

LETTER

Carrying capacity in a heterogeneous environment with habitat connectivity

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Abstract

A large body of theory predicts that populations diffusing in heterogeneous environments reach higher total size than if non-diffusing, and, paradoxically, higher size than in a corresponding homogeneous environment. However, this theory and its assumptions have not been rigorously tested. Here, we extended previous theory to include exploitable resources, proving qualitatively novel results, which we tested experimentally using spatially diffusing laboratory populations of yeast. Consistent with previous theory, we predicted and experimentally observed that spatial diffusion increased total equilibrium population abundance in heterogeneous environments, with the effect size depending on the relationship between r and K . Refuting previous theory, however, we discovered that homogeneously distributed resources support higher total carrying capacity than heterogeneously distributed resources, even with species diffusion. Our results provide rigorous experimental tests of new and old theory, demonstrating how the traditional notion of carrying capacity is ambiguous for populations diffusing in spatially heterogeneous environments.

Keywords

Carrying capacity, consumer–resource model, dispersal experiment, environmental stressor, heterogeneous resource distribution, r - K relationship, spatially distributed population.

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INTRODUCTION

The steady-state upper limit on a population's abundance, often called its carrying capacity, is a key quantity in theoretical and applied ecology (Vasconcellos & Gasalla 2001; Goss-Custard *et al.* 2003; Hayward *et al.* 2007). Its understanding is essential for the design of strategies for management of threatened and invasive species (Pimm & Raven 2000; Pimm *et al.* 2006; Aviron *et al.* 2007). Carrying capacity, for a small uniform patch in space, is considered to be set by a limiting resource, such as energy, light or nutrients. However, most species in nature are distributed over large environmental space (*sensu* Reiners & Driese (2001)) with a heterogeneous (non-uniform) distribution of resources. This is true, for instance, for species whose habitats have been fragmented by human activity (Cosson *et al.* 1999; Fahrig 2003; Marenzi & Gerhardinger 2006; Di Giulio *et al.* 2009). For spatially distributed species, the determinants of total realised asymptotic population abundance (Arditi *et al.* 2015) (abbreviated TRAPA here for convenience) are, surprisingly, more complex than simply the sum of the local carrying capacities over every point in space.

Indeed, mathematical theory predicts that spatial diffusion, a process caused by the undirected local movement of individuals in space, which often provides a very good mathematical description of population movement (Skellam 1951; Okubo

1980; Hastings 1983; Kareiva 1983), has a remarkable effect on TRAPA (see Supporting Information, Appendix A for detailed overview of previous theory). Namely, if resources are heterogeneously distributed over space, then spatial diffusion of individuals can increase the equilibrium total abundance of the global population (Fig. 1). That is, under certain conditions, the TRAPA of a spatially distributed population with diffusion is mathematically predicted to be greater than the sum of local carrying capacities over every point in space. Spatial diffusion increases the number of individuals in the global population. This effect was shown by Holt (1985) for a two-patch model with Pearl–Verhulst logistic population growth, $dN/dt = rN(1 - N/K)$ (Verhulst 1838; Pearl & Reed 1920). If growth in each patch has the same form of density regulation, then when the r 's and K 's (maximum per capita growth rates and carrying capacities, respectively) differ for the two patches (i and j) and $K_i > K_j$ and $r_i/K_i > r_j/K_j$, the total population at equilibrium can exceed the sum of the carrying capacities of the two patches; that is, $N_i^* + N_j^* > K_i + K_j$, in the limit of a very high diffusion rate between the two patches. Holt (1985) showed that this result holds even with source–sink populations, where one patch (the sink) is not self-sustaining ($r_j \leq 0$ and $K_j = 0$). These surprising results were called 'somewhat paradoxical' by Arditi *et al.* (2015). Lou (2006) extended these results to a continuous spatial setting by using a reaction–diffusion model, and

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again arrived at the same paradoxical conclusion (see also DeAngelis *et al.* (2016)).

Most puzzling, these mathematical results imply that if the local carrying capacities are distributed over space in a heterogeneous manner, the TRAPA of a diffusing population can exceed that attained by the population in a homogeneous environment with the same total sum of local carrying capacities (see Supporting Information Appendix A). Put differently, with diffusion, a heterogeneous environment is predicted to hold more individuals than a homogeneous one.

In general, these results show that TRAPA with diffusion is not the same as the summed local carrying capacities, and that, in certain cases, diffusion increases population abundance. Importantly, though, Arditi *et al.* (2015) and DeAngelis *et al.* (2016) proved using the Pearl–Verhulst model that diffusion increases TRAPA only if there exists a positive relationship between the phenomenological logistic parameters r and K (see Mallet (2012) for review of evidence for a positive r - K relationship). With a negative r - K relationship, diffusion is mathematically predicted to decrease the TRAPA (Arditi *et al.* 2015). This requirement can be understood as follows (see also Fig. 1, Supporting Information and Arditi *et al.* 2015; DeAngelis *et al.* 2016): with a positive r - K correlation, highly productive patches (high K) are able to compensate for much of the emigration (high r). The population flux to low productivity patches (low r and K) exceeds their losses to emigration. Thus, with a positive r - K correlation, diffusion can “overfill” low productivity patches while not appreciably depleting high productivity patches, thus leading to higher TRAPA. With a negative r - K relationship, high K patches

have low r , and fail to recover as much from losses due to diffusion.

Despite the broad implications of these theoretical predictions, rigorous empirical tests are lacking. Previous research with duckweed (*Lemnoideae*), which manipulated diffusion between adjacent patches of a linear five-patch system with different initial nutrient levels, provides some corroboration for the effect of diffusion on TRAPA (Zhang *et al.* 2015). While this work provided important insights, there are a number of areas in need of refinement. For example, this work did not directly measure the r - K relationship, or was this relationship experimentally manipulated to test the theoretical prediction that a positive r - K correlation is necessary for the predicted effect. Furthermore, precise control of nutrient levels, small patch number and low throughput proved to be limitations in this work. More generally, testing these mathematical predictions in the field or laboratory is complicated by the fact that real populations are usually limited by resources that are exploitable and renewed. The classic logistic model used in previous theory is phenomenological rather than mechanistic, and its population parameters (e.g. K and r) are assumed fixed, such that they are not influenced by feedback from exploitation by consumers. In reality, these parameters emerge from the interaction of consumers with exploited renewable resources (Tilman 1982), necessitating modification of previous theory.

Therefore, our objectives here are twofold. First, we analyse a mechanistic consumer–resource model to determine analytically whether it produces the same results as those described above for the phenomenological logistic model. Second, we

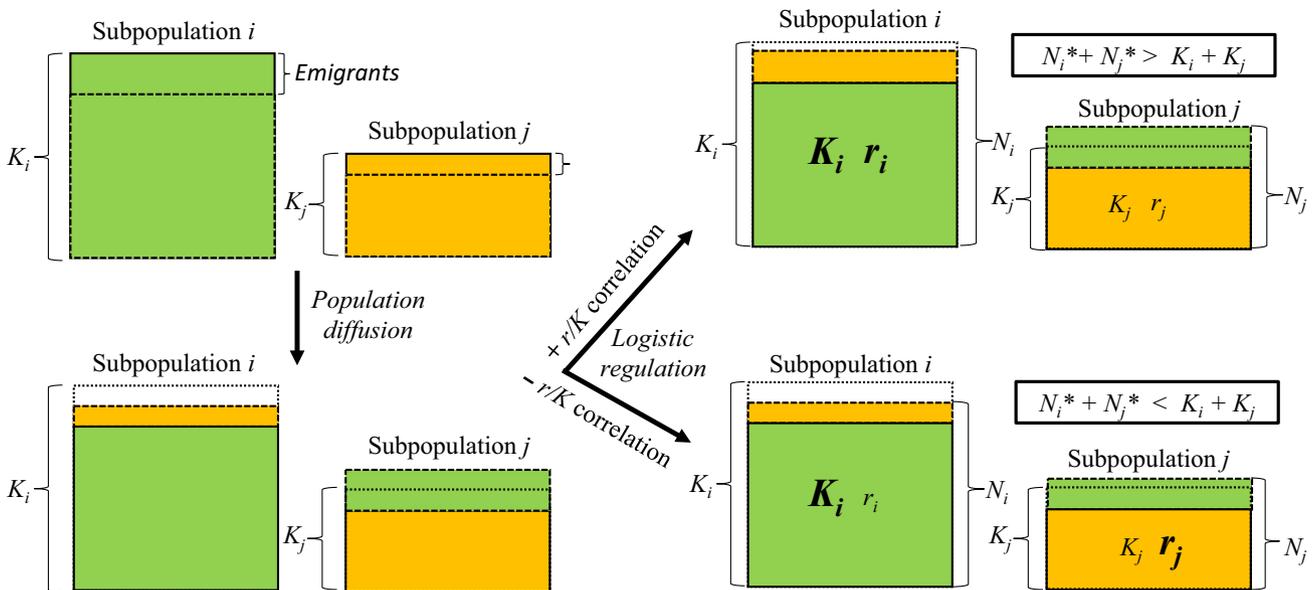


Figure 1 A schematic illustrating the possible total realised population abundance (TRAPA) of a diffusing population described by a Pearl–Verhulst logistic model, $\frac{dN}{dt} = r(1 - \frac{N}{K})N$, where the r 's and K 's (maximum per capita growth rates and carrying capacities) differ for the two patches and $K_j > K_i$. A fraction of each subpopulation is exchanged at each time step, leaving the i th subpopulation below, and j th subpopulation above, its respective carrying capacity. How each subpopulation responds to the flux of individuals depends on the r - K relationship. If positive, then subpopulation i rebounds quickly to diffusive losses and subpopulation j remains “overfilled”, such that $N_i^* + N_j^* > K_i + K_j$. If negative, then subpopulation i does not rebound and subpopulation j declines towards its carrying capacity faster because of relatively high r , with the net result that TRAPA is below the sum of local carrying capacities, $N_i^* + N_j^* < K_i + K_j$. Note that this schematic is meant merely as a guide to intuition. See Supporting Information Appendix for mathematical demonstration.

test theoretical predictions using high-throughput experimental methods in spatially diffusing laboratory populations of the heterotrophic budding yeast, *Saccharomyces cerevisiae*, limited by a single essential nutrient. In these experiments, we manipulate diffusion rate, resource distribution and the r - K correlation. We test mathematically and experimentally three hypotheses suggested by the earlier mathematical results based on the phenomenological logistic model. Hypothesis 1: when a consumer exists in a region with a heterogeneously distributed input of exploitable renewed limiting resource, the TRAPA can reach a greater abundance when it diffuses than when it does not. Hypothesis 2: the higher TRAPA in a heterogeneous environment with diffusion is associated with a positive relationship of growth rate and carrying capacity (e.g. r and K). Hypothesis 3: a consumer population diffusing in an environmental space with a heterogeneously distributed input of exploitable renewed limiting resource can reach a greater TRAPA than a population diffusing (or not) in a space with the same total input of resources distributed homogeneously.

MATERIALS AND METHODS

Mathematical model

We first propose a mechanistic model describing a diffusing population of consumers in an environment with a heterogeneously distributed limiting resource (a nutrient) that is exploited by the consumer and externally renewed. A general pair of equations for the consumer–resource system (yeast–tryptophan in our experiments; see Experimental Methods) is as follows:

$$\frac{\partial u(x, t)}{\partial t} = D \frac{\partial^2 u(x, t)}{\partial x^2} + \frac{r_{\max} n(x, t) u(x, t)}{k + n(x, t)} - m(x) u(x, t) - g(x) u(x, t)^2 \quad (1a)$$

$$\frac{dn(x, t)}{dt} = N_{\text{input}}(x) - \eta n(x, t) - \frac{r_{\max} n(x, t) u(x, t)}{\gamma(k + n(x, t))} \quad (1b)$$

where $u(x, t)$ is the consumer population abundance, $n(x, t)$ is the nutrient concentration, D is the diffusion rate, r_{\max} is the asymptotic growth rate under infinite resources, k is the half-saturation coefficient, defined as the nutrient concentration where $r = r_{\max}/2$, $m(x)$ is the mortality rate, $g(x)$ is the density-dependent loss rate, $N_{\text{input}}(x)$ is the nutrient input, η is the loss rate of nutrient from the system and γ is the yield, as individuals per unit nutrient.

For convenience, two special cases of this model were analysed, which are intended to provide plausible representations of our experimental nutrient–yeast dynamics:

Model 1 : $\eta = 0, m(x) = 0, g(x) > 0$ for all x

Model 2 : $\eta = 1, m(x) > 0, g(x) = 0$ for all x .

Model 1 assumes that nearly all of the nutrients are taken up by the yeast and that dead yeast cells are assumed to accumulate at a rate $g(x)u(x, t)^2$. The yeast cells are not

physically lost, but they stop reproducing, with no recycling of nutrients. Model 2 is a chemostat type model.

We test these models mathematically in the limit that $D \rightarrow \infty$, in which the consumer population is well mixed on the environmental space, and for D close to zero, and use computer simulations of the model to test the analytic results.

Four situations are assumed for which we calculate the TRAPA: (1) Heterogeneous distribution of nutrient inputs with no diffusion of the population, (2) heterogeneous distribution of nutrient inputs with diffusion, (3) homogeneous distribution of the same total nutrient inputs with no diffusion and (4) homogeneous distribution of the same nutrient inputs with diffusion.

Simulations of discretised model

We augment our analytical and experimental investigations with simulations. We simulate a one-dimensional discrete-space, or “patch”, version of equations (1a,b). These simulations are intended to mirror our experimental design (Experimental Methods), in which a yeast population is spatially distributed over 12 subpopulations, linearly arrayed and linked by nearest-neighbour diffusion (Fig. 2). The model for $n = 12$ patches is as follows:

$$\frac{dU_i}{dt} = \frac{r_{\max} N_i}{k + N_i} - m_i U_i - g_i U_i^2 - D \left(U_i - \frac{1}{2} U_{i-1} - \frac{1}{2} U_{i+1} \right) \quad (2a)$$

$(i = 1, 12)$

$$\frac{dN_i}{dt} = N_{\text{input},i} - \eta N_i - \frac{r_{\max} N_i U_i}{\gamma(k + N_i)} \quad (i = 1, 12) \quad (2b)$$

where no diffusion is assumed between the two end patches (1 and 12). The analysis is performed assuming continuous-time dynamics, whereas in the experiment, diffusing events are performed at discrete time intervals. In Supporting Information, Appendix B, we show with simulations that the continuous-time model is a good approximation to the discrete-time experiment.

Experimental methods

To test these hypotheses and to validate our spatial consumer–resource model, we conducted laboratory experiments in spatially distributed, single-strain populations of budding yeast, where yeast serve as the consumer and the amino acid tryptophan as the exploited, renewable resource.

Yeast strains

We used an auxotrophic, haploid (*mat-a*) strain of the budding yeast, *Saccharomyces cerevisiae* (*yJDV111*), provided as a generous gift from M. Mueller and A. Murray (Harvard University) (Muller *et al.* 2014). Briefly, this strain was constructed in a prototrophic W303 background by replacement of the tryptophan biosynthesis pathway gene *TRP2* with an antibiotic resistance cassette, *KANMX*. This strain can synthesise all amino acids except tryptophan (throughout denoted as “Trp”), and therefore it requires growth medium

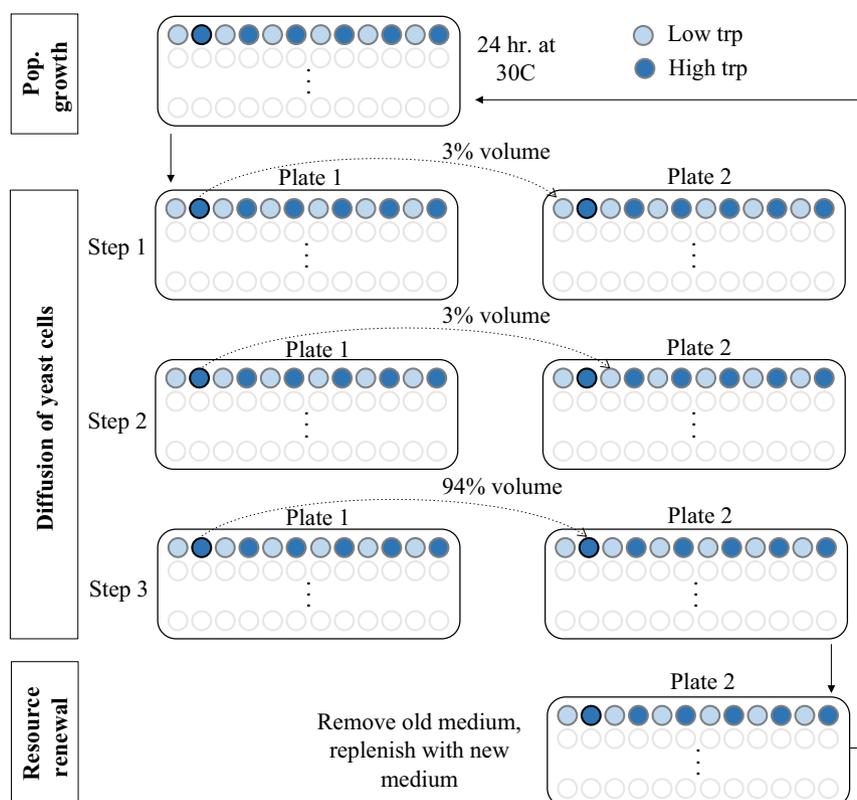


Figure 2 Schematic of experimental diffusion protocol. Represented is a single spatially distributed “population” composed of one row of 12 wells in a 96-well microtitre plate. Each circle is a single well. Colour of a well represents nutrient (Trp) concentration. The initial yeast population had 24 h growth at 30 °C, followed by a diffusion event from the original plate (plate 1) to a new empty plate (plate 2), in which 3% volume in each well was transferred to the well on the left in plate 2 (step 1) and another 3% to the right well of plate 2 (step 2). The remaining volume was transferred to the same well in plate 2 (step 3). After the diffusion and transfer, plate 2 was centrifuged to create a yeast pellet at the bottom in each well, old media were removed and fresh media were added (step 4). The yeast population underwent another 24 h growth, and the previous processes were repeated, up until Day 9.

supplemented with Trp for population growth. In all experiments, cells were propagated vegetatively (i.e. no sexual reproduction).

Culture medium

All growth medium consisted of 0.74 g L⁻¹ of Complete Synthetic Medium minus Trp (CSM -Trp) (Sunrise Science) and 20 g L⁻¹ dextrose. This base medium was then supplemented with different concentrations of Trp in order to manipulate the supply of the limiting resource in the growth media. In order to manipulate growth parameters independently of the resource level, we applied varying sublethal doses of the macrolide eukaryotic antibiotic cycloheximide (throughout denoted as Cyh) to the growth media. Cyh is a translation inhibitor that interferes with translation elongation by binding ribosomes, thus retarding *r*. We reasoned that, for a given Trp level, Cyh would slow *r* but have a smaller relative effect on *K*, which is set by the resource supply. Thus, Cyh would provide a means to experimentally manipulate the relationship between *r* and *K*.

Spatially distributed populations and diffusion

A single “population” was composed of a single row of 12 wells (“subpopulations”) in a 96-well microtitre plate (Fig. 2). Thus, populations were spatially distributed over a

1-dimensional environmental space of 12 subpopulations potentially linked by nearest-neighbour diffusion. Importantly, our experiments were designed such that consumers (yeast cells) diffuse between wells, but nutrients do not. Each 12-well population had either a heterogeneous or homogeneous distribution of resources (Trp). A heterogeneous environment was designed by alternating Trp concentrations between 0.7 mg L⁻¹ (“low” nutrients) and 44.0 mg L⁻¹ (“high” nutrients). A homogeneous environment was designed as having a uniform amount of Trp of 22.2 mg L⁻¹ (“medium” nutrients) in each of the 12 wells, which is within half a per cent of the average of high and low nutrients. In each of the heterogeneous and homogeneous environments, we further had four concentrations of Cyh: 0 nM, 50 nM (“low”), 200 nM (“medium”) and 400 nM (“high”). Each treatment was replicated four times.

Experiments were initiated from an overnight culture of the strain grown in YPD (20 g L⁻¹ yeast extract, 10 g L⁻¹ peptone, 20 g L⁻¹ dextrose) plus tetracycline and ampicillin to prevent bacterial contamination. The saturated culture was washed three times with sterile water and resuspended in the appropriate growth medium. One hundred and twenty-eight microlitres was transferred into each well of a 96-well plate, and then diluted by a factor of 2¹⁰ using a Biomek FXP liquid handling robot, providing an initial population abundance of ~10⁵ cells in each well.

Every 24 h, plates were removed from the incubator and subjected to either a diffusion or a sham-diffusion protocol. The plate was shaken on a plate shaker to disperse the cell pellet. Cell population in each well was quantified every 24 h as the optical density (OD600) measured with a microplate photometer (Tecan Infinite M200 Pro) three times, taking the average of the three technical replicates. Using the liquid handling robot, 3% volume was aspirated from each well and then transferred to a new plate ("Plate 2" in Fig. 2). For the diffusion treatment, this volume was dispensed into Plate 2 in the adjacent position to the left (e.g. from column 2 in Plate 1 into column 1 in Plate 2), thus generating 3% diffusion to the left. For the sham-diffusion treatment, the volume was dispensed into Plate 2 at the same position (e.g. from column 2 in Plate 1 into column 2 in Plate 2), such that no diffusion of cells occurred (Fig. B1 in Supporting Information Appendix C). This was repeated again, but with the 3% volume dispensed into the adjacent position to the right (e.g. from column 2 in Plate 1 to column 3 in Plate 2) to generate diffusion of cells to the right. Again, for the sham-diffusion treatment the volume was dispensed into Plate 2 in the same position as Plate 1. Then, the remaining volume in Plate 1 was dispensed into Plate 2 in the same position (e.g. column 2 in Plate 1 into column 2 in Plate 2), constituting the non-diffusing fraction of the population. Thus, diffusion steps in our protocol did not dilute the populations. Note also that we did not implement a wrap-around boundary, such that the two perimeter columns of the plate (columns 1 and 12) only received diffused population from one, adjacent column.

To renew the resource and to ensure that resources do not diffuse with consumer cells, we removed old medium using the liquid handling robot (after centrifuging Plate 2 at 710g for 5 minutes to pellet cells). Fresh medium was then dispensed/renewed into each well, and the plate was shaken on a plate shaker to resuspend cells before incubating at 30 °C for 24 h. This procedure was repeated for 9 days, at which point the population densities for all treatments had approached an asymptote.

Statistical methods

The total population (TRAPA) was calculated as the sum of OD600 over all of its subpopulations (i.e. wells) (Fig. 2). The diffusion effect was measured by comparing TRAPA between diffusion and sham-diffusion conditions for each treatment. Among-treatment differences in TRAPA were tested for statistical significance using the nonparametric Mann–Whitney U test because of the non-normal distribution of the data. To determine if boundary subpopulations, which only donate and receive individuals from a single neighbouring subpopulation, bias our results in any way, we ran our statistical analysis with and without excluding boundary wells. Specifically, we analysed the data with all subpopulations, and then with all subpopulations excluding the two boundary subpopulations (wells in columns 1 and 12), the four boundary subpopulations (wells in columns 1, 2, 11 and 12) and the six boundary subpopulations (wells in columns 1, 2, 3, 10, 11 and 12). As we found no difference in the significance level (for $P < 0.05$) between these cases (Table B1 Supporting Information

Appendix C), and as we found no evidence of a boundary effect in simulations (Fig. B5 Supporting Information Appendix C), we chose to report throughout the main text results with the single-boundary subpopulations (wells in columns 1 and 12) excluded.

To estimate r and K , we used daily measurement of OD600 in each individual well of our "sham-diffusion" treatments from day 1 to day 9, for three Trp concentrations at four levels of Cyh in all combinations. These r and K values were estimated by fitting the time-course data to both the standard logistic growth equation ($N(t) = KN_0 \exp(rt)/(K + N_0 (\exp(rt) - 1))$) and the Gompertz growth equation ($N(t) = K(N_0/K)^{\exp(-rt)}$) (Paine *et al.* 2012) using custom fit in MATLAB R2015a. $N(t)$: yeast population at time t , K : carrying capacity, N_0 : initial population, r : intrinsic growth rate, t : time. To describe the measured relationships between r and K , we used Model II regression because both r and K are measured values with random errors (Sokal & Rohlf 1995). The Model II regression was calculated by ordinary least square (OLS) method instead of major-axis (MA) method because the data distribution is not bivariate normal, and the relationship between r and K is linear (Legendre 1998). We assumed a linear function ($r = \beta_{r,K} K + a$) to calculate the goodness of fit for the r vs. K relationship. The overall goodness of fit is based on the coefficient of determination (R^2).

RESULTS

Mathematical results

Both mathematical and numerical analysis of equations (2a,b) are presented in detail in Supporting Information Appendices D and E.

Hypothesis 1 states that when a consumer exists in an environmental space with a heterogeneously distributed input of exploited renewable limiting resource, the total steady-state population (TRAPA) diffusing can reach a greater abundance than if non-diffusing. Mathematical expressions for Total population, heterogeneous, no diffusion ($TRAPA_{\text{hetero, no diffusion}}$) and Total population, heterogeneous, diffusion ($TRAPA_{\text{hetero, diffusion}}$) are shown in Box 1, Supporting Information Appendix D. It is proven in Supporting Information Appendix D, for $D \rightarrow \infty$, for both Models 1 and 2, over a range of parameter values relevant to the experiment that

$$TRAPA_{\text{hetero, diffusion}} > TRAPA_{\text{hetero, no diffusion}}$$

Numerical results confirm the analytic results (for small values of D as well), and are shown in Figure 3 for Model 1 and in Supporting Information Appendix D for both models. Thus, Hypothesis 1 is mathematically confirmed.

Hypothesis 2 states that the higher population abundance (TRAPA) in a heterogeneous environment with diffusion is greater than without diffusion when there is a positive relationship of growth rate and carrying capacity. Hypothesis 2 is mathematically confirmed for Models 1 and 2 by determining the relationship between growth rate and carrying capacity across the patches and showing it is positive when the $TRAPA_{\text{hetero, diffusion}} > TRAPA_{\text{hetero, no diffusion}}$ (Supporting Information Appendices D and E).

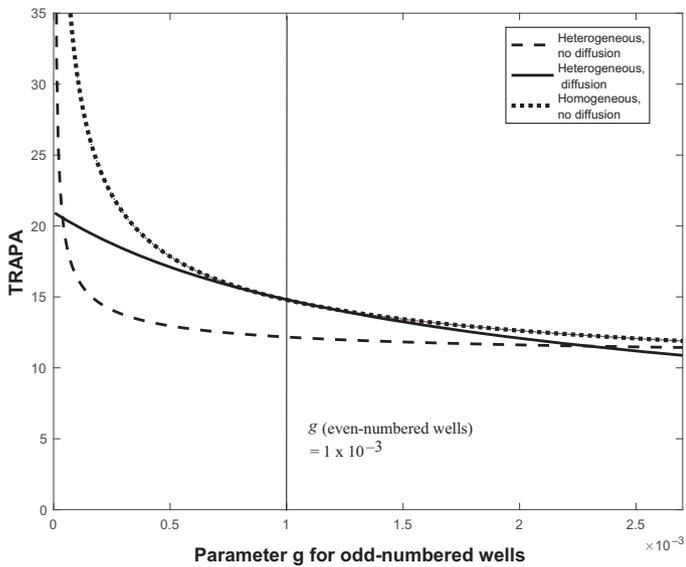


Figure 3 Total population, heterogeneous, no diffusion ($TRAPA_{hetero, no\ diffusion}$, dashed curve), Total population, heterogeneous with diffusion ($TRAPA_{hetero, diffusion}$, solid curve) and Total population, homogeneous no diffusion ($TRAPA_{homogeneous, no\ diffusion}$, dotted curve) as functions of the g_i value for the low nutrient input wells, $g_{low\ nutrient}$ (odd-numbered wells) for fixed value of the $g_i = 0.001$ for the high nutrient input wells, $g_{high\ nutrient}$ (even-numbered wells), for $D \rightarrow \infty$. For simplicity, the other parameters have been set to $r = 0.1$, $k = 0.1$ and $\gamma = 0.01$. The heterogeneous distribution is as follows: $N_{input_i} = (0.02, 0.6, 0.02, 0.6, 0.02, 0.6, 0.02, 0.6, 0.02, 0.6, 0.02, 0.6, 0.02, 0.6)$, and the homogeneous distribution is as follows: $N_{input_i} = (0.31, 0.31, 0.31, 0.31, 0.31, 0.31, 0.31, 0.31, 0.31, 0.31, 0.31, 0.31, 0.31, 0.31)$.

Hypothesis 3 states that a population, $TRAPA_{hetero, diffusion}$, diffusing in an environmental space with a heterogeneously distributed input of exploited renewable limiting resource can reach a greater steady-state abundance (TRAPA) than a population either diffusing ($TRAPA_{homogeneous, diffusion}$) or not diffusing ($TRAPA_{homogeneous, no\ diffusion}$) in an environmental space with the same total input of resources spread homogeneously in the space. Hypothesis 3 is mathematically *disproven* in Supporting Information Appendices D and E; that is, it is shown that

$$TRAPA_{homogeneous, diffusion} = TRAPA_{homogeneous, no\ diffusion} \\ \geq TRAPA_{hetero, diffusion}$$

This is the main novel mathematical result of our study. Numerical results confirm these results for both models, shown in Fig. 3 for Model 1 and in Supporting Information Appendix D for both models. Therefore, the model with an exploited, renewable resource produces the opposite of a key result of the phenomenological logistic models in which growth rates and carrying capacities were fixed (Supporting Information Appendix A). The implications of this are discussed later.

Experimental results

In a heterogeneous environment, we found that spatial population diffusion (D) significantly increased TRAPA compared

to the sham-diffusion treatment (ND) for the two lowest Cyh treatments (Fig. 4a, b) (0 nM Cyh: $P = 0.03$; 50 nM Cyh: $P = 0.03$). Daily abundances over the 9-day course of the experiment are shown in Fig. B2 in the Supporting Information Appendix C. Total population abundance began to noticeably deviate between diffusion and sham diffusion in the 0-nM and 50-nM heterogeneous treatments starting at day 4 and increased progressively for the remaining time points of the 9-day experiment. This result empirically supports Hypothesis 1, and experimentally validates our mathematical results above. In contrast, in the high Cyh concentration treatments (200 and 400 nM), there was a weakened impact of diffusion on increasing TRAPA, resulting in no significant difference in TRAPA between diffusion and sham-diffusion treatments (Fig. 4c, d) (200 nM Cyh: $P = 0.49$; 400 nM Cyh: $P = 0.11$). Ultimately, we found that the average $ratio_{D/ND}$ of diffusion to sham-diffusion TRAPA (± 1 SD) was higher at low Cyh (0 nM: $ratio_{D/ND} = 1.13 \pm 0.02$; 50 nM: $ratio_{D/ND} = 1.05 \pm 0.01$) than high Cyh (200 nM: $ratio_{D/ND} = 0.99 \pm 0.00$; 400 nM: $ratio_{D/ND} = 1.02 \pm 0.00$).

Why does the effect of diffusion on TRAPA in heterogeneous environments differ between low and high Cyh treatments? Recall that we used Cyh in order to manipulate the r - K relationship. A strong positive r - K relationship is predicted by theory to determine the direction and magnitude of the effect of diffusion on TRAPA in heterogeneous environments (Arditi *et al.* 2015; DeAngelis *et al.* 2016). We found a positive linear regression coefficient, $\beta_{r,K}$, of r on K under our experimental conditions for each of the four different levels of cycloheximide (Fig. 5), but that the r and K relationship was 1.6- to 3-fold larger with 0- and 50-nM Cyh than with 200 and 400 nM (0 nM: $\beta_{r,K} = 1.77$, $R^2 = 0.99$; 50 nM: $\beta_{r,K} = 1.63$, $R^2 = 0.99$; 200 nM: $\beta_{r,K} = 0.99$, $R^2 = 0.96$ and 400 nM: $\beta_{r,K} = 0.59$, $R^2 = 0.74$) (Fig. 5). Similar results were found based on Gompertz growth fitting in Fig. B3 in the Supporting Information Appendix C. This result empirically supports Hypothesis 2 that the sign and strength of the relationship between r and K are associated with TRAPA being higher than total carrying capacity in a heterogeneous environment with diffusion. We suspect that this positive relationship was mediated by the joint effect of the limiting resource (Trp) on these two growth parameters. Specifically, lower concentrations of limiting resource led to both lower yield (low K) due to the lower overall abundance of growth-supporting nutrient, and to lower encounter rates between nutrient molecules and cells, which caused slower growth (low r). Our results indicate that the addition of the toxic stressor, Cyh, not only reduced r , as expected from its mode of action (i.e. translation inhibition), but also reduced K , although to a lesser relative degree than it reduced r . The molecular and physiological mechanisms by which a translation inhibitor can reduce yield are unclear, and suggest a deeper biological effect on the r and K relationship than resource mediation alone.

When diffusion increases TRAPA, where do the “extra” individuals reside? To address this, we analysed abundance in each well individually. The insets in Fig. 4a, b show the averages of the final subpopulations at day 9 for the five low nutrient wells and five high nutrient wells for both the diffusion and sham-diffusion treatments in the 0-nM and 50-nM

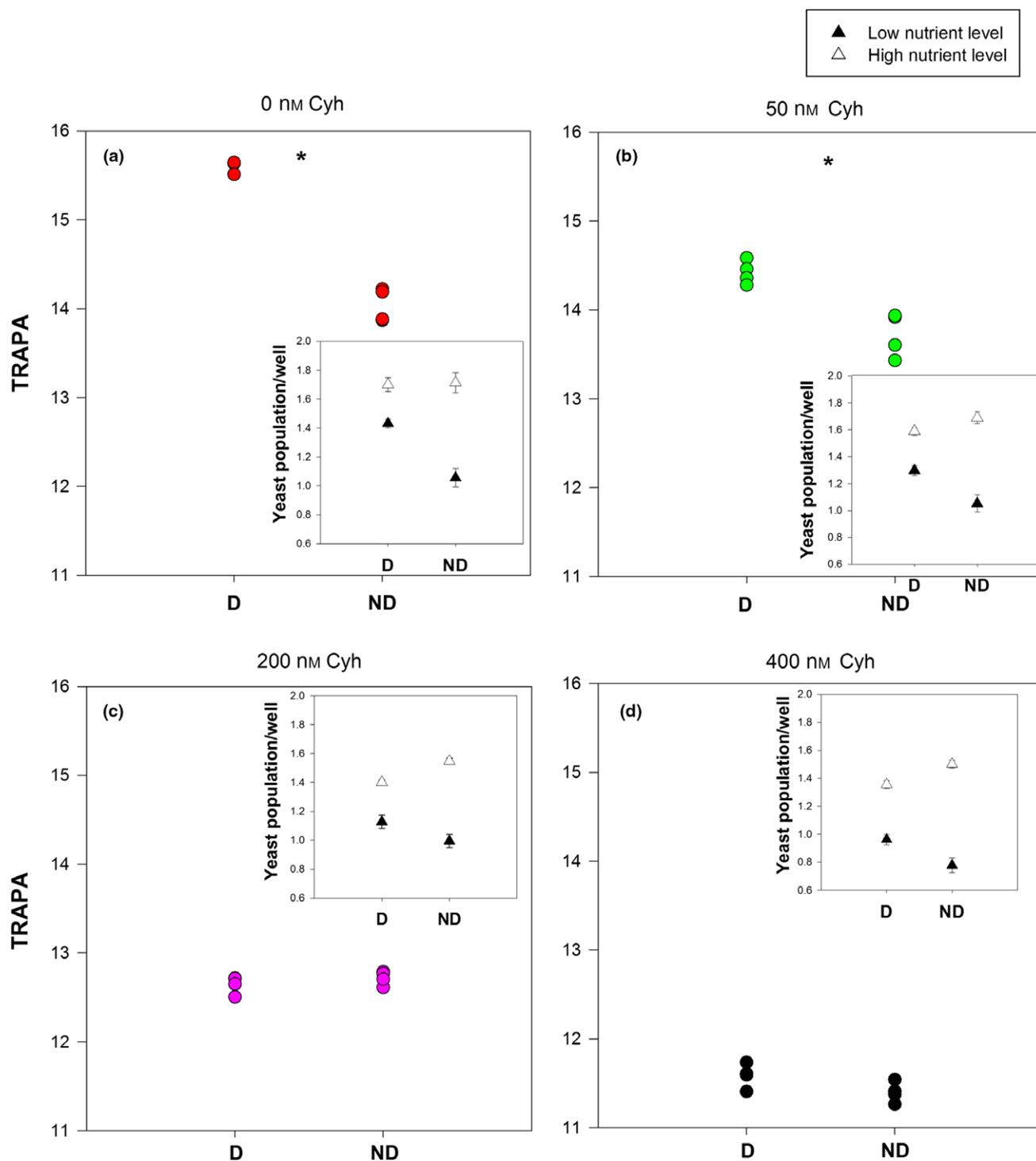


Figure 4 The final total yeast population (OD600) with four replicates with diffusion (D) and without diffusion (ND), at four levels of Cyh in the heterogeneous scenario: a. 0 nM, b. 50 nM, c. 200 nM, d. 400 nM. The diffusion rate is 0.06. Insets a, b, c, d: show the average final subpopulation in the low nutrient condition; that is, averaged over the five wells with lowest nutrients (filled triangle) and in the high nutrient condition; that is, averaged over the five wells with highest nutrient levels (unfilled triangle), with diffusion (D) and without diffusion (ND), with four levels of Cyh in the heterogeneous scenario (0 nM, 50 nM, 200 nM and 400 nM). Asterisks above the dots in a and b represented there was significantly higher final total yeast population with diffusion than without diffusion ($P < 0.05$). Cyh: cycloheximide, Trp: tryptophan. OD 600: optical density.

Cyh cases. We found that low nutrient wells consistently had higher averages of the final subpopulation at day 9 with diffusion than without diffusion (filled triangles in Fig. 4c, d), however, high nutrient wells had lower averages with diffusion

than without diffusion (unfilled triangles in Fig. 4c, d). These experimental results therefore indicate that in the 0-nM and 50-nM Cyh treatments, diffusion increased TRAPA as the loss in abundance in high nutrient subpopulations due to diffusion

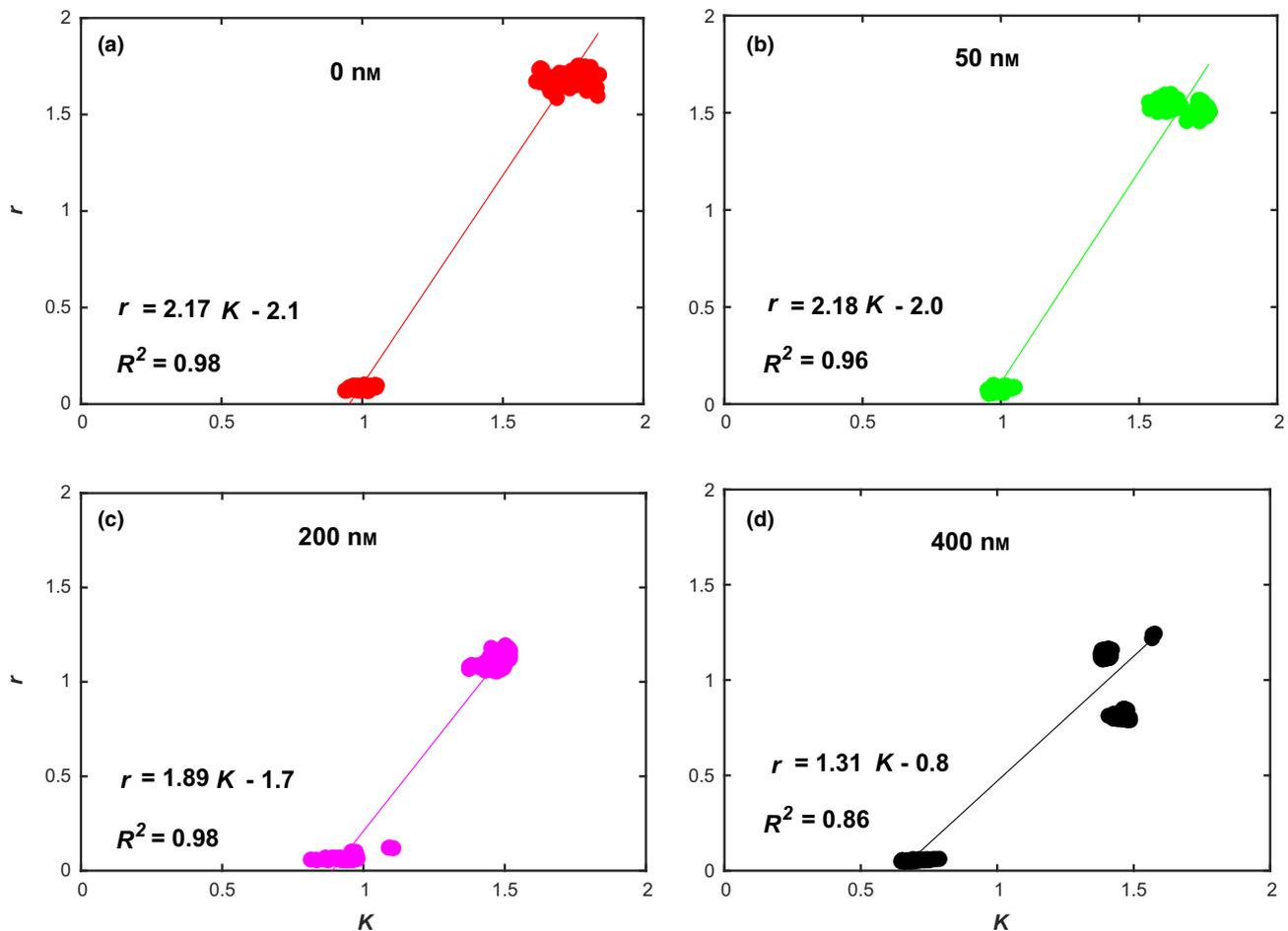


Figure 5 The relationship between per capita growth rates (r) and carrying capacity (K). (a) (b), (c) and (d): The r and K were calculated from sham-dispersal data at the three Trp concentrations used in the non-dispersal experiment with four Cyh concentrations (results of the latter two concentrations converged). The solid lines represent the fitted line of the data points, using linear function ($r = \beta_{r,K} K + a$), to calculate the goodness of fit for the r and K relationship.

was more than compensated by an increase in abundance in the low nutrient subpopulations.

In contrast, in the homogeneous case, there was no statistically significant difference in the final total population with and without diffusion. This was consistent among all four Cyh levels (Fig. 6) (0 nM Cyh: $P = 1.00$; 50 nM Cyh: $P = 0.89$; 200 nM Cyh: $P = 0.69$; 400 nM Cyh: $P = 0.89$). We found no difference between the abundance of individual wells between diffusion and sham-diffusion treatments (filled triangles in Fig. 6), as one would expect. Therefore, we found similar mean $ratio_{D/ND}$ across the four Cyh levels (0 nM: $ratio_{D/ND} = 1.00 \pm 0.01$; 50 nM: $ratio_{D/ND} = 1.00 \pm 0.01$; 200 nM: $ratio_{D/ND} = 1.00 \pm 0.00$; 400 nM: $ratio_{D/ND} = 1.00 \pm 0.00$).

Finally, we found that the TRAPA in the homogeneous environment treatments (with and without diffusion) exceeded that of the heterogeneous treatments with diffusion (compare Figs 6 and 4). We thus empirically reject Hypothesis 3, which, based on previous mathematical theory using the phenomenological logistic equation, predicted that TRAPA would be higher in heterogeneous environments with diffusion than in a homogeneous environment with the same total resource level.

This empirical result, then, supports our mathematical prediction based on an explicit consumer–resource model (Fig. 3).

DISCUSSION

Both the experiments with yeast diffusing in a heterogeneous environment of exploitable renewed resources (Fig. 4) and the mathematical analysis (Supporting Information Appendices D and E) support our Hypotheses 1 and 2. Namely, a consumer population with a positively related growth rate and carrying capacity can reach a greater total steady-state abundance (TRAPA) when diffusing in space than when not diffusing. The empirical results showed a consistent and significantly higher (up to 10%) total yeast population with diffusion than without diffusion in the heterogeneous case; that is, the total population with diffusion exceeded the summation over the local observed carrying capacities. The effect size in our experiments is therefore on the order of the diffusion rate ($D = 6\%$), consistent with previous theory in the weak diffusion limit by DeAngelis *et al.* (2016). Previous theory by Holt (1985) predicted dispersal could increase TRAPA by around

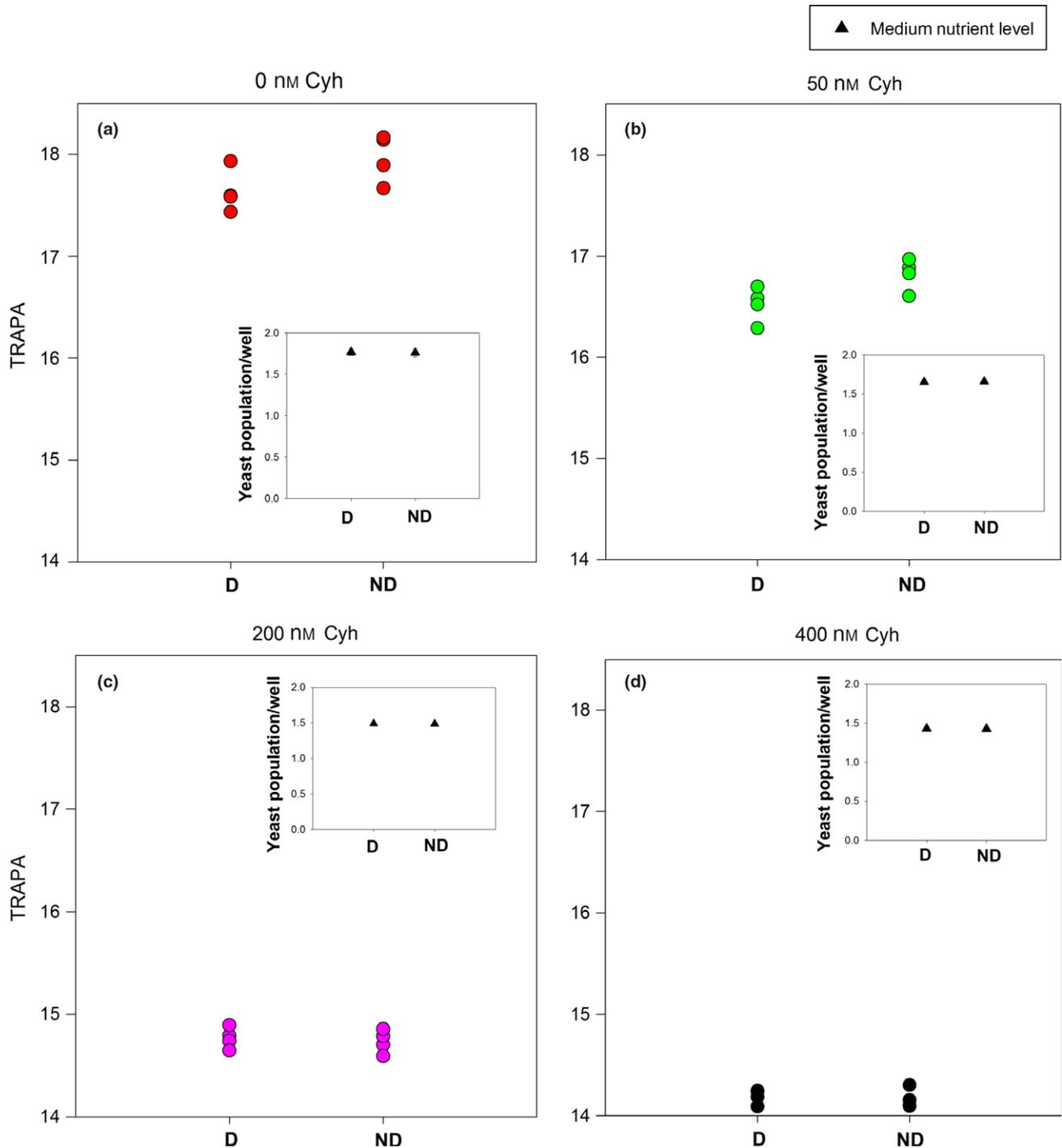


Figure 6 The final total yeast population (OD600) with four replications with diffusion (D) and without diffusion (ND), at four levels of Cyh in the homogeneous scenario: a. 0 nM, b. 50 nM, c. 200 nM, d. 400 nM. The diffusion rate is 0.06 per day. Insets a, b, c and d: the average final subpopulation in medium nutrient well (filled triangle), with diffusion (D) and without diffusion (ND), at four levels of Cyh in the heterogeneous scenario (0 nM, 50 nM, 200 nM and 400 nM). Cyh: cycloheximide, Trp: tryptophan. OD 600: optical density.

100%, but his analysis was done in the strong diffusion limit with particular choices of parameters. In general, these results suggest that if the resource inputs are heterogeneous by nature, thus creating heterogeneous carrying capacities, diffusion may be more advantageous for the population rather than no diffusion.

However, both experiments (compare Figs 6 and 4) and the mathematical analysis (Appendix D and E) reject Hypothesis 3, which stated that a diffusing population will reach a higher population abundance (TRAPA) when a resource is heterogeneously distributed over environmental space than when the same amount of resource is homogeneously distributed. This

result would seem to contrast with the theoretical results of Holt (1985), Lou (2006), Arditi *et al.* (2015) and DeAngelis *et al.* (2016), who showed mathematically that diffusion allows the population in a heterogeneous system to exceed that attained in the system with the same, but homogeneously distributed, carrying capacity. However, those earlier mathematical results were derived with models in which the carrying capacities varied with spatial location yet were fixed in that the population did not have a feedback effect on those values. Sometimes that assumption is justified, as in some cases space alone may be the main limiting factor, as for some intertidal populations (Paine 1966). But in many cases, some exploitable renewed resource is limiting, so the feedback of the population on that resource must be taken into account.

Our results revealed a positive relationship between carrying capacity and per capita growth rate when population growth was manipulated by changing the resource supply (the concentration of amino acid, tryptophan). Previous theoretical work (Arditi *et al.* 2015; DeAngelis *et al.* 2016) predicted that a positive r - K relationship is necessary for diffusion to increase TRAPA in a heterogeneous environment, and our empirical results support these predictions (Fig. 5). Importantly, the growth inhibitor, by reducing the positive relationship of the r vs. K , reduced the positive effect of diffusion on population abundance in the heterogeneous environment. As far as we are aware, this is the first rigorous experimental support that the r vs. K relationships determined whether a diffusing population could exceed in abundance the sum of the local carrying capacities.

Given its potential implications, the generality of a positive r - K relationship deserves further empirical and mathematical investigation. Mallet (2012) argues that r and K should, in general, be proportional. A meta-analysis by Hendriks *et al.* (2005) of 128 environmental toxicology studies, in taxa including phytoplankton, insects and primarily microcrustaceans, found a consistently positive r - K relationship when growth was experimentally manipulated by a stressor, primarily toxins. Alternatively, the r - K relationship we measured emerges when growth is manipulated via the resource supply. The positive r - K relationship we observe likely, in part, to be mediated by the effect of resource supply on both parameters. Higher resource concentration increases the encounter rate between consumer and resource, which increases r , and also increases the total available supply of resource, which increases K . However, deeper physiological mechanisms are also clearly at work in causing this correlation, particularly when manipulated by stressors as in the studies analysed by Hendriks *et al.* (2005). For example, our inability to predict the effect of a growth inhibitory drug, Cyh, on carrying capacity highlights the need for future theoretical work on the mechanistic/physiological basis of an r - K correlation. Theory linking physiology to population-level phenomena should be brought to bear on this question. We note, for example, that the Metabolic Theory of Ecology appears to predict a positive, linear r - K correlation (combining equations 9 and 11 in Brown *et al.* (2004))

In general, carrying capacity is a key concept in ecology. Our work highlights the fact that, in spatially distributed species, this core ecological concept becomes rather

complicated, a fact stressed by others (Mallet 2012; Arditi *et al.* 2015). Appreciating these complexities may be essential for applying ecological theory to the management of natural populations, where knowledge of the factors determining total population abundance in fragmented landscapes is paramount.

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AUTHORSHIP

JDVD and DLD designed research. JDVD designed experiments. BZ, AK, AR and KMLM conducted the experiment and collected data. BZ, AK, LZ, DLD and JDVD analysed the data. DLD and WN contributed to the mathematical analysis and proof. BZ, AK, DLD, WN and JDVD wrote the first draft. All authors contributed to revisions of the manuscript.

DATA ACCESSIBILITY STATEMENT

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.9sm68>

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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