cells having greater access to resources compared to smaller ones. Previous studies on our artificially selected lineages support this hypothesis. We found that the nutrient utilisation of our lineages coevolved with size, with large-evolved cells being able to assimilate and store more nutrients to supplement periods of resource limitation (Malerba *et al.* 2018b). Large-selected cells have also evolved 'supercharged' photosystems, modifying the organisation, composition and concentration of their photosynthetic pigments, with an overall higher energy production compared to small-selected cells (Malerba *et al.* 2018a). Specifically, large-selected cells had higher pigment concentration (+ 61% chlorophyll *a* and + 105% chlorophyll *b*) compared to small-selected cells, resulting in higher quantum yield and O_2 production (both per cell and per volume). Hence, coevolution between size and resource acquisition altered the relationship between metabolic rate and demography in ways that current theory struggles to explain.

The mechanistic explanation for why metabolism affects demography should apply at all scales of biological organisation – indeed this is a key part of the appeal of metabolic theories. Instead, we found that metabolism does not drive demography in the way theory predicts within species, even though our approach precludes potentially confounding factors such as differences in life history, which hamper among-species comparisons (Kozlowski & Weiner 1997; Glazier 2005). By considering not only metabolism but also photosynthesis and net energy use, we also tested more energy rates than earlier studies and still could not reconcile most predictions with observations. Moreover, we found that the predictive power of metabolic scaling relationships often degraded with evolutionary time – more parameters scaled the way they 'should' after 300 generations than after 400 generations. That evolution can alter size-scaling relationships within a relatively short period of evolutionary time suggests that metabolism alone is not a strong driver of the size–demography relationships observed among species. We do not suggest that metabolism is unimportant, instead we suggest that it does not mechanistically determine among-species patterns. Overall, our results indicate that among-species size–demography relationships are not driven directly by metabolic scaling, but other factors that covary with size and metabolism among species (e.g. population density, generation time, growth rate) may drive these effects.

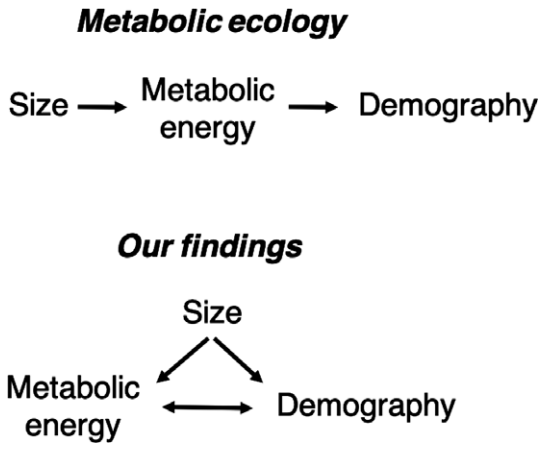


Figure 5 Schematic representation of the different theoretical underpinnings between the classic view and the interpretation of our findings. The classic view from metabolic theory holds that body size determines the rate at which organisms use energy, which in turn determines the demography of a population. We instead propose that both size and metabolism determine population dynamics.

We suggest a revision to the way the relationships between size, metabolism and demography are usually conceptualised. While classic theory did not predict the scaling of demography in our evolved lineages, size still strongly affected both metabolism (Fig. 2) and demography (Fig. 3). Thus, there are strong links between size, energy and demography, it is just that the link between energy and demography might be less direct than commonly thought. Figure 5 summarises our proposed reframing of how size, energy and demography relate to each other. Rather than metabolism driving the demography of a species directly – with size only affecting demography indirectly via metabolic rate – our results instead suggest that both size and metabolism determine population dynamics (Fig. 5). An important next step will be to determine whether size evolution in multicellular animals yields similar findings.

Our results extend the range of demographic parameters that are affected by body size. Size explained nontrivial levels of variation in demographic parameters that were expected by theory to be size independent (such as total energy usage and productivity rates). The size dependency of these parameters further suggests that size affects demography in ways that have not been anticipated. In particular, we were surprised to see that our lineages deviated from the 'Energy Equivalence rule' (Damuth 1981) at 200 and 400 generations. The total energy usage of populations at carrying capacity (E_{tot}) actually increased with size at these two time-points. This relationship emerged because our evolved lineages deviated from the predicted scaling of $M^{-\beta}$ for population density at carrying capacity (K_{pop}). As expected K_{pop} declined with size, but it did not decline as steeply as metabolic theory would predict, such that E_{tot} was not size independent. Somehow, populations of larger cells can generate and use more energy than predicted based on standard theory. If this covariance between size and demography also exists within species in nature, there are worrying consequences of current size declines on global change and carbon cycles.

At the interface between nonliving and living systems, phytoplankton cells are responsible for about half the carbon fixation on the planet and form the base of virtually all aquatic ecosystems (Field *et al.* 1998). However, various human activities are causing many phytoplankton species to decrease in size and future global warming conditions are predicted to

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further accelerate the shrinking of phytoplankton cells (Atkinson *et al*. 2003; Daufresne *et al*. 2009; Gardner *et al*. 2011; Forster *et al*. 2012). Theories based on among-species comparisons predict that smaller species should have greater intrinsic growth rates and population densities at carrying capacity than larger ones (Brown *et al*. 2004). Yet, current trends in body size reduction are acting within species – as is the case for our study – not across species. Assuming our laboratory cultures are representative of natural phytoplankton communities, we would instead predict that the maximum intrinsic rate of population increase (r), maximum biomass productivity (r_{bio}) and maximum biomass at carrying capacity (K_{bio}) all decrease with decreasing cell size, reducing the capacity of phytoplankton to fix carbon and support food chains. It seems that among-species patterns are insufficiently informative regarding within-species processes and we encourage more studies (e.g. Bernhardt *et al*. 2018) that seek to understand the consequences of size changes within species for population dynamics.

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AUTHORSHIP

All authors contributed to designing the study. MEM. conducted the experiment, collected the data and carried out statistical analyses. MEM. and DJM. wrote the initial draft of the manuscript. Both authors gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.g11gs40.

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