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DEGREES AWARDED:

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CHAPTER I

INTRODUCTION

A reference to an image can be done like this one, to Figure C.1. You might also want to cite an article like this: [103].

FIGURE 1.1. This is a short description that goes in the Table of Figures. This is an extension of that description, which may include cascading turns of phrase, which appears directly below the figure as expected, but not in the table of figures which appears at the beginning of this exceedingly amazing dissertation.

CHAPTER II

BACTERIAL COHESION PREDICTS SPATIAL DISTRIBUTION IN THE LARVAL ZEBRAFISH INTESTINE

Are there general biophysical relationships governing the spatial organization of the gut microbiome? Despite growing realization that spatial structure is important for population stability, inter-bacterial competition, and host functions, it is unclear in any animal gut whether such structure is subject to predictive, unifying rules, or if it results from contextual, species-specific behaviors. To explore this, we used light sheet fluorescence microscopy to conduct a high-resolution comparative study of bacterial distribution patterns throughout the entire intestinal volume of live, larval zebrafish. Fluorescently tagged strains of seven bacterial symbionts, representing six different species native to zebrafish, were each separately mono-associated with animals that had been raised initially germ-free. The strains showed large differences in both cohesion—the degree to which they auto-aggregate—and spatial distribution. We uncovered a striking correlation between each strain's mean position and its cohesion, whether quantified as the fraction of cells existing as planktonic individuals, the average aggregate size, or the total number of aggregates. Moreover, these correlations held within species as well; aggregates of different sizes localized as predicted from the pan-species observations. Together, our findings indicate that bacteria within the zebrafish intestine are subject to generic processes that organize populations by their cohesive properties. The likely drivers of this relationship, peristaltic fluid flow, tubular anatomy, and bacterial growth and aggregation kinetics, are common throughout animals. We therefore suggest that the framework introduced here, of

biophysical links between bacterial cohesion and spatial organization, should be useful for directing explorations in other host-microbe systems, formulating detailed models that can quantitatively map onto experimental data, and developing new tools that manipulate cohesion to engineer microbiome function.

Introduction

Dense and diverse communities of microbes reside in the intestines of humans and other animals. Their large impact on processes ranging from digestion to disease progression [1, 2, 3] motivates a great deal of work aiming to uncover determinants of community composition and function. Because of the size and anatomy of the gut, and because of the remarkable number of microbial species that coexist within it—hundreds to thousands in humans—it is widely believed that spatial organization plays an important role in orchestrating community structure [4, 5]. In support of this, for example, recent studies have shown that distinct groups of bacteria inhabit the lumenal space of the intestine compared to the dense mucus layer lining the epithelium [6] and that distinct taxa are found in different regions along the length of the digestive tract [7]. The drivers of spatial organization are most often considered to be anatomical, as above, or biochemical, for example caused by variation in pH or the concentrations of nutrients, oxygen, or antimicrobial peptides [8].

Here, we suggest and demonstrate that the biophysical character of the microbes themselves, namely the degree to which they are planktonic or aggregated, can be a strong predictor of their populationÕs overall position within the intestine. In macroscopic ecological contexts, such relationships between morphology and spatial distribution are well known. For example, animal body

mass is greater in colder regions (Bergmann's rule), likely due to the scaling of surface driven heat loss with size; phytoplankton aggregation is correlated with position in the water column, due to buoyancy [9]; and seed mass varies robustly with latitude, for reasons that are still unclear [10].

It remains an open question whether gut microbes are governed by broad, pan-species principles linking cellular behavior and large-scale distribution, or whether spatial structure is contingent on context- and species-specific interactions. Investigating this requires high-resolution imaging within live animals in a controlled setting, which has only recently become possible. Uncovering such principles would demonstrate that despite the biochemical complexity of the vertebrate microbiota, general biophysical principles governing the architecture of gut microbial communities may exist.

Our study makes use of larval zebrafish (Fig. 1A, 1B), a model organism of particular utility to investigations of host-microbe interactions due to its anatomical and physiological similarities to other vertebrates, its optical transparency, and its amenability to gnotobiotic techniques for the creation of fish colonized only by particular microbial species [11, 76, 86, 99]. Zebrafish naturally associate with a diverse intestinal microbiome containing hundreds of bacterial species [15, 75] that influence a wide range of host processes [17, 18, 19, 20]. Earlier work on the dynamics of two native zebrafish bacterial symbionts [76] and a human-derived pathogen [86] showed associations between cellular growth mode, specifically whether the bacteria are planktonic or aggregated, and spatial distribution, specifically the location of the population along the length of the intestine, but the robustness and generality of this association remains unexplored.

As detailed below, we find across multiple bacterial strains, and even within strains, that greater aggregation is strongly correlated with more posterior localization.

Materials and Methods

Bacteria: All bacterial strains used in this study are listed in Table 1. Each strain was previously engineered via Tn7-mediated insertion to constitutively express either dTomato or sfGFP fluorescent reporters from a single chromosomal locus [77]. Archived stocks of bacteria were maintained in 25% glycerol at -80¡C. Prior to experiments, bacteria were directly inoculated from frozen stocks into 5ml lysogeny broth (LB) media (10g/L NaCl, 5g/L yeast extract, 12g/L tryptone, 1g/L glucose) and grown for 16h (overnight) shaking at 30¡C.

Animal care and gnotobiology: All experiments with zebrafish were done in accordance with protocols approved by the University of Oregon Institutional Animal Care and Use Committee and following standard operating procedures [96]. Wild-type (AB x TU strain) zebrafish were derived germ-free and colonized with bacterial strains as previously described [97] with slight modification (Supplemental Text).

Live imaging: Live imaging of larval zebrafish was conducted using a home-built light sheet fluorescence microscope previously described in detail [99]. The full volume of the intestine (approximately 1200x300x150 microns) is captured in four sub-regions that are registered in software following imaging. An entire intestine sampled with 1-micron steps between planes is imaged in less than 1 minute. All images were taken with an exposure time of 30ms and an excitation laser power of 5mW at 488 nm and 561 nm wavelengths.

Image analysis: Three-dimensional image stacks were analyzed using a pipeline described in detail in [99], with minor changes (Supplemental Text). The goal of the analysis is to identify the location and size of all bacterial clusters, ranging from individual, planktonic cells to large multicellular aggregates. Small objects are identified using a spot detection algorithm calibrated to over count spots, which are then filtered using a trained classifier (Supplemental Text). Large objects are segmented using a graph-cut algorithm [24], typically seeded with a mask obtained by intensity thresholding. The number of cells per multicellular aggregate is estimated by dividing the total fluorescence intensity of the aggregate by the average intensity of single cells in the same fish host. In cases where single cells are sparse or absent, the average is taken across all single cells for that strain. From these estimates we compute the 1D center of population mass, i.e. the mean position weighted by population density at each position, normalized by intestine length ("population center"), and also calculate the various cohesion metrics discussed in the text.

Data: To maximize statistical power, we combined newly acquired data with a recently published image dataset obtained under identical conditions [77]. The recently published data had been subjected to prior analysis to estimate overall bacterial abundances, but was reanalyzed here from scratch using the methods described above and in the Supplemental Text. The combined dataset consisted of N=6 fish per strain, except for *Plesiomonas* ZOR0011, which had N=3 fish. The output of our computational pipeline, a text file containing the size and location of every bacterial cluster, with identifiers for strain, fish, and dataset, is included in Supplemental Data File 1, with details on its format in the Supplemental Text. In

addition, a spreadsheet with the cohesion and distribution metrics plotted in Figure 2 is included in Supplemental Data File 2.

Experimental Design

To investigate this putative relationship, we analyzed seven bacterial strains representing six different species (Table 1). All were isolated from zebrafish intestines, where they are common and abundant [75]. Each species was previously engineered to constitutively express fluorescent proteins [77]. To deduce relationships governing species morphology and its interaction with the gut environment, we examined animals that were sufficiently developed to exhibit stereotypical intestinal transport, and sufficiently young to permit germ-free derivation and handling. We first raised larvae germ-free and allowed them to be colonized with individual bacterial strains by inoculation of the aqueous medium for 24 hours starting at four days post-fertilization (dpf) (Methods and Supplemental Text). During this developmental stage, until roughly 7 dpf, larvae need not be fed and are sustained on yolk-derived nutrients, enabling maintenance under gnotobiotic conditions. The larval intestine is, however, highly active beginning at 3.5 days [25] and exhibits a range of motility patterns, including coordinated peristalsis-like movements that are controlled by the enteric nervous system and can act on resident bacteria [76, 87]. After the colonization period, three-dimensional image stacks were acquired using a custom-built light sheet fluorescence microscope described in detail elsewhere [99]. The images span the entire larval intestine, roughly 200 x 200 x 1000 microns in extent, with single-bacterium resolution. Additional details are provided in Methods.

strain	median abundance
Aeromonas veronii ZOR0001	8.4×10^2
Aeromonas caviae ZOR0002	1.2×10^3
Enterobacter cloacae ZOR0014	3.5×10^3
Plesiomonas ZOR0011	4.6×10^3
Pseudomonas mendocina ZWU0006	3.5×10^2
Vibrio cholerae ZOR0036	1.6×10^3
Vibrio cholerae ZWU0020	2.0×10^4

TABLE 2.1. **Bacterial strains and imaging-derived estimates of mono-association abundances *in vivo***. Abundances were estimated from 3D image stacks using the computational pipeline described in Methods and Supplementary Text

Results

Imaging multiple fish per strain revealed a broad spectrum of growth modes and bacterial distributions, ranging from the highly planktonic populations of *Vibrio cholerae* ZWU0020 located within the anterior ÒbulbÓ (Fig. 1C, top; Supplemental Movie 1) to the almost entirely aggregated populations of *Enterobacter cloacae* ZOR0014 located within the midgut (Fig. 1C, bottom). Most populations displayed intermediate mixtures of cellular growth modes and spatial distributions, similar to that of *Aeromonas caviae* ZOR0002 (Fig. 1C, middle; Supplemental Fig. 1, Supplemental Movies 2-4). As with observations of *Aeromonas* strains in earlier work [76], bacterial aggregates were dense, compact, and cohesive. The predominant difference in spatial position between species was their location along the longitudinal axis of the intestine. We observed no strains, for example, that localized along the radial axis, lining the gut epithelium.

We computationally identified each individual bacterium and aggregate in each three-dimensional image stack, and also determined the number of cells in each aggregate [99] (see Methods). For each population, we computed the center of

mass along the longitudinal axis of the intestine, normalized by the total intestinal length, to represent its spatial distribution. We also enumerated the fraction of the population present as planktonic cells to represent the strainÕs growth mode. Plotting each strain's planktonic fraction versus its population center shows a clear and striking correlation (Fig. 2A). Linear regression of $\log_{10}$-transformed planktonic fraction ($\log_{10} f_p$) against center of mass position (x_c) gives a coefficient of determination of $R^2 = 0.91$, and best-fit parameters

$$\log_{10} f_p = (0.81 \pm 0.32) + (-5.4 \pm 0.8)x_c. \tag{2.1}$$

Making use of our image segmentation of bacterial aggregates, we examined the relationship between mean object size and position. Defining a cluster as any group of bacteria (so that an individual bacterium is a cluster of size one), we find a strong correlation between each species' average cluster size (mean cells per cluster, C_c) and its center of mass (Fig. 2B, $R^2 = 0.79$);

$$\log_{10} C_c = (-0.74 \pm 0.47) + (4.9 \pm 1.1)x_c. \tag{2.2}$$

Because C_c is proportional to the total number of cells and inversely proportional to the number of clusters per fish (n_c), the relationship in Fig. 2B could be caused by a dependence on either or both of these factors. However, the total population of each species, save for *V. cholerae* ZWU0020, is roughly similar (Table 1); in contrast, n_c is strongly negatively correlated with position (Fig. 2C, $R^2 = 0.88$). Regression gives

$$\log_{10} n_c = (4.7 \pm 0.5) + (-6.6 \pm 1.1)x_c. \tag{2.3}$$

The slope, -6.6 ± 1.1, is close to the negative of the slope of the C_c vs x_c relationship (4.9 ± 1.1), as would be expected if $C_c \sim 1/n_c$ with the overall population being species-independent. Together, the C_c vs x_c and n_c vs x_c relationships confirm the lack of a global correlation between abundance and location and imply instead that local interactions relate the size and positioning of aggregates.

We next asked if the relationship between aggregation and intestinal distribution we found between strains could be detected within individual strain populations, which would further support its biophysical generality. For this, we considered only clusters of two or more cells because individual cells dominate each dataset. For each strain, excluding *V. cholerae* ZWU0020 because it shows almost no aggregation (Fig. 2A), we combined measurements of cluster size and intestinal position from all specimens. We restricted our analysis to the anterior half of the intestine because the distal half rarely contained substantial populations (likely due to frequent intestinal expulsion), limiting our statistical power in that region. Regressing $\log_{10}$-transformed sizes of aggregates against their position (Fig. 3, small circles and dashed trendlines), we found a positive correlation between aggregate size and aggregate position for each of the six strains (Table 2). Finding this relationship within strains, as well as across strains, suggests a generic mechanism that spatially segregates bacterial cells based on their cohesive properties, resulting in the localization of small aggregates to the anterior of the intestine and larger aggregates to the posterior.

Discussion

Harnessing the natural variation displayed by native zebrafish symbionts and the spatial insights made possible by 3D live imaging, we have uncovered a quantitative relationship between bacterial cell behavior and large-scale spatial organization throughout the intestine. We found that across species and strains, the degree to which bacterial populations are aggregated, a biophysical characteristic we term "cohesion", correlates strongly with their mean position along the intestine. Moreover, looking within strains we were able to detect further signatures of the cohesion-distribution correlation: namely, the size and location of individual aggregates are also correlated. These findings suggest that the relationship between cohesion and spatial structure represents a general principle that manifests across both taxonomic and cellular scales. Intriguingly, the diverse species and strains we examined each have well-defined characteristics, while together they span the range from almost wholly planktonic to almost wholly aggregated, with the corresponding range of intestinal locations. Each of these bacterial strains is a zebrafish gut symbiont, and so it is reasonable to suspect that traits such as degree of aggregation have evolved to enhance their fitness in the intestinal environment, or are responses to features of that environment. Through these traits, the bacteria have the capacity to influence how the intestine shapes their populations.

The specific mechanisms driving bacteria to aggregate may be complex. One can imagine, for example, simple adhesion of individual cells into clumps, assembly of aggregates through chemotaxis, and enchainment of daughter cells after division [72]. We note that specific motility behaviors do not in themselves explain aggregation; our data show that populations of both motile and non-motile strains [77] are capable of generating aggregates. These aggregates are perhaps

reminiscent of *in vitro* surface-mediated biofilms, but are distinct in that they form three-dimensional clumps in the intestinal lumen rather than lining the walls, and there is in general no simple mapping between strains' highly context dependent *in vitro* biofilm formation and *in vivo* aggregation. Nonetheless, investigating potential mechanisms of aggregation would be a valuable target for future studies, especially with an aim towards designing perturbations of cohesion.

We posit that the mechanism underlying the cohesion-distribution relationship emerges from the interplay between physical properties of the intestinal environment, especially its shape and peristaltic activity, and the cellular lifestyles of resident bacteria. As in all vertebrates, the larval zebrafish intestine is roughly tubular with a corrugated surface of villi, and transports and mixes contents using coordinated, periodic peristaltic contractions [28]. Earlier work looking solely at *A. veronii* ZOR0001 found aggregated microbes pushed and sporadically ejected by these contractions [76]; such forces more generally affect all aggregated bacteria. Theoretical studies of particle suspensions under low Reynolds number peristaltic flow also show spatial segregation of planktonic and aggregated cells [29]. These observations suggest that it should be possible to construct models that quantitatively match in vivo measurements and that offer predictions relevant for other animals, including humans. The development of such models will be challenging, as they must combine fluid dynamics, anatomy, and the nucleation, growth, and transport properties of bacterial aggregates. Aggregation kinetics are quantifiable from in vivo time-series imaging [99], and ongoing work, from both imaging and modeling, suggests that a robust, pan-species characterization of cluster dynamics is possible.

Even in the absence of such detailed models, however, it is reasonable to believe that the general relationship uncovered here will occur in larger systems, such as the human gut. Peristaltic transport, a tube-like geometry, and bacterial growth are universal features of all animal intestines. Given that Reynolds and Stokes numbers are low in both the zebrafish intestine and the much larger human intestine, we expect that the flow fields and particle transport that result from peristaltic contractions will be similar across scales. This similarity has already allowed quantitative comparisons of microbial compositions driven by pH and flow rates between in vitro fluidic devices and the human microbiome [30]. Therefore, the longitudinal segregation of bacterial clusters by size that we observed here may be a generic consequence of peristaltic activity. Moreover, the finer-scale structure of crypts and folds affords still further possibilities for spatial structuring driven by the associated flow fields and bacterial cohesion. Host anatomy, diet, and biochemical heterogeneity will likely complicate this picture, but we suggest that a general trend connecting bacterial morphology and intestinal position is reasonable to expect and intriguing to search for.

The relationship between cohesion and spatial distribution described here offers a framework for precision microbiome engineering. For example, by manipulating cohesion it may be possible to selectively displace bacterial populations from certain regions of the intestine or to remove them entirely. Reflecting this point, it was recently shown in a murine *Salmonella* vaccine model that antibody-mediated enchaining of bacterial cells led to aggregation and intestinal expulsion [72]. In addition, peristaltic activity can change in response to diet, therapeutic drugs, infection, and a range of chronic diseases. Therefore, elaborating the link between cohesion, spatial structure, and flow may help explain

diseases that result from microbial imbalances, and inspire methods for countering such changes in community composition through the targeted alteration of bacterial aggregation.

Author Contributions

BS, TW, KG, and RP designed the research. TW and EW performed gnotobiotic procedures. BS performed the imaging. BS, TW, and RP analyzed the data. All authors wrote the manuscript.

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CHAPTER III

STATIONARY MOMENTS, DIFFUSION LIMITS, AND EXTINCTION TIMES FOR LOGISTIC GROWTH WITH RANDOM CATASTROPHES

A central problem in population ecology is understanding the consequences of stochastic fluctuations. Analytically tractable models with Gaussian driving noise have led to important, general insights, but they fail to capture rare, catastrophic events, which are increasingly observed at scales ranging from global fisheries to intestinal microbiota. Due to mathematical challenges, growth processes with random catastrophes are less well characterized and it remains unclear how their consequences differ from those of Gaussian processes. In the face of a changing climate and predicted increases in ecological catastrophes, as well as increased interest in harnessing microbes for therapeutics, these processes have never been more relevant. To better understand them, I revisit here a differential equation model of logistic growth coupled to density-independent catastrophes that arrive as a Poisson process, and derive new analytic results that reveal its statistical structure. First, I derive exact expressions for the model's stationary moments, revealing a single effective catastrophe parameter that largely controls low order statistics. Then, I use weak convergence theorems to construct its Gaussian analog in a limit of frequent, small catastrophes, keeping the stationary population mean constant for normalization. Numerically computing statistics along this limit shows how they transform as the dynamics shifts from catastrophes to diffusions, enabling quantitative comparisons. For example, the mean time to extinction increases monotonically by orders of magnitude, demonstrating significantly higher extinction risk under catastrophes than under diffusions. Together, these results provide

insight into a wide range of stochastic dynamical systems important for ecology and conservation.

3.1. Introduction

Stochastic fluctuations are important drivers of ecological and evolutionary processes [31, 32, 33, 34]. Understanding their consequences is essential for ecological management, as well as for explaining observed patterns of biodiversity [32]. Given that data is often limited, general principles of stochastic population dynamics derived from the mathematical analysis of minimal models can be immensely useful [32, 35]. For example, in classic work [36] Beddington and May derive for a stochastic logistic growth model how harvesting yields become less predictable as harvesting rates increase, a phenomenon that was suggested by historical fisheries data at the time [37]. Extensions of this analysis have led to threshold harvesting strategies that are proven optimal for a wide class of stochastic growth models that include extinction [38]. Beyond harvesting theory, analytically tractable models have led to diverse ecological and evolutionary insights [31, 33, 39].

In these types of analyses, stochasticity is often modeled by coupling growth to a Gaussian noise process, leading to stochastic differential equations that are amenable to well established tools from diffusion theory [32, 40]. However, large, abrupt catastrophes are not captured by Gaussian models and are better modeled by discontinuous stochastic processes. These catastrophes are increasingly observed in a variety of ecological systems. On global scales, ecological catastrophes have already been observed as the result of rapid warming and are expected to become more frequent as the climate continues to change [41]. At the opposite extreme, the intestinal microbiomes of humans and other animals are observed to undergo abrupt compositional changes following perturbations, such as antibiotic treatments [42, 43, 45, 78]. At all scales, efforts to understand and manipulate

ecological systems would greatly benefit from general, quantitative principles of how perturbations and catastrophes shape population statistics.

I address this issue here by analytically and numerically studying a single-species model of logistic growth coupled to discontinuous, multiplicative jumps that arrive as a Poisson process, introduced in [46] and referred to here as the Logistic Random Catastrophe (LRC) model (Figure 1A). Using the method of moment equations [47], I derive exact expressions for the stationary moments of the population distribution, neglecting the possibility of extinction. These results provide a direct look into the statistical structure of the LRC model, revealing a single, effective catastrophe parameter that largely controls ensemble statistics. This effective parameter was recently observed empirically in computer simulations and aided the analysis of experimental data, but there was no theoretical basis for its existence [45].

With this insight, I then turn to an old and fundamental problem: which dynamics, intermittent random catastrophes or continuous stochasticity, poses a higher risk of extinction? For models of exponential growth up to a hard wall carrying capacity in the presence of either multiplicative Gaussian noise, called environmental stochasticity, or random, multiplicative Poisson catastrophes, Lande [48] derives how the mean time to extinction scales as a power law in the carrying capacity for positive long-run growth rate, with the exponent depending on the details of the particular model. This similarity in scaling behavior implies similar extinction risk in a qualitative sense, but it remains unclear how to construct a meaningful quantitative comparison, since the noise parameters of the two models describe distinct processes.

To circumvent this issue, I propose a method that treats the models not as distinct processes, but as extreme versions of the same process. Using functional generalizations of the Central Limit Theorem [49] and drawing inspiration from renormalization methods in theoretical physics [50, 51], I analytically construct the diffusion analog of the LRC model, referred to here as the Logistic Environmental Stochasticity model (Figure 1B), in the limit of infinitely frequent, infinitesimal catastrophes, such that the stationary mean of the process remains constant. In this way, the problem of quantitatively comparing two distinct models is traded for the more straightforward problem of computing statistics of one model as a function of parameters, specifically, along a particular limit in parameter space. I apply this method to the comparison of extinction times and find that the mean time to extinction increases monotonically along this limit by orders of magnitude in a wide region of parameter space, implying significantly higher risk of extinction under random catastrophes dynamics in general.

Taken together, these results highlight the power of analytically tractable models of stochastic population dynamics. The expressions derived here aid the analysis of experimental and observational data, inform the design of computer simulations, and reveal deep connections between distinct stochastic processes relevant for a wide range of ecological systems.

3.2. Background on the Logistic Random Catastrophe model

Hanson and Tuckwell [46] introduce an ideal minimal model for the study of random catastrophes in isolation from additional complications: single-species logistic growth coupled to constant fraction catastrophes that arrive as a Poisson process, referred to here as the Logistic Random Catastrophe (LRC) model. The

LRC model can be written analytically as an Itô Stochastic Differential Equation (SDE):

$$dX_t = rX_t\left(1 - \frac{X_t}{K}\right)dt - (1-f)X_{t^-}dN_t. \tag{3.1}$$

The first term on the right hand side, of order dt, encodes deterministic logistic growth with growth rate r and carrying capacity K. The second term encodes random catastrophes with the use of a differential Poisson process, dN_t, which is equal to one if a catastrophe happens at time t and zero otherwise. Poisson catastrophes arrive with a constant probability per unit time, λ, and have a size set by f, the fraction of the population remaining after catastrophe. The notation $X_{t^-}dN_t$ indicates the Itô integration convention [52]. By including logistic growth, the LRC model captures realistic density-dependent regulation; by including catastrophes of constant fraction, it captures the realistic feature that larger populations can experience larger losses, assuming that all individuals are equally susceptible to the disturbance. Despite its simplicity, much about the statistical structure of the LRC model remains mysterious, due to the combined complications of the discontinuous Poisson process and nonlinear logistic growth.

3.3. Results

3.3.1. Deriving exact expressions for LRC stationary moments

I present here exact results for the stationary moments of the LRC model in absence of extinction, derived with the method of moment equations. The method of moment equations turns a stochastic differential equation into an deterministic differential equation for the moment in question by averaging. For nonlinear SDEs this results in a hierarchy of moment equations, in which each

moment is coupled to higher moments, that generally cannot be solved exactly. However, in the absence of extinction, this hierarchy reduces in the steady state to an algebraic recursion relation, which in the case of the LRC model is a simple relation between $\mathbb{E}[X^{n+1}]$ and $\mathbb{E}[X^n]$ (Appendix A). This recursion relation can be iterated to express each moment just as a function of the mean. The mean in turn can be computed independently from the steady state condition on $\ln X$ (Appendix A), resulting in

$$\mathbb{E}[X]_{LRC} = K\left(1 + \frac{\lambda}{r}\ln f\right). \tag{3.2}$$

(recall that $f \in (0,1)$, so $\ln f$ is negative for $f < 1$). This then determines all higher moments:

$$\mathbb{E}[X^n] = K^n\left(1 + \frac{\lambda}{r}\ln f\right)\prod_{m=1}^{n-1}\left(1 - \frac{\lambda(1-f^m)}{mr}\right), \qquad n \geq 2. \tag{3.3}$$

The stationary variance is readily computed to be

$$\mathrm{Var}[X]_{LRC} = K^2\frac{\lambda}{r}\left(-\ln f - (1-f)\right)\left(1 + \frac{\lambda}{r}\ln f\right). \tag{3.4}$$

These results agree well with simulations, as shown in Figure 2A in the form of cumulants [53], which generally provide more intuitive information than moments. The solid lines show the time evolution of the first 4 cumulants, C_n, of the LRC model, computed via stochastic simulation of the Poisson process with no absorbing state representing extinction (Materials and Methods). The dashed lines are the analytic results, computed from the expressions for the moments in equation (3) [53]. Each cumulant asymptotes to the analytic value.

3.3.2. A single, effective catastrophe parameter largely controls LRC moments

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These analytic results suggest that the parameter combination $\lambda \ln f$ plays an important role in determining population statistics. To investigate its role, I computed the response of the first four stationary cumulants of the LRC model to simultaneous, reciprocal scaling of λ and $\ln f$ via a dimensionless scale factor, β. Specifically, parameters were scaled according to

$$\lambda' = \beta\lambda, \qquad \ln f' = \beta^{-1} \ln f. \tag{3.5}$$

In regions of parameter space where statistics depend only on the effective parameter $\lambda \ln f$, curves of cumulants as a function of β will be flat. The results are shown Figure 2B for β ranging from $10^{-2} - 10^{3}$. Stationary moments were computed for each value of β using the analytic results derived above and converted to cumulants [53]. The stationary mean is invariant under this scaling, as indicated by equation (2). Higher order cumulants are approximately invariant for low values of β, which correspond to rare, large catastrophes, but decay to zero for large values of β, which correspond to frequent, small catastrophes.

The behavior of LRC cumulants under scalings of β can be understood by considering different limits of the two timescales of the model, namely the scales of growth and of catastrophe. Consider a population that started from carrying capacity and then experienced a catastrophe. The timescale of recovery back to carrying capacity, t_R, can be estimated by the time to reach half the carrying capacity, which is given by $-r^{-1} \ln f$. The typical time between catastrophes,

t_C, is given by λ^{-1}. The regime $t_C \ll t_R$ corresponds to large β, specifically, $\beta \gg (-r^{-1}\lambda \ln f)^{-1/2}$. As β increases, the size of the jumps becomes small fast enough to result in a deterministic limit, a dynamic analog of the law of large numbers. To see this, recall that for a Poisson process, all cumulants are equal to the mean, λt, just as all cumulants of a Poisson distribution are equal to the mean. The n^{th} cumulant of the scaled process $(1-f)N_t$ is therefore $(1-f)^n \lambda t$. The limit $\beta \to \infty$ corresponds to $\lambda' \to \infty$ and $f' \to 1$, such that $\lambda' \ln f'$ is constant. In this limit, $-(1-f)$ is well approximated by $\ln f$, so, higher cumulants of the scaled process decay as $\beta^{-(n-1)}$, resulting in a deterministic model.

The other regime, $t_C \gg t_R$, corresponds to small β, specifically, $\beta \ll (-r^{-1}\lambda \ln f)^{-1/2}$. In this regime, since catastrophes are rare and populations can recover from them, only a single catastrophe per population needs to be considered. A population at time t will be at carrying capacity unless a catastrophe occurred recently enough that the population is still recovering, *i.e.*, if the catastrophe occurred in the interval $(t - t_R, t)$. The number of trajectories to have a collapse in this interval is governed by a Poisson distribution with mean $\lambda t_R = -r^{-1}\lambda \ln f$. The fact this this distribution is invariant under scalings of β suggests that cumulants of the full LRC model in this limit are as well, though further analysis is needed make this connection rigorous.

Finally, when $t_C \sim t_R$, the statistics of the LRC model are governed by a more complicated interaction of growth and collapse timescales, and the invariance under β scalings is lost; moments depend on λ and f independently via equation (3).

The effective parameter $\lambda \ln f$ has an intuitive interpretation: It is the correction to the long-run growth rate due to catastrophes, and has been previously

identified as an important quantity in a variety of related models [48, 52, 54]. Its existence has important consequences for analyzing experimental data. As was done in [45], fitting ensemble statistics with the effective catastrophe parameter reduces the number of parameters that needs to be estimated. In fact, attempting to fit both the rate (λ) and size (f) independently results in highly unconstrained parameter estimates [45] and should be avoided. The analytic results derived here put the use of the effective parameter, $\lambda \ln f$, on firmer ground and explicitly delineate the range of its validity.

3.3.3. The diffusion limit shows that random catastrophes pose higher extinction risk than environmental stochasticity

I now consider the problem of quantitatively comparing extinction risks in the LRC model and its environmental stochasticity analog, referred to here as the Logistic Environmental Stochasticity (LES) model. The LES model can be written as an SDE,

$$dX_t = rX_t\left(1 - \frac{X_t}{K}\right) + \sigma X_t \circ dB_t, \tag{3.6}$$

with B_t the standard Brownian motion process [40, 52], whose intervals are independent, Gaussian distributed variables with $\mathbb{E}[B_t] = 0$ and $\mathrm{Var}[B_t] = t$, and σ setting the strength of the noise. Historically, there has been no obvious way of quantitatively comparing extinction risk between the two models across parameter space, since the noise parameter σ and the catastrophe parameters, a rate λ and size f, describe distinct, model-specific processes [32, 48, 55].

To circumvent this issue, I propose an approach in which the LES model is viewed not as a distinct process, but as a special case of the LRC model. This notion has been expressed qualitatively for decades [48, 55], but, to my knowledge,

has never been made explicit. This can be done using a functional generalization of the Central Limit Theorem (CLT) [49], which says that fluctuations of the Poisson process about its mean converge in distribution to Brownian motion in the limit of infinite jump rate and infinitesimal jump size. In Appendix B, it is shown that the relevant limits are

$$\ln f(N_t - \lambda t) \xrightarrow{\lambda\to\infty,\ f\to 1} \sqrt{\lambda}\ln f B_t, \tag{3.7}$$

such that $\sqrt{\lambda}\ln f$ is constant. Consequently, the mean drift of the scaled Poisson process diverges as $\sqrt{\lambda}$, the variance remains finite and all higher cumulants go to zero. These are functional analogs to what happens when a Poisson distribution limits to a Gaussian in the classical CLT. In this case, the diverging drift - which is proportional to effective catastrophe parameter $\lambda \ln f$ discussed above - is a manifestation of the fact that catastrophes are unidirectional, whereas noise in the LES model is bidirectional. To obtain a non-trivial limiting process, this drift must be subtracted off manually before taking limits. This subtraction can be absorbed into a rescaling of the growth rate and carrying capacity, similar to renormalization methods in theoretical physics [50], such that the final transformation from the LRC model to the LES model involves rescaling all four LRC model parameters.

The complete transformation from the LRC to LES model will be parameterized by a dimensionless scale parameter, α. The prescription is as follows. Start from an LRC model together with a target LES noise strength σ and fix $\lambda \ln^2 f = \sigma^2$. Then transform the LRC parameters according to

$$\lambda' = \alpha\lambda, \quad \ln f' = -\sigma/\sqrt{\lambda'},$$

$$r' = r\left(1 - \frac{\lambda' \ln f'}{r}\right), \ \ K' = K\left(1 - \frac{\lambda' \ln f'}{r}\right). \tag{3.8}$$

It is shown analytically in Appendix B that in the limit $\alpha \to \infty$, the LRC model $LRC(r', K', \lambda', f')$ gets mapped to an LES model $LES(r_{ES}, K_{ES}, \sigma)$, with $\sigma = \lambda \ln^2 f$, $r_{ES} = r(1 + (2r)^{-1}\sigma^2)$, and $K_{ES} = K(1 + (2r)^{-1}\sigma^2)$. In this way, the stationary mean of the process without extinction remains constant throughout this transformation, fixed at K. This is chosen as a convenient way to normalize the effects of noise. By adding constant offsets, the transformation can be tuned to preserve other properties (Appendix B). This transformation is shown visually in Figure 3A, which depicts numerical results for the stationary distribution of the LRC model (in log variables for visual reasons) being transformed with α increasing on the interval $(1, 75)$ (Materials and Methods). The distribution approaches that of the target LES model, shown in green in panel (vi) .

With this transformation, the question of relative risks of extinction under the LRC model and the LES model was revisited. The mean time to extinction, τ, was computed via stochastic simulation of the LRC model for various values of α (Figure 3B, bottom), rescaling LRC model parameters according to equation (8) for each α (Materials and Methods). The LRC model extinction time (purple circles) increases with increasing α and asymptotes to the LES model extinction time (green square). Computed numerically, the stationary population mean in the absence of extinction does indeed remain constant throughout the transformation (Figure 3B, top). The conclusion is again that there exists a significantly higher risk of extinction under random catastrophe dynamics than under environmental stochasticity dynamics.

This conclusion is robust across parameter space. Plotting the beginning and end points of the curve in Figure 3B for various values of carrying capacity reproduces the asymptotic power law behavior described by Lande for simpler

models [48] (Figure 3C), though the exponents obtained by linear fitting (Materials and Methods) are smaller for the LRC model across a wide range of effective noise strengths (Figure 3D). Note that because the growth rate is rescaled in this procedure, as long as the original growth rate r is positive, the long-run growth rate [48] is positive for all values of σ. The conclusion is also insensitive to the initial starting population, x_0, as the mean time to extinction becomes independent of x_0 above a critical threshold (see [32, 46] and Supplementary Figure 1). In addition to this method based on the diffusion limit, an alternative approach, in which the stationary means of the LRC and LES models equated simply by mapping $\sigma^2 = -2\lambda \ln f$, leads to the same conclusion (Supplementary Figure 2, Appendix C).

3.4. Discussion

This work presented new results for the Logistic Random Catastrophe (LRC) model, a model that serves both as a foundation for understanding the ecological consequences of random catastrophes and as an empirical model that describes real data [45, 46]. Exact analytic results for its stationary moments were derived using the method of moment equations. These expressions revealed that ensemble statistics are largely controlled by a single parameter that combines the average catastrophe rate and size, which is both a fundamental insight into the model's statistical structure and a useful result for the analysis of ecological data [45]. They also revealed the similarity in structure between the LRC model and its Gaussian noise counterpart, the Logistic Environmental Stochasticity (LES) model, which was exploited to construct the latter as a limit of the former. The mean time to extinction increased monotonically along this limit by orders of magnitude in

relevant regions of parameter space, indicating higher extinction risk under random catastrophe dynamics in general.

This heightened extinction risk has implications for the prioritization of conservations efforts in the face of different types of stochasticity. In the absence of detailed information, the results derived here imply that a system subject to random catastrophes should be considered to have higher risk of extinction than a comparable system experiencing strong environmental stochasticity. Moreover, for systems subject to random catastrophes, estimates of extinction risk obtained using Gaussian noise models will likely be underestimates.

In addition, given that large fluctuations appear to be intrinsic to intestinal microbiota [42, 43, 45], the enhanced extinction risk reported here may be important for understanding the evolution of functional redundancy across symbiotic taxa and of host biochemical networks that sense fluctuating microbial products. For example, it was recently discovered that a certain zebrafish intestinal bacterial species produces a compound that triggers the expansion of beta cells in the pancreas [56] during larval development. The same bacterial species has also been shown to exhibit random catastrophe dynamics driven by peristaltic contractions [45] during this developmental period. Given these large fluctuations, the question arises as to how the biochemical pathway that mediates this interaction evolved to remain sensitive yet robust, so to ensure proper timing of development. One possibility is that the sensing system effectively computes a type of running time average on the signal to smooth out large fluctuations. The diffusion limit of random catastrophes discussed here, with its roots in in the Central Limit Theorem, can viewed precisely as a kind of running average, or

coarse-graining, and so may have a direct biological realization in these types of sensing systems.

The diffusion limit method derived here is readily applied to the study of other statistics of the LRC model. It can also be easily adapted to other Markov models, including multi-species models [49, 54]. These generalizations allow for more computations, analogous to extinction times in Figure 3B, that could provide useful insight. For one example, it would be useful to revisit optimal control problems relevant for ecological management in the presence of random catastrophes, such as the harvesting strategies for fisheries considered in [57], and study how optimal policies evolve when discontinuous jumps limit to continuous environmental stochasticity. For another, evolutionary studies of bet hedging in the presence of catastrophes [58] could be directly mapped to the analogous problem in the presence of continuous noise [33], connecting ecological and evolutionary dynamics relevant for a wide variety of systems.

3.5. Materials and Methods

All code was written in MATLAB and is available at INSERT URL

3.5.1. LRC and LES model simulations

Sample paths of the differential Poisson process were generated as Bernoulli trials [52]. These paths were then used in the numerical integration of the LRC model. For all calculations except for the diffusion limit calculations in Figure 3, the logistic growth equation was integrated with the Euler method between jump times, at which the population was reduced by a factor of f. The LES model was integrated with a straightforward application of the Milstein method [59].

3.5.2. The diffusion limit

In the diffusion limit, jump sizes approach the size of deterministic growth in one numerical timestep. So, the deterministic contribution of order Δt must be retained, resulting in a more straightforward Euler-type integration scheme. In this case, an adaptive timestep is used, scaling $\Delta t' = \Delta t/\sqrt{\alpha}$, identically to $\ln f$, which sets the size of the jump. This scaling will lead to numerical artifacts when the probability of catastrophe in one timestep, $\lambda'\Delta t'$, approaches unity. Since $\lambda' = \alpha\lambda$, this will occur at $\alpha_c \sim (\lambda\Delta t)^{-2}$, and so can be put off by starting with a sufficiently small time step.

3.5.3. Extinction times

Extinction times were computed by straightforward stochastic simulation, following population trajectories from an initial population, x_0 until they reached the extinction threshold, x^*. To extract the exponent, ν, of the asymptotic relationship $\tau \sim K^\nu$, a linear fit to log-transformed variables was done for the larger half of the carrying capacity values, typically 5 data points.

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Appendix

.7. Detailed calculation of stationary moments

In this section an expression for the n^{th} stationary moment for the LRC model is derived. The approach is analogous for the LES model and since the results are already known [60], a detailed derivation isn't given, though one remark is made on the application of this method to diffusion processes.

.7.1. LRC model

Before beginning, the chain rule for jump processes [52] is stated without proof, for reference. Let X_t by a general process given by

$$dX_t = f(X_t, t)dt + h(X_{t^-}, t^-)dN_t \tag{.71}$$

with f and h deterministic functions, N_t a Poisson process with rate λ, and t^- denoting the Itô convention as in the main text. Further let $Y_t \equiv F(X_t, t)$ be a transformed process. Then Y_t is governed by

$$dY_t = \left(\partial_t F(X_t, t) + f(X_t, t)\partial_{X_t} F(X_t, t)\right) dt + \Delta Y_{t^-}^{jump} dN_t \tag{.72}$$

with $\Delta Y_{t^-}^{jump} \equiv F(X_{t^-} + h(X_{t^-}, t^-)) - F(X_{t^-}, t^-)$.

Now recall the LRC model,

$$dX_t = rX_t \left(1 - \frac{X_t}{K}\right) dt - (1 - f)X_t dN_t. \tag{.73}$$

The first step is to change variables to X_t^n using the stochastic chain rule for jump SDEs. The result is

$$dX_t^n = nrX_t^n\left(1 - \frac{X_t}{K}\right)dt - (1 - f^n)X_t^n dN_t. \tag{.74}$$

Then, each term in this SDE is averaged. The expectation of $X_{t^-}^n dN_t$ can be factored: $\mathbb{E}[X_{t^-}^n dN_t] = \mathbb{E}[X_{t^-}^n]\mathbb{E}[dN_t] = \mathbb{E}[X_{t^-}^n]\lambda dt$. Intuitively, this is because the two processes appear mutually independent. The Poisson process has independent increments, and since the Itô convention was used, $X_{t^-}^n$ is independent of N_t, which occurs in the future. This is certainly true for a discrete time model, but care must be taken in the continuous limit.

A more rigorous argument can be made using the Dominated Convergence Theorem. The case $n = 1$ is considered without loss of generality. Consider X_j, a discrete partition of the continuous time process X_t, such that $X_j \to X_t$ in probability. Then, sums of X_j converge in probability to integrals, in particular,

$$\sum_j X_{j-1}\Delta N_j \to \int_T X_{t^-} dN_t, \tag{.75}$$

where ΔN_j is a partition of the Poisson process. The Dominated Convergence Theorem says that if X_t is dominated by an integrable function on the interval T,

$$\mathbb{E}\left[\sum_j X_{j-1}\Delta N_m\right] \to \mathbb{E}\left[\int_T X_{t^-} dN_t\right] \tag{.76}$$

in probability. Since populations in the LRC model are bounded by the carrying capacity for all time, this is always valid. The expectation of the sum is

straightforward, leading to the result,

$$\mathbb{E}\left[\int_T X_{t^-} dN_t\right] = \int_T \mathbb{E}[X_{t^-}]\lambda dt, \tag{.77}$$

from which the infinitesimal version follows as a special case.

Factoring the expectation results in an ODE for the n^{th} moment. In the steady state, this becomes the recursion relation

$$\mathbb{E}[X^{n+1}] = K\left(1 - \frac{\lambda(1-f^n)}{nr}\right)\mathbb{E}[X^n]. \tag{.78}$$

Defining

$$c_n \equiv \left(1 - \frac{\lambda(1-f^n)}{nr}\right), \tag{.79}$$

the n^{th} moment can be expressed in terms of the mean as

$$\mathbb{E}[X^n] = K^{n-1}\left(\prod_{m=1}^{n-1} c_m\right)\mathbb{E}[X]. \tag{.710}$$

To complete the recursion relation, the mean must be computed independently. This is accomplished by changing variables to $\ln X_t$ using the chain rule for jump processes:

$$d\ln X_t = r\left(1 - \frac{X_t}{K}\right)dt + \ln f dN_t, \tag{.711}$$

which in the steady state gives an expression for the stationary mean,

$$\mathbb{E}[X] = K\left(1 + \frac{\lambda}{r}\ln f\right). \tag{.712}$$

Plugging this back into equation (A10) gives the final result

$$\mathbb{E}[X^n] = K^n \left(1 + \frac{\lambda}{r} \ln f\right) \prod_{m=1}^{n-1} \left(1 - \frac{\lambda(1-f^m)}{mr}\right). \tag{.713}$$

Evaluating this equation for $n = 2$ leads to the expression for the variance in the main text:

$$\mathrm{Var}[X]_{LRC} = K^2 \frac{\lambda}{r} \left(-\ln f - (1-f)\right) \left(1 + \frac{\lambda}{r} \ln f\right). \tag{.714}$$

.7.2. LES model

The derivation is analogous for the LES model, except that Itô's chain rule for diffusion processes is used. Since the results are already known [60], derived with traditional methods, a detailed computation will not be given. However, one remark worth making concerns the expectation of $X_{t^-} dB_t$. The intuitive argument outlined for the LRC model - that since the Itô convention was employed the expectation of the product can be factored - gives the correct answer in this case, but is in fact not generally valid. Essentially, for processes governed by equations of the form

$$dX_t = f(X_t, t)dt + g(X_{t^-}, t^-)dB_t, \tag{.715}$$

the integral $\int g(X_{t^-}, t^-)dB_t$ can acquire non-zero expectation if the function g grows too quickly. A classic example is the CEV model of quantitative finance [61], which is of the form $f(X_t, t) = X_t$ and $g(X_t, t) = X_t^\gamma$ for $\gamma > 1$. However, one can use the fact that the exponential version of the LES model, i.e. $K \to \infty$, is a well known SDE for which $\mathbb{E}\left[\int X_{t^-} dB_t\right] = 0$. This model is known as Geometric Brownian Motion and describes asset prices in the Black-Scholes model of quantitative finance [61]. Since paths of the exponential model almost surely

dominate paths of the LES model, $\int X_{t^-} dB_t$ for the LES model inherits the martingale property from the exponential case, which implies zero expectation.

Following the same procedure as for the LRC model, factoring expectations of $X^n_{t^-} dB_t$, results in

$$\mathbb{E}[X^n]_{LES} = K^n \left(1 - \frac{\sigma^2}{2r}\right) \prod_{m=1}^{n-1} \left(1 + \frac{(m-1)\sigma^2}{2r}\right). \tag{.716}$$

Special cases of this include

$$\mathbb{E}[X]_{LES} = K \left(1 - \frac{\sigma^2}{2r}\right) \tag{.717}$$

and

$$\mathrm{Var}[X]_{LES} = \frac{K^2\sigma^2}{2r} \left(1 - \frac{\sigma^2}{2r}\right). \tag{.718}$$

.8. The diffusion limit and the Central Limit Theorem

This section contains details of the construction of the LES model from the LRC model in the limit of infinitely frequent, infinitesimal catastrophes, referred to here as the diffusion limit. The complete transformation involves all four LRC model parameters and is specified as follows. Let α be a scale parameter, $LRC(r, K, \lambda, f)$ an LRC model, and σ be the target noise-strength parameter of the limiting LES model. Fix $\lambda \ln^2 f = \sigma^2$, and scale

$$\lambda' = \alpha\lambda, \quad \ln f' = -\sigma/\sqrt{\lambda'}$$

$$r' = r\left(1 - \frac{\lambda' \ln f'}{r}\right) = r\left(1 + \frac{\sigma\sqrt{\lambda}}{r}\sqrt{\alpha}\right),$$

$$K' = K\left(1 - \frac{\lambda' \ln f'}{r}\right) = K\left(1 + \frac{\sigma\sqrt{\lambda}}{r}\sqrt{\alpha}\right). \tag{.819}$$

The claim is that in taking the limit $\alpha \to \infty$, the LRC model $LRC(r', K', \lambda', f')$ gets mapped to an LES model $LES(r_{ES}, K_{ES}, \sigma)$, with $\sigma^2 = \lambda \ln^2 f$, $r_{ES} = r(1 + (2r)^{-1}\sigma^2)$, and $K_{ES} = K(1 + (2r)^{-1}\sigma^2)$, such that the stationary means of both models are equal. I first motivate the form of this transformation, which involves all four LRC model parameters, by studying the behavior of the stationary moments. I then show how the precise form of these limits, namely $\lambda \to \infty$, $f \to 1$, such that $\lambda \ln^2 f \to$ const., follows from functional generalizations of the Central Limit Theorem (CLT), in which a scaled, compensated Poisson process limits to Brownian motion. Finally, I show analytically how the full transformation maps the LRC model into the LES model.

.8.1. Motivation

As discussed in the main text, taking the limits $\lambda \to \infty$, $f \to 1$, such that $\lambda \ln f \to$ const. is analogous to the law of large numbers, leading to a deterministic limit. The correct limits instead are $\lambda \to \infty$, $f \to 1$, such that $\lambda \ln^2 f \to$ const., which I show below is analogous to the CLT. To motivate the final four parameter transformation, let us first consider the behavior of the LRC variance under these limits:

$$\begin{aligned} \mathrm{Var}[X] &= K^2 \frac{\lambda}{r}(-\ln f - (1-f))\left(1 + \frac{\lambda}{r}\ln f\right) \\ &\xrightarrow{\text{limits}} K^2 \frac{\lambda \ln^2 f}{2r}\left(1 + \frac{\lambda}{r}\ln f\right) \\ &= K^2 \frac{c^2}{2r} - K^2 \frac{c^3}{r^2}\sqrt{\lambda}. \end{aligned} \tag{.820}$$

with $c = \text{ const } = -\sqrt{\lambda}\ln f$. In taking the limit $f \to 1$, the relation $(-\ln f - (1 - f)) \to 2^{-1}\ln^2 f$ was used, based on a 2^{nd} order Taylor expansion.

The variance diverges, but a part of it remains finite. The finite piece of the variance in this limit is exactly the variance of an LES model with $\sigma^2 = \lambda \ln^2 f$ and increased growth parameters $K_{ES} = K(1 + (2r)^{-1}\sigma^2)$, and $r_{ES} = r(1 + (2r)^{-1}\sigma^2)$. Looking at the behavior of the mean in this limit leads to the same conclusion. This suggests that this limit does take the LRC model into an LES model, but one that is accompanied by a noise-induced drift that diverges as $\sqrt{\lambda}$. This is divergence should be expected, as it reflects the unidirectionality of jumps in the LRC model, which is absent in the LES, analogous to the divergence of the mean of a Poisson distribution when it limits to a Gaussian. To obtain a non-trivial limiting process, this drift needs to be subtracted off, for example, by adding a term $-\lambda \ln f dt$ to the LRC model SDE. This is equivalent to rescaling the growth rate and carrying capacity each by a factor of $(1 - r^{-1}\lambda \ln f)$, leading to the full four parameter transformation.

.8.2. Functional Central Limit Theorems

The form of the limits $\lambda \to \infty$, $f \to 1$, such that $\lambda \ln^2 f \to$ const., is a direct consequence of the CLT. The classical CLT says that given a set of n random variables, $\{\xi_j\}$, that are identically and independently distributed (i.i.d.) with mean μ and finite variance σ^2, the sum of the deviations of these variables from their mean, when rescaled by $\sqrt{n}$, tends in distribution to a Gaussian variable as $n \to \infty$:

$$\lim_{n\to\infty} \frac{\sum_j \xi_j - n\mu}{\sqrt{n}} = \eta \sim \mathcal{N}(0, \sigma^2) \tag{.821}$$

where $\mathcal{N}(\mu, \sigma^2)$ is a Gaussian distribution with mean μ and variance σ^2. The condition that the variables ξ_j follow identical distributions can be relaxed, but we focus on this restricted case here.

A vast body of mathematical literature concerns the construction of generalization of the CLT to stochastic processes. One important generalization, which we will employ in the study of the Poisson process, is Donsker's theorem [49]. Donsker's theorem dictates the limit of a sequence of stochastic process, $X_t^{(n)}$, constructed from sums of i.i.d. random variables $\tilde{\xi}_j$ with zero mean and finite variance via

$$X_t^{(n)} \equiv \frac{1}{\sqrt{n}} \sum_{j=1}^{[ns]} \tilde{\xi}_j, \quad ns \equiv t. \tag{.822}$$

Here we have introduced time as multiples of a unit s, such that $t = ns$, and [...] denotes the integer part. Donsker's theorem says that as $n \to \infty$ with $s \to 0$ such that $ns \to t$ for arbitrary t, the processes $X_t^{(n)}$ converge in law to Brownian motion,

$$X_t^{(n)} \to B_t. \tag{.823}$$

Donsker's theorem can be used to show the convergence of the compensated Poisson process to Brownian motion in particular limits. The idea is to write the Poisson process as a sum of intervals which themselves are i.i.d. random variables that meet the criteria for Donsker's theorem, and then scale the jump size and rate in the ways that map onto the $n \to \infty$ limit. This approach is based on a method known as finite dimensional convergence, which is only applicable to processes with independent increments [49].

Consider breaking a scaled, compensated Poisson process, $\epsilon\tilde{N}_t$ into a sum of finite intervals,

$$\epsilon\tilde{N}_t = \epsilon \sum_{j=1}^{[ns]} \Delta\tilde{N}_j. \tag{.824}$$

From inspection, we see that the appropriate mapping is $\lambda \to n\lambda$, $\epsilon = \sigma\lambda^{-1/2}$, in which case

$$\epsilon\tilde{N}_t = \sigma \sum_{j=1}^{[ns]} \frac{\Delta\tilde{N}_j/\sqrt{\lambda}}{\sqrt{n}}. \tag{.825}$$

Donsker's theorem can then be applied with $\tilde{\xi}_j \equiv \Delta\tilde{N}_j/\sqrt{\lambda}$, resulting in

$$\epsilon\tilde{N}_t \xrightarrow{\lambda\to\infty,\ \epsilon\to 0} \sqrt{\lambda}\epsilon B_t. \tag{.826}$$

In the LRC model, collapse size is forced to zero by taking $f \to 1$. This still leaves room for how exactly λ and f should map on to σ. One choice would be to take $\epsilon = -(1-f)$, such that $\sigma^2 = \lambda(1-f)^2$. In this case, a quick calculation shows that the limiting process would be an LES model with unchanged growth parameters, (r, K), and consequently a reduced stationary mean of $K(1-(2r)^{-1}\sigma^2)$, whereas the original LRC process, after removing the divergence of $\lambda \ln f$, has a stationary mean of K. Alternatively, one could take $\epsilon = \ln f$, such that $\sigma^2 = \lambda \ln^2 f$. This is the case examined above, which results in an LES model with an unchanged stationary mean, but altered growth parameters. Since our present goal is to normalize the effect of noise to construct a fair comparison of extinction risk, the latter choice is more appropriate.

.8.3. Convergence of LRC to LES

I now discuss the convergence of the LRC model to the LES model via the convergence of the Poisson process to Brownian motion discussed above. General conditions for the convergence of a pure jump Markov process to a diffusion are given in [49]. Rather than verify these general conditions here, I'll take a more intuitive approach that exploits the simplicity of the present models and uses the fact that both the LRC model and the LES model possess unique, strong solutions, as follows from special cases of a general result derived in [54]. This allows us to uniquely define a sequence of processes $X'_t(\alpha) \equiv F[\tilde{N}_t(\alpha); r(\alpha), K(\alpha), \lambda(\alpha), f(\alpha)]$, where F is the solution to the LRC model depending on parameters r, K, λ, and f, and the limit of the sequence $X^*_t \equiv \lim_{\alpha\to\infty} X'_t(\alpha)$. Existence and uniqueness of solutions to both models allows us in principle to take the limit and then invert the solution, recovering a diffusion SDE. In practice, we can take the limit directly in the context of the LRC model SDE. To begin, recall the LRC model,

$$dX_t = rX_t\left(1 - \frac{X_t}{K}\right)dt - (1-f)X_{t^-}dN_t. \tag{.827}$$

Let us expand $(1-f)$ in powers of $\ln f$ to second order and write the Poisson process in terms of its mean and compensated process.

$$dX_t = rX_t\left(1 - \frac{X_t}{K}\right)dt + \left(\ln f + \frac{1}{2}\ln^2 f\right)X_t\lambda dt + \left(\ln f + \frac{1}{2}\ln^2 f\right)X_{t^-}d\tilde{N}_t + \mathcal{O}(\lambda \ln^3 f) \tag{.828}$$

Now let us absorb the mean drift of the Poisson process as scaling factors for the growth rate and carrying capacity

$$dX_t = r\left(1+\frac{\lambda}{r}\ln f+\frac{\lambda}{2r}\ln^2 f\right)X_t\left(1+\frac{X_t}{K\left(1-\frac{\lambda}{r}\ln f+\frac{\lambda}{2r}\ln^2 f\right)}\right)dt + \left(\ln f+\frac{1}{2}\ln^2 f\right)X_{t^-}d\tilde{N}_t+\mathcal{O}(\lambda\ln^3 f). \quad (.829)$$

Now we apply the transformation [19] with α finite and evaluate r' in terms of r and K' in terms of K. This has the effect of canceling all $\lambda\ln f$ terms, as intended.

$$dX'_t(\alpha) = r\left(1+\frac{\lambda'}{2r}\ln^2 f'\right)X'_t\left(1-\frac{X'_t}{K\left(1+\frac{\lambda'}{2r}\ln^2 f'\right)}\right)dt + \left(\ln f'+\frac{1}{2}\ln^2 f'\right)X'_{t^-}d\tilde{N}'_t+\mathcal{O}(\lambda\ln^3 f) \quad (.830)$$

where primed variables depend on α. Before taking the $\alpha\to\infty$ limit, we can identify $\lambda'\ln^2 f'$ as σ^2, a finite constant independent of α,

$$dX'_t(\alpha) = r\left(1+\frac{\sigma^2}{2r}\right)X'_t\left(1-\frac{X'_t}{K\left(1+\frac{\sigma^2}{2r}\right)}\right)dt+\left(\ln f'+\frac{1}{2}\ln^2 f'\right)X'_{t^-}d\tilde{N}'_t+\mathcal{O}(\lambda\ln^3 f). \quad (.831)$$

We can now evaluate the $\alpha\to\infty$ limit, knowing how $\tilde{N}_t$ transforms: $\ln f' d\tilde{N}_t\to\sigma dB_t$ in law, $\ln^2 f' d\tilde{N}_t\to 0$, resulting in

$$\lim_{\alpha\to\infty} dX'_t(\alpha) = dX^*_t = r\left(1+\frac{\sigma^2}{2r}\right)X^*_t\left(1-\frac{X^*_t}{K\left(1+\frac{\sigma^2}{2r}\right)}\right)dt+\sigma X^*_{t^-}dB_t. \quad (.832)$$

The limiting process is an LES model with increased growth parameters $r_{LES} = r(1 + (2r)^{-1}\sigma^2)$ and $K_{LES} = K(1 + (2r)^{-1}\sigma^2)$. Comparing this model to the transformed LRC model of [31] using the analytic results for the stationary mean equations (A12) and (A17) reveals that the two models do indeed have the same stationary mean.

.9. An alternative mapping that equates stationary means

The stationary means of the LRC and LES models can also be equated by using the same growth rate and carrying capacities and mapping $\sigma^2 = -2\lambda \ln f$, as is clear from equations (A12) and (A17). This mapping provides an alternative method of quantitatively comparing the two models, though one that is perhaps less meaningful than the diffusion limit approach. It can be understood intuitively by plotting the time evolution of the mean population of both models in the presence and absence of extinction (Supplementary Figure 2A). In the absence of extinction, both models asymptote to the same value. In the presence of extinction, the LRC model average decays to zero faster than the LES model average, indicating higher extinction risk. Computed directly, the mean times to extinction for the LRC model are significantly shorter than for the LES model (Supplementary Figure 2B), supporting the conclusions of the diffusion limit-based method.

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APPENDIX A

SUBLETHAL ANTIBIOTICS COLLAPSE GUT BACTERIAL POPULATIONS BY ENHANCING AGGREGATION AND EXPULSION

Antibiotics induce large and highly variable changes in the intestinal microbiome even at sublethal concentrations, through mechanisms that remain elusive. Using gnotobiotic zebrafish, which allow high-resolution examination of microbial dynamics, we found that sublethal doses of the common antibiotic ciprofloxacin cause severe drops in bacterial abundance. Contrary to conventional views of antimicrobial tolerance, disruption was more pronounced for slow-growing, aggregated bacteria than for fast-growing, planktonic species. Live imaging revealed that antibiotic treatment promoted bacterial aggregation and increased susceptibility to intestinal expulsion. Intestinal mechanics therefore amplify the effects of antibiotics on resident bacteria. Microbial dynamics are captured by a biophysical model that connects antibiotic-induced collapses to gelation phase transitions in soft materials, providing a framework for predicting the impact of antibiotics on the intestinal microbiome.

Introduction

Antibiotic drugs induce large, long-lasting, and disease-associated alterations in the composition of the intestinal microbiota [62, 63, 64]. Even at concentrations well below the minimum inhibitory levels of many bacteria, antibiotics can lead to major and highly variable changes in the gut microbiome through mechanisms that remain mysterious [63, 64, 65]. Sublethal antibiotics can also significantly alter animal physiology; the intentional growth enhancement of livestock is a well-known

example that may involve microbiome-mediated pathways [63]. Low concentrations of antibiotics are often present in the environment as byproducts of unchecked agricultural and biomedical use, generating public health concerns associated with the emergence of drug resistance [66] as well as more direct impacts on human health [67]. It is therefore crucial to uncover mechanisms by which sublethal antibiotics reshape resident gut microbial communities. Understanding why particular bacterial strains are resilient or susceptible to antibiotic perturbations may allow us to predict the consequences of environmental contamination and may enable tailoring of antibiotic treatments as a therapeutic tool for manipulating the intestinal microbiome.

Conventional wisdom regarding bacterial responses to antibiotic drugs, derived largely from in vitro assays, holds that drug tolerance is facilitated by low growth rates and biofilm formation [68, 69]. Recent work suggests that microbes in the vertebrate gastrointestinal tract adopt a variety of growth and aggregation phenotypes [70, 71, 72, 73], raising the question of whether antibiotic susceptibility in the gut bears the same relationship to kinetics and physical structure as in less dynamic environments, or whether the strong mechanical activity and large fluid flows present in the intestine [74] lead to fundamentally different rules.

To investigate the in vivo response of gut bacteria to low-dose antibiotic exposure, especially the relationship between susceptibility and bacterial behavior, we conducted live imaging-based studies of larval zebrafish (Fig. 1A, 1B), spanning the entire intestinal volume with spatial and temporal resolutions not attainable in humans or other model vertebrates. We focused our study on two native zebrafish bacterial isolates, both frequently found in the intestine [75], that we identified as representing extremes of growth and aggregation phenotypes [71].

The first, *Vibrio cholerae* ZWU0020, hereafter referred to as "*Vibrio*", exists in the larval zebrafish intestine primarily as dense populations of highly motile and planktonic individuals (Fig. 1C, SI Movie 1). *Vibrio* grows rapidly, with an in vivo doubling time of approximately 1 hour (exponential growth rate of 0.8 ± 0.3 1/hr) [76]. The second, *Enterobacter cloacae* ZOR0014, hereafter referred to as "*Enterobacter*" primarily forms large, dense bacterial aggregates with small sub-populations of non-motile planktonic cells (Fig. 1D, SI Movie 2) [77] and has an in vivo doubling time of approximately 2.5 hours (exponential growth rate of 0.27 ± 0.05 1/hr) (SI Appendix, Fig. S1). To delineate and quantify antibiotic responses independent of inter-bacterial competition, we studied *Vibrio* and *Enterobacter* separately in hosts that were initially raised germ-free (Materials and Methods). We assessed response dynamics of each bacterial population after treatment with the antibiotic ciprofloxacin, a broad spectrum fluoroquinolone that interferes with DNA replication by inhibiting DNA gyrase. Ciprofloxacin is widely administered therapeutically and has been used as a model antibiotic in studies of human microbiome disruption [78]. Furthermore, ciprofloxacin is often detected in environmental samples at ng/ml concentrations that are sublethal but capable of perturbing bacterial physiology [79, 80].

As detailed below, we discovered that sublethal levels of ciprofloxacin lead to major reductions in intestinal abundance of both *Vibrio* and *Enterobacter* that could not be predicted from in vitro responses alone. In contrast to conventional wisdom, the slow-growing and highly aggregated *Enterobacter* was impacted far more severely than the fast-growing, planktonic *Vibrio*. Changes in bacterial abundances were driven primarily by clearance from the intestine by peristaltic-like fluid flow, which impacts aggregated bacteria more severely than planktonic

cells. Exposure to sublethal levels of ciprofloxacin shifted both species to a more aggregated state, but for *Enterobacter* this state was unsustainable and led to population collapse and extinction. Quantitative image-derived population data motivate and are well fit by physical models originally used to describe colloidal growth and polymer gelation, implying an antibiotic-induced phase transition in bacterial community physical structure and revealing a general framework for understanding and predicting intestinal antibiotic perturbations.

Results

Low-dose ciprofloxacin increases bacterial aggregation and intestinal expulsion

For both *Vibrio* and *Enterobacter*, we empirically determined a ciprofloxacin dosage that induced clear changes in bacterial physiology and behavior in vitro, but that was below the apparent minimum inhibitory concentration. We first describe results of antibiotic exposure, in vitro and in vivo, for the *Vibrio* species.

From an initial survey of dose-response in rich media, we identified 10 ng/mL ciprofloxacin as an appropriate exposure for *Vibrio* populations. Growth of *Vibrio* in lysogeny broth in the presence of 1 ng/ml ciprofloxacin closely resembles that of the untreated control, while a concentration of 100 ng/ml is largely inhibitory (SI Appendix, Fig. S2A). An intermediate concentration of 10 ng/ml leads to a stable, intermediate optical density. Viability staining (Materials and Methods) after 6 hours of incubation with 10 ng/ml ciprofloxacin identifies 30-80% of cells as alive (SI Appendix, Fig. S3A and S3B), again consistent with this antibiotic concentration being sufficient to perturb the bacterial population without overwhelming lethality. Growth in the presence of 10 ng/ml ciprofloxacin induces marked changes in cell morphology and motility: treated cells exhibit filamentation,

making them considerably longer (mean ± std. dev. 5.3 ± 3.1 μm) than untreated *Vibrio* (2.9 ± 0.9 μm) (SI Appendix, Fig. S2B). Swimming speed was also reduced compared to untreated cells (mean ± std. dev. 11.4 ± 7.2 μm/s, untreated 16.9 ± 11.1 μm/s) (SI Appendix, Fig. S2C, SI Movies 3 and 4). We note also that 10 ng/ml ciprofloxacin is comparable to levels commonly measured in environmental samples [79].

While useful for illuminating the appropriate sub-lethal concentration to further examine, experiments in rich media conditions are not an optimal assay for comparison of in vitro and in vivo antibiotic treatments, as the chemical environments are likely very dissimilar. We therefore assessed effects of ciprofloxacin on bacterial populations in the aqueous environments of the flasks housing the larval zebrafish in comparison to populations in the intestines. In the flask water, as in the intestine, the only nutrients are fish-derived. Oxygen levels are comparable to those in the larval gut, due to fast diffusion and the animals' small size. Bacteria in flask water therefore constitute a useful baseline against which to compare antibiotic impacts on intestinal populations.

Vibrio was associated with germ-free zebrafish at 4 days post-fertilization (dpf) by inoculation of the aqueous environment at a density of 10^6 cells/ml (Materials and Methods) and allowed to colonize for 24 hours, which based on previous studies provides ample time for the bacterial population to reach its carrying capacity of approximately 10^5 cells/gut [76]. Animals and their resident *Vibrio* populations were then immersed in 10 ng/ml ciprofloxacin for 24 or 48 hours, or left untreated (Fig. 2A and 2B). *Vibrio* abundances in the gut were assayed by gut dissection and plating to measure colony forming units (CFUs) (Materials and Methods). Abundances in the flask water were similarly assayed

by plating. We quantified the effect of the antibiotic treatment by computing the ratio of bacterial abundances in the treated and untreated cases, resulting in a normalized abundance (Fig. 2C). After a 24 hour treatment, $\log_{10}$-transformed abundances in the flask water dropped by 0.98 ± 0.4 (mean $\pm$ std. dev.) compared to untreated controls, or one order of magnitude on average. In contrast, $\log_{10}$-transformed intestinal abundances showed a more severe reduction of 1.75 ± 0.88 (Fig. 2C), or a factor of approximately 60 on average, suggesting that the intestinal environment amplifies the severity of ciprofloxacin treatment. For the 48 hour treatment, the declines in flask water and intestinal abundances were similarly severe (Fig. 2C). In terms of absolute abundances, pooled data from 24 and 48 hour treatments gives a mean $\pm$ std. dev. of the $\log_{10}$-transformed *Vibrio* population of 3.1 ± 0.9 ($n = 40$), compared to 4.9 ± 0.5 ($n = 42$) for untreated specimens (Fig. 2D). Unpooled data are similar (SI Appendix, Fig. S3E, S3F).

To assess the possibility that the intestine makes *Vibrio* more susceptible to ciprofloxacin-induced cell death, we embedded larval zebrafish in a 0.5% agarose gel, which allowed collection of expelled bacteria. After staining expelled bacterial cells with the viability dyes SYTO9 and propidium iodide, we imaged ejected material. We found no detectable difference between ciprofloxacin-treated and untreated populations (Fig. 2F, insets). Similarly sizeable fractions of viable and non-viable cells are evident in both ciprofloxacin-treated and untreated populations; however, co-staining of zebrafish host cells hindered exact quantification (SI Appendix, Fig. S4). This result suggests that the ciprofloxacin-induced population decline observed in vivo occurs independent of overt cell death and is a consequence of the response of living bacteria to the intestinal environment. We further note that the dose-response of the intestinal *Vibrio* abundance (SI Appendix, Fig. S5)

mirrors the dose-response of the in vitro growth rate, implying that the larval gut does not significantly alter or concentrate ciprofloxacin. This is also consistent with the widespread use of zebrafish larvae as a pharmacological screening platform, as water soluble chemicals readily enter and leave the animal [81, 82].

To investigate the causes of ciprofloxacin's disproportionately large impact on in vivo bacterial abundance, we used light sheet fluorescence microscopy to directly monitor *Vibrio* populations within the intestine over several hours as they responded to antibiotic exposure. Three-dimensional time-lapse imaging revealed that within hours of ciprofloxacin treatment, large numbers of bacteria became depleted from the anterior-localized planktonic and motile population (SI Movies 5 and 10). Cells were instead found in the mid and distal regions of the gut, where they appeared to be condensed into large multicellular aggregates prior to being expelled from the gut altogether (SI Movies 5 and 11). After 10 hours of exposure, *Vibrio* populations in ciprofloxacin-treated hosts contained large, 3D aggregates localized to the posterior of the intestine, a feature not observed in untreated controls (Fig. 2E and 2F) nor in all previous characterizations of this strain [71, 76]. We note also that in vitro, antibiotic-treated *Vibrio* does not form large aggregates (SI Appendix, Fig. S3 and S6, SI Movie 4)

To determine whether the bacterial aggregation observed in vivo stems from a fundamentally different response to antibiotics at the single-cell level or different large-scale consequences of similar cell-level response, we generated in *Vibrio* a genetically encoded fluorescent reporter of the SOS pathway (SI Appendix, Fig. S7, Materials and Methods), a DNA damage repair pathway induced by genotoxic agents such as ciprofloxacin [83, 84]. Genes in the SOS regulon halt replication and enable DNA repair, and also affect motility and biofilm formation [80, 85]. In

vitro, we found that treatment with 10 ng/ml ciprofloxacin strongly induced *recN*-based SOS reporter activity, with a heterogeneous response across individual cells (SI Appendix, Fig. S3C and S3D). Within the intestine, SOS reporter activity was also heterogeneous, appearing in both planktonic and aggregated cells. Planktonic cells that were SOS-positive appeared more filamented and less motile compared to SOS-negative cells within the same host (SI Movie 6). The activation of the SOS reporter in vitro and in vivo by ciprofloxacin (SI Movie 6 and Fig S3C and S3D) suggests that in both cases a canonical SOS response is involved in the perturbation of *Vibrio* physiology.

Together, these data begin to reveal a mechanism by which the intestine amplifies the effect of low-dose ciprofloxacin. Individual *Vibrio* cells first undergo an SOS response that is associated with changes in cellular morphology and behavior. In the context of the mechanical activity of the intestine, these molecular and cellular-level changes then give rise to population-level aggregation and spatial reorganization throughout the entire length of the intestine, with the population shifting its center of mass posteriorly (Fig. 2G, $n = 4$ per case). This process culminates in the expulsion of large bacterial aggregates from the host, causing a precipitous decline in total bacterial abundance (Fig. 2H).

Low-dose ciprofloxacin suppresses small cluster reservoirs associated with intestinal persistence

In contrast to *Vibrio*, *Enterobacter* is slower growing, non-motile, and naturally forms dense aggregates within the zebrafish intestine. *Enterobacter* populations have an in vivo growth rate of 0.27 ± 0.05 h^{-1} (mean $\pm$ std. dev, SI Appendix, Fig. S1), compared to 0.8 ± 0.3 h^{-1} for *Vibrio* [76]. Based on

conventional notions of antibiotic tolerance, we hypothesized that *Enterobacter* would be less affected by ciprofloxacin treatment than the fast growing, planktonic *Vibrio*. However, as detailed below, we found this prediction to be incorrect; *Enterobacter* exhibits an even greater response to low-dose ciprofloxacin.

We first established in vitro that 25 ng/ml ciprofloxacin produces similar effects on *Enterobacter* growth as did 10 ng/ml exposure on *Vibrio*. With the identical inoculation procedure used for *Vibrio*, $\log_{10}$-transformed *Enterobacter* abundance in the flask water dropped by 1.2 ± 0.4 (mean $\pm$ std. dev.) compared to untreated controls after 24 hours, and dropped by 1.8 ± 0.2 after 48 hours (Fig. 3A). These values match well the values for *Vibrio*: 0.98 ± 0.37 for 24 hours, 1.81 ± 0.5 for 48 hours. Assays in rich media show a similarly reduced density between the two species (SI Appendix, Fig. S8) and an even lesser degree of cell death and damage in vitro for *Enterobacter* as compared to *Vibrio*, with a viable fraction of approximately 95% (SI Appendix, Fig. S9A and S9B). As with *Vibrio*, in vitro growth measurements and viability staining both imply that low-dose ciprofloxacin treatment of *Enterobacter* induces growth arrest rather than widespread lethality.

Strikingly, low-dose ciprofloxacin treatment of fish colonized with *Enterobacter* (Materials and Methods) resulted in even greater reductions in abundance than in the case of *Vibrio*, with the majority of populations becoming nearly or completely extinct during the assay period (Fig. 3A and 3B). Inoculation, treatment, dissection, and plating were performed as for *Vibrio* (Materials and Methods). Compared to untreated controls, $\log_{10}$-transformed intestinal abundances were reduced by 2.3 ± 1.1 after 24 hours, and by 3.2 ± 1.0 after 48 hours (Fig. 3A). These reductions in intestinal abundances greatly exceeded the reductions of bacterial abundances in the flask water (Fig 3A). In terms of absolute abundances,

pooled data from 24 and 48 hour treatments gives a mean ± std. dev. of the $\log_{10}$-transformed *Enterobacter* population of 1.5 ± 1.0 ($n = 40$), compared to 4.0 ± 1.0 ($n = 39$) for untreated specimens (Fig. 3B); unpooled data are similar (SI Appendix, Fig. S9C and S9D).

Live imaging of intestinal populations at single time points revealed approximately 40% of treated hosts to be devoid or nearly devoid of *Enterobacter*, consistent with the plating-based measurements. In hosts that contained appreciable bacterial populations we observed a clear difference between treated and untreated specimens: *Enterobacter* populations in ciprofloxacin-treated hosts contained fewer small bacterial clusters and fewer individual planktonic cells than untreated controls (Fig. 3C and 3D). We quantified this distinction using computational image analysis to identify each cluster (Materials and Methods), defining a single cell as a cluster of size one. Bacterial populations in ciprofloxacin-treated animals contained ∼80x fewer clusters than untreated animals (Fig. 3C). Viability staining showed that there were no obvious differences in the viable fractions of bacteria expelled from the intestines of untreated and treated hosts (Fig. 3D, insets, SI Appendix, Fig. S10). As with *Vibrio*, these observations suggested that the reduction in *Enterobacter*'s intestinal abundance was independent of cell death.

Previous studies of other naturally aggregated bacterial species have revealed that large bacterial aggregates are highly susceptible to expulsion from the gut [76, 86]. To establish whether this is also the case for *Enterobacter* in the absence of low-dose ciprofloxacin treatment, we performed time-lapse 3D imaging (Materials and Methods). Indeed, in 2 out of 5 hosts imaged for 3.5 hours each, we observed events in which the largest bacterial aggregate was abruptly expelled from the

intestine (Fig. 4A and SI Movie 7). These time-lapse movies also showed clear examples of cluster aggregation (SI Movie 8), in which single cells and small aggregates appear to come together and fuse, a process that is likely due to the rhythmic intestinal contractions that occur between frames. Importantly, smaller aggregates and planktonic cells that preferentially localize to the intestinal bulb are relatively undisturbed during these expulsion events, save for a few clusters that become incorporated into the large mass during its transit (SI Movie 7).

Our observations suggest an explanation of how low-dose ciprofloxacin can lead to dramatic drops in *Enterobacter* abundance that moreover illuminates the more general question of how naturally aggregating bacterial species can persist in the vertebrate gut in spite of transport-driven expulsion. We provide both a qualitative and a quantitative description of the relevant dynamics, beginning with the following conceptual model: single cells of *Enterobacter* replicate to form small clusters, which then aggregate to form larger clusters under the influence of intestinal flow. Large clusters are transported by the rhythmic contractions of the gut [76, 86, 87] and are stochastically expelled from the host [76, 86]. The individual bacteria and small clusters that remain within the intestine serve as a reservoir that reseeds the next population, and the process of replication, aggregation, and expulsion repeats. Therefore, persistence within the intestine requires processes that generate single cells or small clusters, otherwise transport will eventually lead to extinction. This reseeding could take the form of (i) immigration of new cells from the environment, (ii) passive fragmentation of clusters, or (iii) active fragmentation in which single cells break away from a cluster surface during cell division. Immigration from the environment likely occurs even in established populations, but measurements in larval zebrafish suggest very low rates

of immigration [88]. We therefore suspected that more robust mechanisms must promote persistence. Supporting the active fragmentation mechanism, we found in untreated hosts examples of *Enterobacter* populations that contain an abundance of single cells, a single large aggregate, and a lack of mid-sized aggregates (SI Appendix, Fig. S9E). Following low-dose ciprofloxacin treatment, the planktonic cell reservoir associated with resilience to intestinal transport is depleted (Fig. 3C), most likely due to stalled *Enterobacter* division (SI Appendix, Fig. S8), leading to collapse of the resident bacterial population (Fig. 3A and 3B).

A quantitative model of bacterial cluster dynamics

To solidify and test our conceptual picture, we developed a predictive mathematical model of bacterial cluster dynamics. We describe the framework of the model, its validation, and general insights it provides into perturbations and population stability. Drawing on ideas from non-equilibrium statistical mechanics and soft matter physics, we constructed a general kinetic model that describes the time evolution of a collection of bacterial clusters with varying sizes, illustrated schematically in Fig. 4B. We posit that four processes govern cluster dynamics: aggregation, fragmentation, growth, and expulsion. Each is described by a kernel that specifies its rate and size dependence: (1) aggregation of a cluster of size n and a cluster of size m occurs with rate A_{nm}; (2) fragmentation of a cluster of size $n+m$ into clusters of size n and m occurs with rate F_{nm}; (3) growth (due to cell division) of a cluster of size n occurs with rate G_n; (4) expulsion (removal by intestinal transport) of a cluster of size n occurs with rate E_n. Note that condensation of the population into a single massive cluster poises the system for extinction, for any nonzero E_n. The model is non-spatial and is inspired by well established

frameworks for nucleation and growth phenomena such as polymer gelation and colloidal aggregation [89]. For example, sol-gel models describe a transition between dispersed individual units ("sol") and a system-spanning connected network ("gel") in materials capable of polymerization. In the thermodynamic limit of infinite system size, the model can be studied using standard analytic techniques [89]. However, unlike polymer solutions and other bulk systems for which the possible number of clusters is effectively unbounded, our intestinal populations are constrained to have at most a few hundred clusters (Fig. 3C), necessitating the use of stochastic simulations (Materials and Methods).

In its general form, the model encompasses a wide range of behaviors that can be encoded in the various functional forms possible for the rate kernels A_{nm} F_{nm}, G_n, and E_n. Based on our observations and theoretical considerations elaborated in the Materials and Methods section, we made the following assumptions: (1) the rate of aggregation between two clusters is independent of their size, $A_{nm} = \alpha$; (2) fragmentation occurs only by separation of single cells and with a rate that is independent of cluster size, $F_{nm} = \beta$ for $m = 1$ and $F_{nm} = 0$ otherwise; (3) growth is logistic with a global carrying capacity, $G_n = rn(1 - N/K)$ with N the total number of cells, r the per capita growth rate, and K, the carrying capacity; (4) expulsion is independent of cluster size, $E_n = \lambda$. This model contains as special cases various simple models of linear polymers [90] and also resembles recent work modelling chains of *Salmonella typhimurium* cells in the mouse gut [91]. As discussed in the SI Appendix, these choices constitute the minimal model consistent with theoretical constraints and experimental phenomenology. More complex models are of course possible, but the requisite increase in the number of

adjustable parameters would result in a trivial but meaningless ability to fit the observed data.

Even with the assumptions described above, the model needs 5 parameters: rates of aggregation, fragmentation, growth, and dispersal, and a global carrying capacity. However, all of these parameters can be set by experimentally derived values unrelated to cluster size distributions. We measured *Enterobacter*'s per capita growth rate by performing time-lapse imaging of initially germ-free hosts that had been exposed to *Enterobacter* for only 8 hours, capturing the exponential increase of a small founding population (SI Appendix, Fig. S1, SI Movie 9), yielding $r = 0.27 \pm 0.05$ hr^{-1} (mean $\pm$ std. dev across $n = 3$ hosts). We identified expulsion events as abrupt collapses in *Enterobacter* abundance from time-lapse images (Fig. 3C, SI Movie 7) and set the expulsion rate equal to the measured collapse rate, $\lambda = 0.11 \pm 0.08$ hr^{-1} (mean $\pm$ standard error, assuming an underlying Poisson process (Materials and Methods)). The model can be simulated to provide the mean and variance of the $\log_{10}$-transformed abundance distribution at a given time for a given set of parameters. Using this approach, we fit static bacterial abundance measurements from dissection and plating at 72 hours post-inoculation (Materials and Methods) to determine the carrying capacity, K, and the ratio of fragmentation and aggregation rates, β/α. As discussed in the Materials and Methods section, the cluster dynamics should depend primarily on the ratio of β/α rather than either rate separately. This yielded $\log_{10} K = 5.0 \pm 0.5$ and $\log_{10} \beta/\alpha = 2.5 \pm 0.4$.

The model therefore allows a parameter-free prediction of the size distribution of *Enterobacter* aggregates, plotted in Fig. 5A together with the measured distribution derived from three-dimensional images, averaged across 12 untreated

hosts. The two are in remarkable agreement. We also plot, equivalently, the cumulative distribution function $P(\text{size} > n)$, the probability that a cluster will contain greater than n cells, again illustrating the close correspondence between the data and the prediction and validating the model. We emphasize that no information about the cluster size distribution was used to estimate any of the model parameters. We further note that the cluster size distribution is a stringent test of the model's validity. Other cluster models predict different forms, typically with steep tails [90, 91]. The linear chain model of [91], for example, leads to an exponential distribution of cluster sizes that does not match the shallower, roughly power-law form of our data.

The abundance phase diagram and extinction transition

Our kinetic model provides insights into the consequences of low-dose antibiotic perturbations on gut bacterial populations. We consider a general phase diagram of possible growth, fragmentation, aggregation, and expulsion rates, and then situate *Enterobacter* in this space. For simplicity of illustration, we consider a two-dimensional representation with one axis being the ratio of the fragmentation and aggregation rates, β/α, and the other being the ratio of the growth and expulsion rates, r/λ (Fig. 5B). As noted above and in the Materials and Methods section, the model in the regime studied should depend on the ratio β/α rather than on β or α independently. However, the roles of r and λ are not simply captured by their ratio. The expulsion rate nonetheless provides a scale to which to compare the growth rate, r, and we plot Fig. 5B using r/λ calculated for fixed $\lambda = 0.11$ hr^{-1}, the measured value. For completeness, we show a three-dimensional $r, \lambda, \beta/\alpha$ phase diagram as SI Appendix, Figure S11E and S11F. We numerically

calculated the steady state phase diagram of the model (Materials and Methods) and show in Figure 5B the mean log-transformed abundance, $\langle \log_{10}(N+1) \rangle$. The regime of extinction ($N = 0$) is evident (dark purple, with dashed white boundary).

The data-derived parameter values place untreated intestinal *Enterobacter* fairly close to the extinction transition (Fig. 5B). An antibiotic-induced growth rate reduction of approximately 5x is sufficient to cross the boundary to the $N = 0$ regime (i.e. to extinction), moving downward in Fig. 5B. This growth rate reduction, or an equivalent increase in death rate, reflects the conventional view of antibiotic effects. An approximately 300x reduction in the balance between fragmentation and aggregation spurs an alternative path to extinction, moving leftward in Fig. 5B, reflecting a distinct mechanism resulting solely from changes in physical structure. The extinction transition in this direction corresponds to the condensation of the population into a single cluster, reminiscent of gelation phase transitions in polymer systems. As described above, low-dose ciprofloxacin causes a strong reduction in the number of small bacterial clusters, lowering β and possibly also r if fragmentation and individual cell division are linked. Conservatively assuming an equal effect along both axes, and fitting simulations to the 24 hour treatment abundances (Materials and Methods), we find that the antibiotic reduces r and β/α by $\sim$10x. This drives the bacterial system through the phase boundary and well into the extinction regime (Fig. 5B, orange circle), consistent with our observations.

In contrast to *Enterobacter*, treatment of *Vibrio* with ciprofloxacin does not lead to widespread extinction after 48 hours, suggesting that treated populations either lie safely at a new steady state away from the extinction boundary, or are

close enough to the transition so that dynamics are slow. To estimate model parameters for ciprofloxacin-treated *Vibrio*, we performed a two parameter fit of $(\beta/\alpha, r)$ to the 24 hour treatment abundances. Because of *Vibrio*'s large population size ($\sim$ 10^5 clusters), we modified the stochastic simulation procedure using a tau-leaping algorithm (Materials and Methods, SI Appendix, Fig. S12). We indeed find ciprofloxacin-treated *Vibrio* is located close to but safely inside the extinction boundary (Fig. 5B). Untreated *Vibrio* populations show no appreciable multicellular aggregation and are located off-scale far to the upper-right side of the phase diagram (Fig. 5B, arrow).

Discussion

We have discovered that sublethal levels of a commonly used antibiotic can reduce the intestinal abundance of bacterial populations much more severely than would be predicted from in vitro responses, and that this amplification is a consequence of drug-induced changes to the bacterial groups' spatial architecture. Contrary to conventional notions of antibiotic tolerance, largely derived from in vitro studies, reductions in bacterial abundances were greater for the slow-growing, aggregated *Enterobacter* species than for the fast-growing, planktonic *Vibrio*. Live imaging revealed drug-induced increases in bacterial cohesion that, coupled to gut mechanical activity, lead to the expulsion of viable bacterial cells. The microscopic details of this cohesion, likely involving cell wall characteristics, mechanical compression by the gut wall and fluid flows, and perhaps intestinal mucus rheology, remain to be explored.

Notably, the underlying processes of bacterial aggregation and host intestinal transport are found throughout the animal kingdom, suggesting a

general relevance beyond zebrafish that may explain, for example, data on weak antibiotics having strong effects on mammalian microbiomes [63, 64]. Of course, chemical perturbations in more anatomically complex animals or non-gnotobiotic animals that house hundreds of resident bacterial species will undoubtedly involve additional processes beyond those uncovered here. We note, however, that responses to intestinal flow will influence bacterial population dynamics regardless of ecological complexity, and that our choice of model bacterial species spans the extremes of highly planktonic and highly cohesive strains, further implying generality. In the larval zebrafish, enhanced bacterial susceptibility to transport leads to expulsion from the gut. In larger or more complex intestines this may take the form of displacement from one region to a more distal region, with a corresponding shift in local nutrients or competitors, in addition to expulsion from the gut altogether.

The concentrations of ciprofloxacin examined here are commonly found in environmental samples, indicating a potentially widespread perturbation of animal gut microbiota due to antibiotic contaminants. In addition, the expulsion of live, antibiotic-exposed bacteria from animal intestines through the aggregation-based processes described here suggests a potential mechanism for enhanced spread of antibiotic resistance. This possibility is bolstered by our observation that in addition to aggregation, ciprofloxacin-treated cells undergo an active SOS response, which has been shown to promote mutation and horizontal gene transfer [92, 93, 94]. Together, these observations underscore recent concerns about the public health risk posed by antibiotic contaminants in the environment [67].

Our biophysical model of aggregation, fragmentation, growth, and expulsion describes our data well and provides testable predictions. It is remarkable, given

the chemical and morphological complexity of even the larval zebrafish gut, that such a minimal model can accurately predict emergent properties such as the size distribution of bacterial aggregates. That this works is an indication of the power of theories of soft condensed matter physics, whose generality may prove useful in understanding the gut microbiome. Furthermore, our model supplies a framework for a quantitative understanding of gut microbial homeostasis in general. Like recent work modelling antibody-mediated enchaining of *Salmonella* cells in the mouse gut [91], our model implies that the physical processes of bacterial cluster formation and fragmentation play central roles in large-scale microbiota stability. We suggest that our cluster-dynamics model, validated by quantitative agreement between predictions and in vivo data (Fig. 5A), may prove useful in less tractable host species such as mice and humans. Without live imaging or non-invasive sampling, it is challenging to estimate kinetic properties of microbial populations, such as aggregation rates. However, advances in histological sample preparation [95] can preserve bacterial aggregates and yield cluster size distributions; inverting our model, such distributions can reveal the underlying in vivo bacterial kinetics.

Regarding antibiotics, the main prediction of our model is that naturally aggregated, slow growing bacteria will be impacted more severely than fast growing, planktonic species by equivalent low-dose antibiotic perturbations. This is contrary to conventional wisdom that links bacterial tolerance to reduced growth and increased aggregation [68, 69], which stems from studies of antibiotic exposure in static or well-mixed environments. We find that in the intestine, where bacteria can be removed through fluid flow, there exist critical values of aggregation, fragmentation, growth, and expulsion rates, beyond which sustainable colonization becomes impossible (Fig. 5B). Naturally aggregated and slow-growing

species are situated closer to this extinction phase boundary and are therefore more easily driven to population collapse by low-dose antibiotic perturbations. Intriguingly, new meta-omics methods [70] can be used to estimate in vivo growth rates of mammalian gut microbes, which would be interesting to correlate with antibiotic responses. We note in addition that inter-bacterial competition in the gut can be influenced by clustering and susceptibility to intestinal transport [76, 86], suggesting that competition outcomes could be altered by antibiotic treatment if changes in aggregation properties are different for different species. A final prediction of our model is that intestinal transport, which has been linked to microbiota composition [74], will influence the effects of low-dose antibiotic perturbations on microbial community composition. Combining pharmacological manipulations of intestinal transport with antibiotic treatments may therefore lead to novel strategies for precision engineering of the gut microbiome.

Materials and Methods

Animal care

All experiments with zebrafish were done in accordance with protocols approved by the University of Oregon Institutional Animal Care and Use Committee and following standard protocols [96].

Gnotobiology

Wild-type (AB×TU strain) zebrafish were derived germfree (GF) and colonized with bacterial strains as previously described [97] with slight modifications elaborated in the SI Appendix.

Bacterial strains and culture

Vibrio cholerae ZWU0020 and *Enterobacter cloacae* ZOR0014 were originally isolated from the zebrafish intestine [75]. Fluorescently marked derivatives of each strain were previously generated by Tn*7*-mediated insertion of a single constitutively expressed gene encoding dTomato [77]. We note that all plating- and imaging-based experiments performed in this study were done using fluorescently marked strains, which carry a gentamicin resistance cassette, with the exception of experiments in which fluorescent dyes were used to assess viability of cells. Archived stocks of bacteria were maintained in 25% glycerol at -80°C. Prior to experiments, bacteria were directly inoculated from frozen stocks into 5 ml LB media (10 g/L NaCl, 5 g/L yeast extract, 12 g/L tryptone, 1 g/L glucose) and grown for ∼16 hours (overnight) shaking at 30°C.

Culture-based quantification of bacterial populations

Dissection of larval guts was done as described previously [98], with slight modifications elaborated in the SI Appendix. To compare the effect of ciprofloxacin on populations in the intestine and in the flask water, we normalized treated abundances by the corresponding untreated median abundance (Fig. 2C and 3A). To account for variation in untreated bacterial dynamics between weekly batches of fish, we performed the normalization within each batch. Unnormalized data is available in the SI Dataset.

Light sheet fluorescence microscopy of live larval zebrafish

Live imaging of larval zebrafish was performed using a custom-built light sheet fluorescence microscope previously described in detail [99], with slight modifications elaborated in the SI Appendix.

Viability staining of expelled aggregates:

Germ-free larval zebrafish were colonized with wild type *Vibrio* or *Enterobacter* (without fluorescent markers) for 24 hours and then mounted into agarose plugs using small glass capillaries identically to the imaging procedure (above). Individual capillaries were suspended into isolated wells of a 24-well tissue culture plate filled with embryo media containing anesthetic or anesthetic + ciprofloxacin (10 ng/ml for *Vibrio*, 25 ng/ml for *Enterobacter*) and the larvae were extruded from the capillaries. Fish remained mounted for 24 hours, during which expelled bacteria remained caught in the agarose plug. After treatment, fish were pulled back into the capillaries and transferred to smaller wells of a 96 well plate containing embryo media, anesthetic, and the LIVE/DEAD BacLight Bacterial Viability stains SYTO9 and propridium iodide. Fish were stained according to kit instructions, with the exception of the incubation period being extended from 15 to 30 min to account for potential issues with the aggregate nature of the cells [100]. Following staining, fish were pulled again into the capillaries and transferred to the light sheet microscope for imaging. As shown in the SI Appendix, Figures S4 and S10, zebrafish cells stain in addition to bacterial cells, precluding accurate quantification of viable fractions.

Image analysis

Bacteria were identified in three-dimensional light sheet fluorescence microscopy images using a custom MATLAB analysis pipeline previously described [71, 99], with minor changes elaborated in the SI Appendix.

Kinetic model and stochastic simulations

Simulation details are provided in the SI Appendix. In brief, Gillespie's direct method [101] was used to simulate stochastic aggregation, fragmentation, and expulsion events, while growth was treated as deterministic. To simulate *Vibrio* populations, direct stochastic simulation becomes intractable due to the large number of clusters ($\sim 10^5$ single cells). We therefore implemented a modified tau-leaping algorithm [102] that facilitates large simulations. We opted for a straightforward fixed τ method and empirically determined an optimal value of $\tau = 0.001$ h (SI Appendix, Fig. S12A,B). All simulations were written in MATLAB and code is available at INSERT URL

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APPENDIX B

MOTAXIS PAPER HERE

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FIGURE B.1. This is a short description that goes in the Table of Figures. This is an extension of that description, which may include cascading turns of phrase, which appears directly below the figure as expected, but not in the table of figures which appears at the beginning of this exceedingly amazing dissertation.

APPENDIX C

CONCLUSION

A reference to an image can be done like this one, to Figure C.1. You might also want to cite an article like this: [103].

FIGURE C.1. This is a short description that goes in the Table of Figures. This is an extension of that description, which may include cascading turns of phrase, which appears directly below the figure as expected, but not in the table of figures which appears at the beginning of this exceedingly amazing dissertation.

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