Supplementary Materials

Title: DO LARGER INDIVIDUALS COPE WITH RESOURCE FLUCTUATIONS BETTER? AN ARTIFICIAL SELECTION APPROACH

Authors: Martino E. Malerba, Maria M. Palacios, Dustin J. Marshall

Table S1: Anova table with numerator (numDF) and denominator (denDF) degrees of freedom, F-values and P-values (Wald tests) of best-fitting models (following AIC) for maximum specific growth rate (r_{max}) and the maximum total biovolume reached (K) of the cultures. Main effects are the size-selection treatment during artificial selection (Size), the nutrient regime of the culture before starting trials (i.e. nutrient–deplete or nutrient–replete; N-hist), the Size:N-hist interaction, and the initial biovolume density of the culture (ID). The lineage identity was included in all models as a random effect. A random effect with the position of the sample on the well plate was removed from the final model because selected against by AIC model selection.

Maximum growth rate (r_{max})

	numDF	*denDF*	*F-value*	*p-value*
Size	3	27	2203.8	<.0001
N-hist	2	54	231.57	<.0001
ID	1	54	169.20	<.0001
Size:N-hist	4	54	3.1981	0.0198

Maximum total biovolume reached (K)

Size	3	27	140666	<.0001
N-hist	2	54	54.14	<.0001
ID	1	54	20.82	<.0001
Size:N-hist	4	54	5.25	0.0012

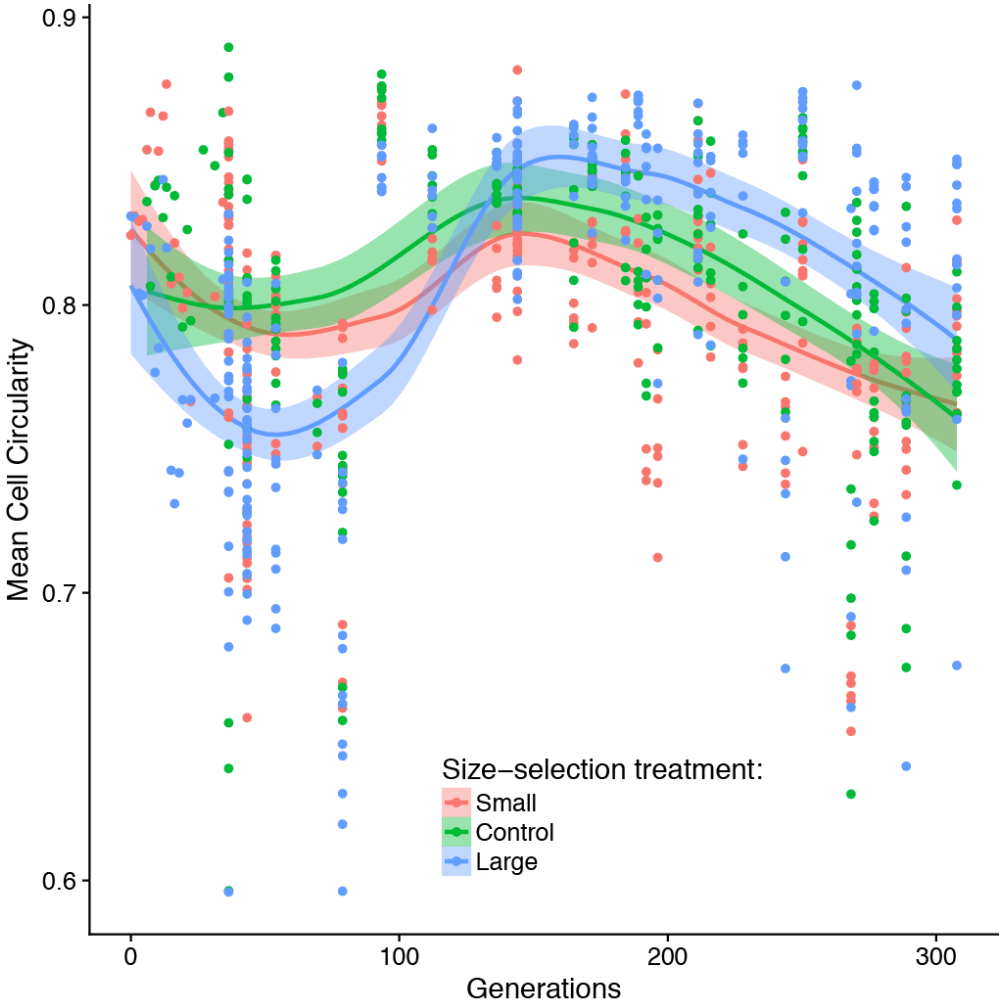


Fig. S1 Trends in cell circularity among lineages that were artificially selected for 300 generations. Each point represents the mean cell circularity of a lineage. Colours represent the size-selection treatment. Lines represent loess smoothers (±95% confidence intervals). Circularity was calculated as $4\pi \times \frac{Area}{Perimeter^2}$, with a value of 1 indicating a perfect circle.

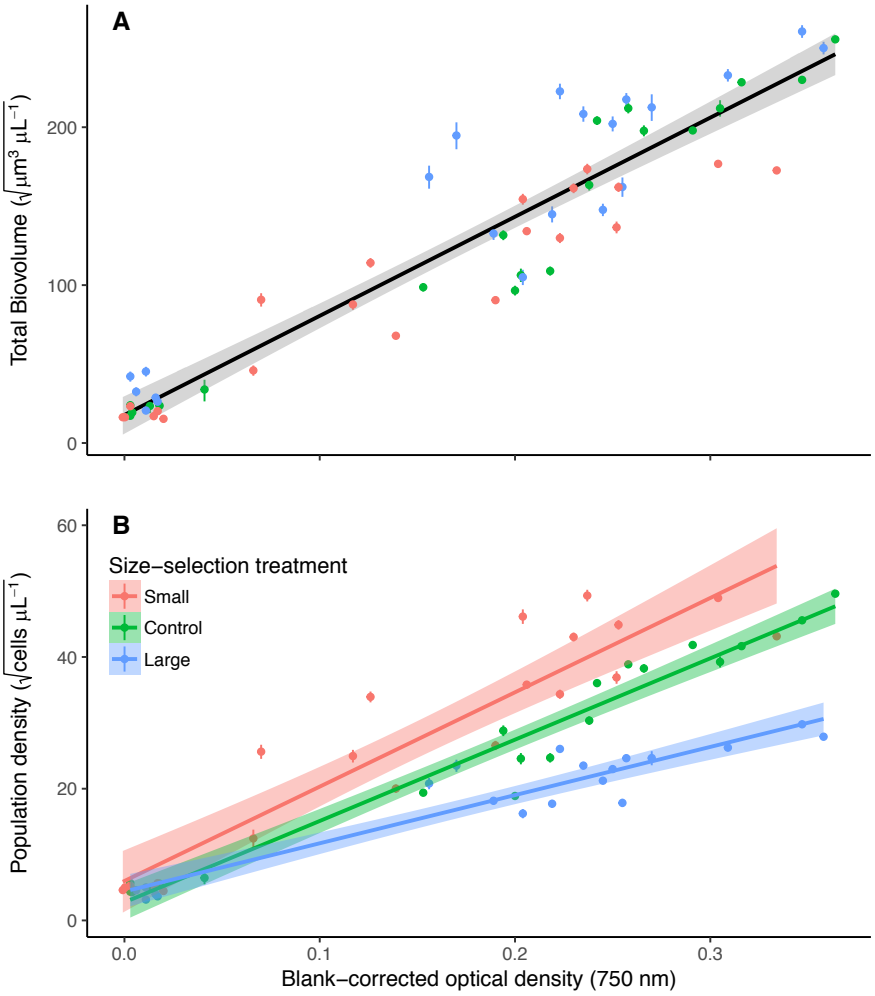
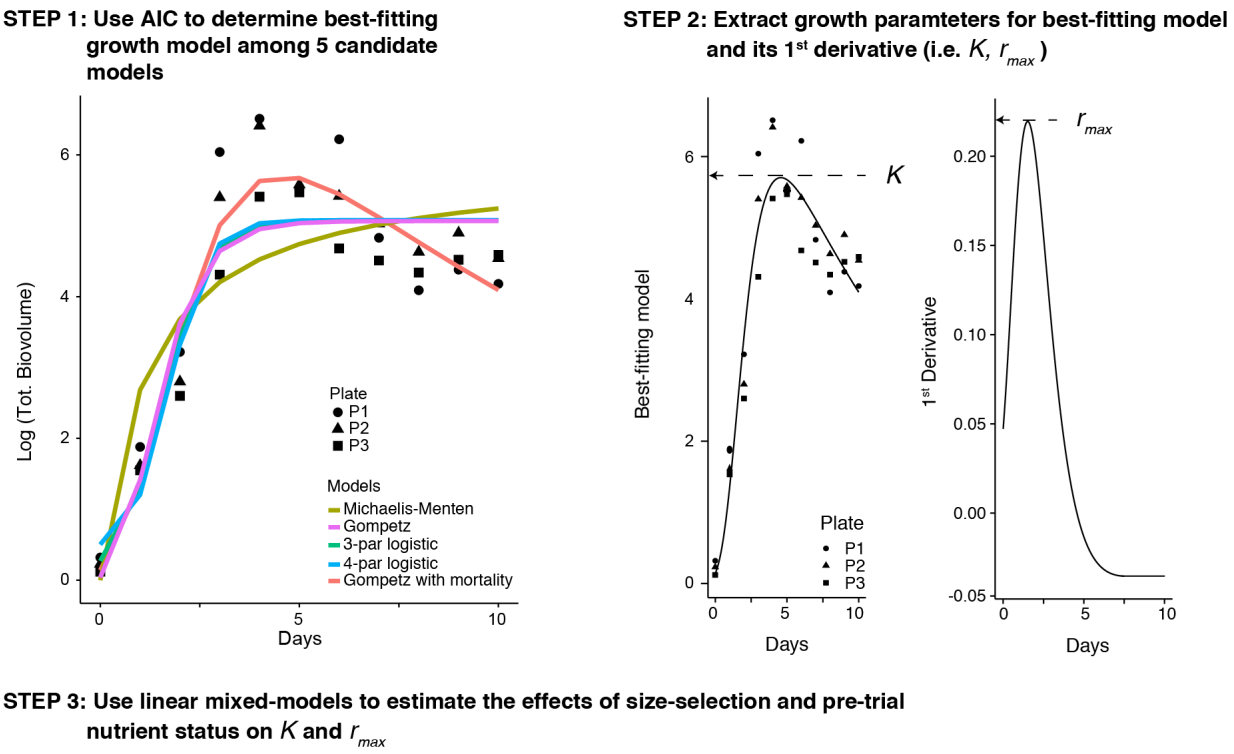


Fig. S2 Calibration curves for each size-selected treatment with blank-corrected optical density (OD). (A) Total biovolume, calculated by multiplying mean cell density by mean cell volume for each size-selected lineage (R^2 = 0.89, $F_{1,60}$ = 468.5, p < 0.001). Model: Total biovolume = (17.77 + 627.61 OD)^2. (B) Population density (R^2 = 0.91, $F_{5,56}$ = 126.9, p < 0.001). Models: Small cell density = (5.99 + 143.25 OD)^2; Control cell density = (2.75 + 123.49 OD)^2; Large cell density = (4.39 + 73.17 OD)^2.



STEP 3: Use linear mixed-models to estimate the effects of size-selection and pre-trial nutrient status on K and r_{max}

Fig. S3 Graphical explanation of the analytical methods. **STEP 1**: For each sample at all combinations of size-selection and pre-trial nutrient history, five different growth models were fitted to the combined blank-corrected optical density of three replicate time-series grown on independent well plates. The performance of each model was compared using Akaike Information Criteria (AIC). **STEP 2**: For the best-fitting model, the maximum biovolume reached (K) was estimated as the maximum value of the model curve. We also calculated the max. rate of change (r_{max}) from the first derivative of the model curve. **STEP 3**: The effects of the experimental treatments (i.e. size and pre-trial nutrient history) on each population growth parameters were estimated using linear mixed models, including the lineage identity as random effect.

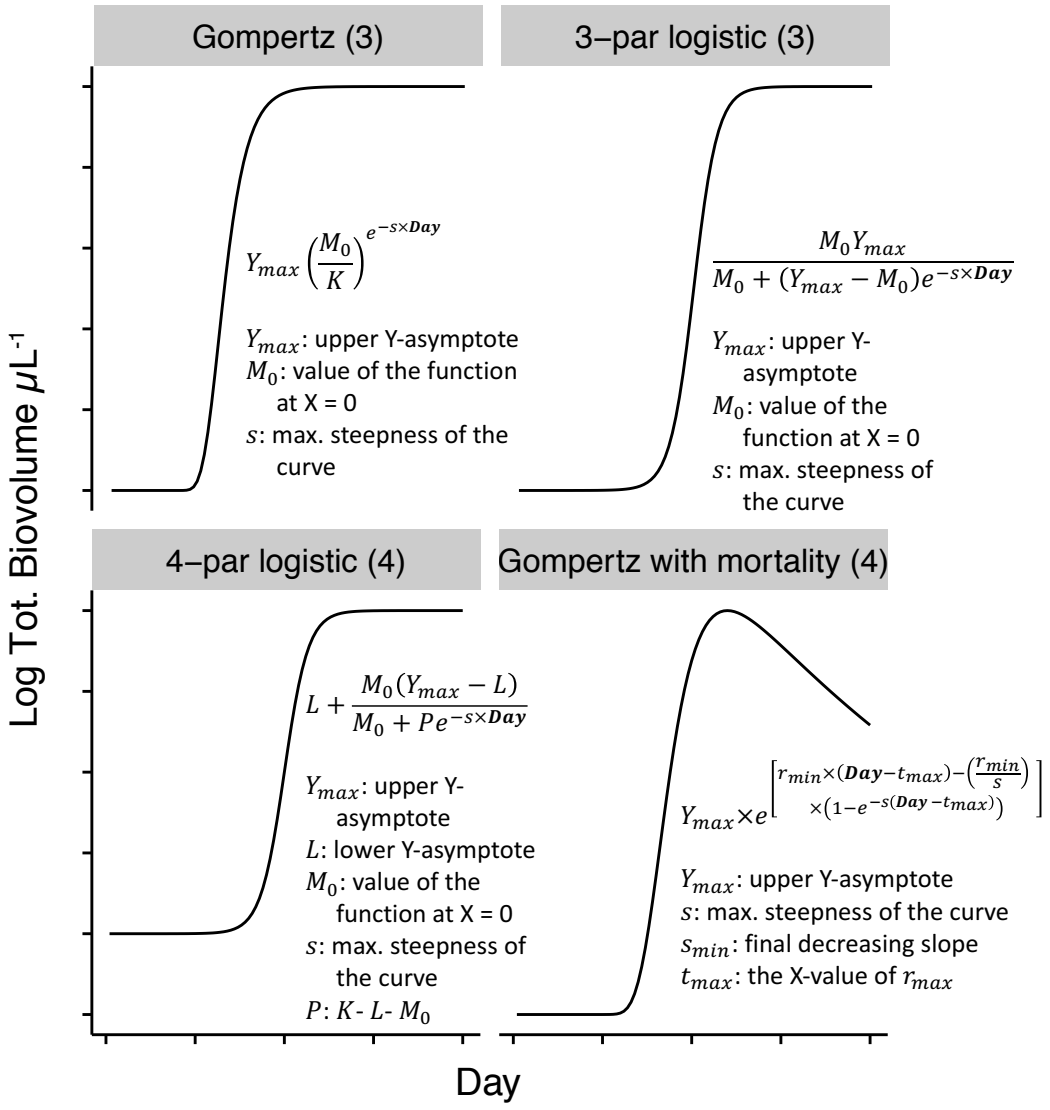


Fig. S4 Shapes and model structures for the four candidate growth models used to fit observations of total biovolume (log µm^3 µL^{-1}) over time. The number of free parameters is indicated in brackets. *Day* (in bold) is the numeric vector of the x-coordinates at which to evaluate the model. All models include parameters quantifying the maximum steepness (*s*) and the upper Y-asymptote (Y_{max}) of the curve. The *Gompertz* model is characterized by a relative growth rate declining exponentially over time and shows an inflection point at around 37% of Y_{max}. The *3-parameter logistic* model also has an exponentially declining relative growth rate but its inflection point occurs at 50% of Y_{max}. The *4-parameter logistic* model is a generalisation of

the *3-parameter logistic* allowing a non-zero lower asymptote for the initial total biovolume (i.e. M_0). Finally, the *Gompertz with mortality* model allows extending the normal *Gompertz* model to include a rate of decline (s_{min}) after reaching Y_{max}. For more details on the *Gompertz with mortality* model, see Werker and Jaggard (1997). For the other four models, see Paine et al. (2012). Also, refer to Fig. S3 for a definition of the specific shape descriptors used to quantify the shapes of the curves across all models. The Michaelis-Menten model was initially tried as a candidate model, but was never favoured by AIC model selection and was therefore removed.

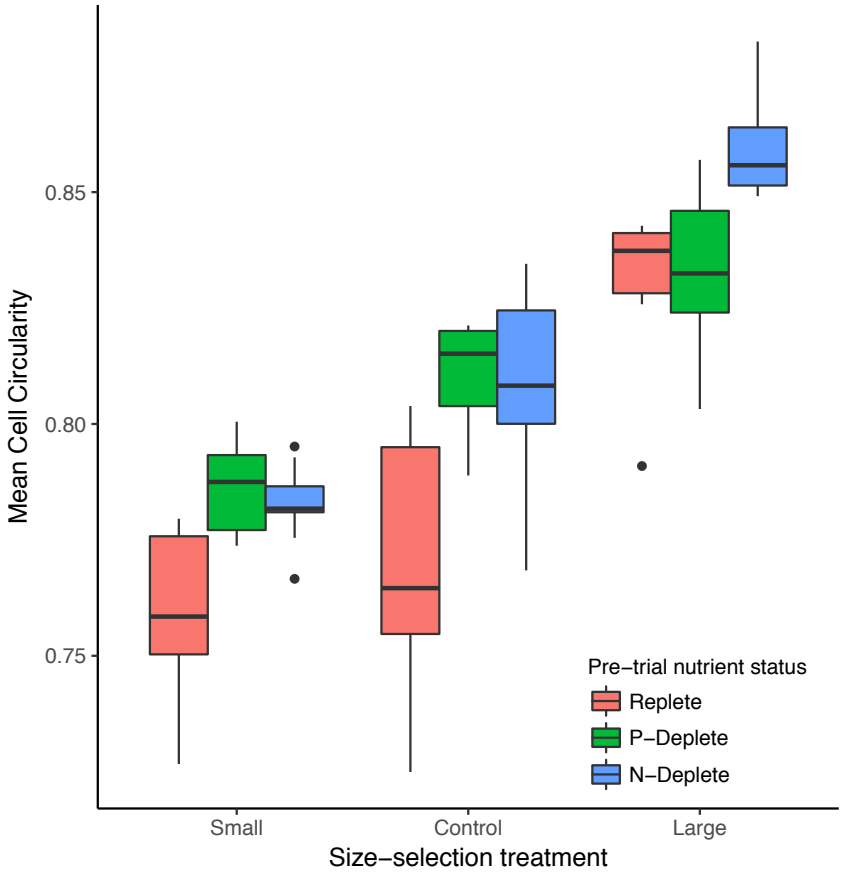


Fig. S5. Mean cell circularity in each experimental lineage as a function of size-selection treatment and pre-trial nutrient status. Large-selected cells were consistently rounder than control and small-selected cells ($F_{2,27} = 119.42$, $p < 0.001$). Circularity was calculated as $4\pi \times \frac{Area}{Perimeter^2}$, with a value of 1 indicating a perfect circle.

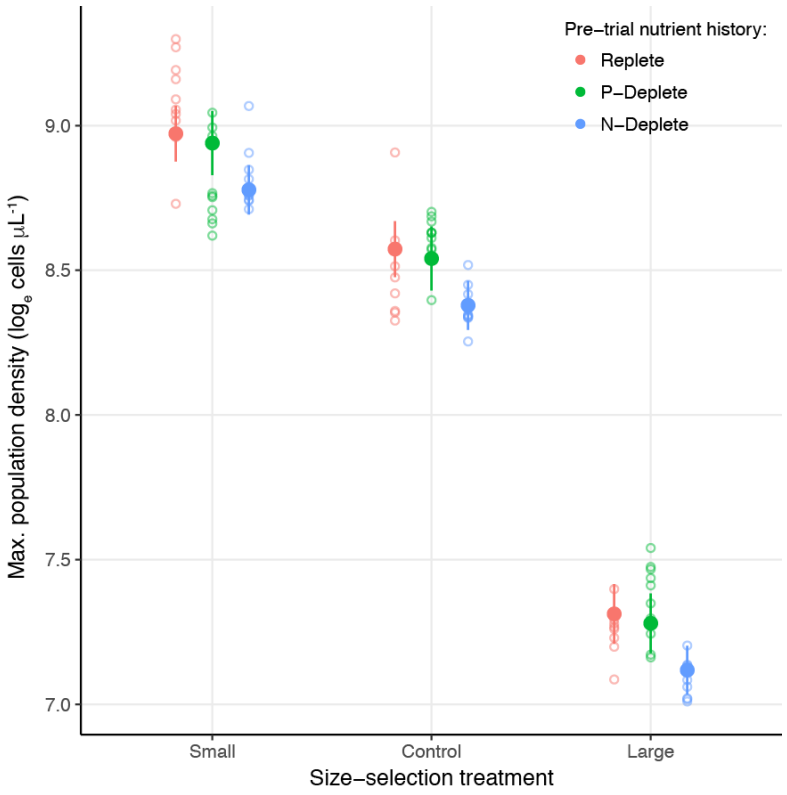


Fig. S6 Maximum biovolume reached (*K*) converted into units of population density, as a function of size-selection treatment and pre-trial nutrient status (see Fig. 3B for *K* in units of total biovolume). When converting *K* from units of total biovolume (µm^3 µL^{-1}) into units of total population density (cells µL^{-1}), small-selected and control cells reached much higher total population densities than large-selected cells. This was mostly a consequence of substantial differences in cell volume among size-selection treatments – few larger cells have equal total biovolume than many smaller cells (see Fig S2).

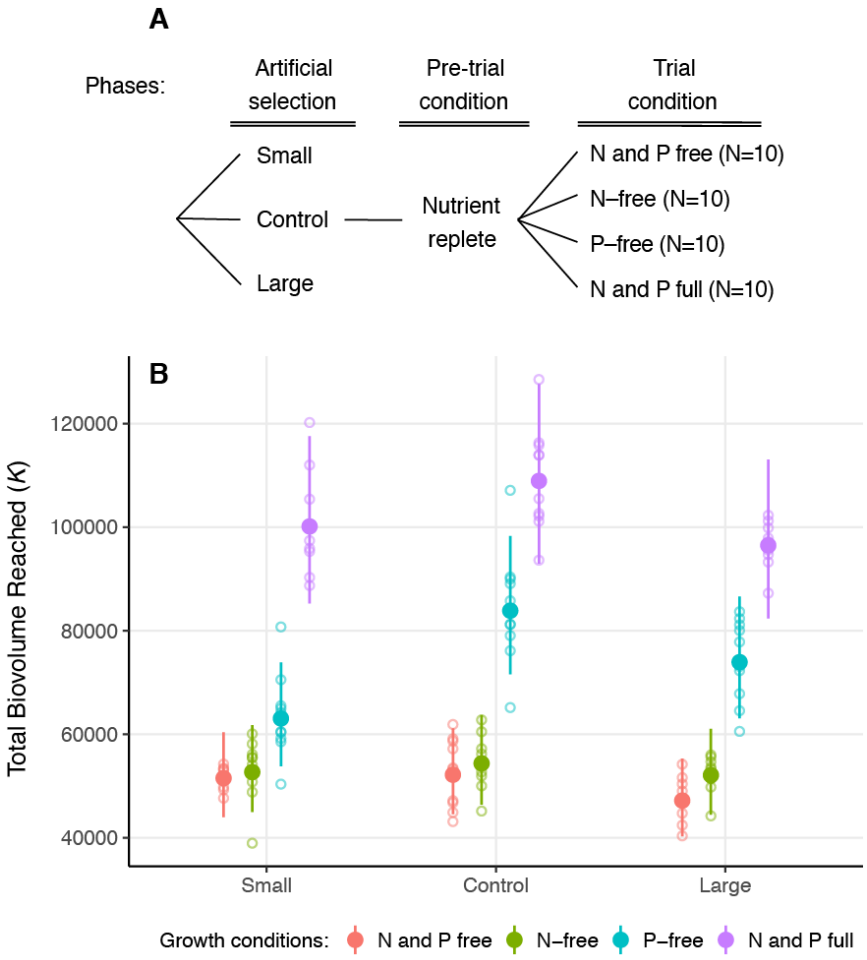


Fig. S7 (A) To further explore the effects of size on the ability of a cell to tolerate periods of nutrient depletion, 10 evolved lineages per size-selection treatment were sampled at nutrient-replete conditions during pre-trial, standardized to the same total biovolume, and resuspended in modified F/2 media that were either without nitrogen and phosphorous (red points), only without nitrogen (green points) or without phosphorous (light blue points), or with both nitrogen and phosphorous (purple points). (B) We used the techniques explained in Fig. S3 to estimate the total biovolume reached at the end of trials. Results showed a significant interaction between size-selection treatment and growth conditions ($F_{6,80} = 3.61$, $p = 0.0032$). Specifically, smaller cells could reach equivalent biomass in N- and P-free environments (overlapping green and light

blue points). Conversely, control and larger cells could reach greater biomass in P-free compared to N-free environments (light blue points higher than green points).