

1 Bacteriophage specificity is impacted by interactions between bacteria.

2
3 Ave T. Bisesi^a, Wolfram Möbius^{b,c}, Carey D. Nadell^d, Eleanore G. Hansen^e, Steven D. Bowden^e,
4 William R. Harcombe^{a,f} #

5
6 ^a Department of Ecology, Evolution and Behavior, University of Minnesota, St. Paul, MN, USA.

7 ^b Living Systems Institute, University of Exeter, Exeter, UK.

8 ^c Department of Physics and Astronomy, University of Exeter, Exeter, UK.

9 ^d Department of Biological Sciences, Dartmouth College, Hanover, NH, USA.

10 ^e Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN, USA.

11 ^f BioTechnology Institute, University of Minnesota, St. Paul, MN, USA.

12
13 **# Corresponding Author**

14 harcombe@umn.edu

15 (0000-0001-8445-2052)

Abstract (233 words)

Predators play a central role in shaping community structure, function, and stability. The degree to which bacteriophage predators (viruses that infect bacteria) evolve to be specialists with a single bacterial prey species versus generalists able to consume multiple types of prey has implications for their effect on microbial communities. The presence and abundance of multiple bacterial prey types can alter selection for phage generalists, but less is known about how interactions between prey shape predator specificity in microbial systems. Using a phenomenological mathematical model of phage and bacterial populations, we find that the dominant phage strategy depends on prey ecology. Given a fitness cost for generalism, generalist predators maintain an advantage when prey species compete, while specialists dominate when prey are obligately engaged in cross-feeding interactions. We test these predictions in a synthetic microbial community with interacting strains of *Escherichia coli* and *Salmonella enterica* by competing a generalist T5-like phage able to infect both prey against P22_{vir}, an *S. enterica*-specific phage. Our experimental data conform to our modeling expectations when prey species are competing or obligately mutualistic, although our results suggest that the *in vitro* cost of generalism is caused by a combination of biological mechanisms not anticipated in our model. Our work demonstrates that interactions between bacteria play a role in shaping ecological selection on predator specificity in obligately lytic bacteriophages and emphasizes the diversity of ways in which fitness trade-offs can manifest.

Importance (140 words)

There is significant natural diversity in how many different types of bacteria a bacteriophage can infect, but the mechanisms driving this diversity are unclear. This study uses a combination of mathematical modeling and an *in vitro* system consisting of *Escherichia coli*, *Salmonella*

41 *enterica*, a T5-like generalist phage, and the specialist phage P22*vir* to highlight the connection
42 between bacteriophage specificity and interactions between their potential microbial prey.
43 Mathematical modeling suggests that competing bacteria tend to favor generalist bacteriophage,
44 while bacteria that benefit each other tend to favor specialist bacteriophage. Experimental results
45 support this general finding. The experiments also show that the optimal phage strategy is
46 impacted by phage degradation and bacterial physiology. These findings enhance our
47 understanding of how complex microbial communities shape selection on bacteriophage
48 specificity, which may improve our ability to use phage to manage antibiotic resistant microbial
49 infections.

Introduction

Predators can impose top-down control of ecosystems, impacting species abundances, community structure, and community function [1]. For example, in marine environments, lytic bacteriophages (phages), the viral predators of bacteria, are critical drivers of microbial populations and nutrient cycling, lysing up to 40% of phytoplankton biomass per day [2]. The diet breadth of predators – how many different prey species they can consume – is an important component of how top-down control shapes an environment (Figure 1A) [3-8]. Specialist predators often drive limit cycles with their prey, while generalists are more likely to stabilize prey populations through the emergence of apparent mutualisms, in which the presence of one prey species reduces the burden of predation on the other [9-14]. Diversity in specificity is widespread in microbial communities, where some phages are generalists that can prey upon bacterial species across multiple genera, while others specialize on a single serovar [15-16]. The determinants of phage specificity are likely complex, particularly in coevolving communities, as bacterial prey develop mechanisms either to block phage adsorption (e.g. loss of receptors) or prevent phage replication (e.g. CRISPR, superinfection immunity) [17] and phage evolve to overcome these resistance mechanisms, often with pleiotropic costs for host range expansion [18]. Identifying the key forces shaping predator diet breadth, as well as their relative importance, would therefore have substantial consequences for our ability to predict the long-term dynamics of multitrophic microbial communities.

The composition of the prey community is one force known to impact predator specificity. The evolution of generalist predators often requires prey heterogeneity to provide opportunities for diversification [19-24]. While it has been suggested that prey diversity could reduce the incidence of predator generalism given the demands of engaging in coevolutionary arms races with multiple species [25], microbial studies have shown that the presence of multiple

bacterial strains is sufficient to select for generalists [19, 26]. Assuming a heterogeneous prey environment, optimal foraging theory provides several additional predictions of how the structure of prey communities might shape predator diet breadth. First, it suggests that absolute prey densities alter selection on predator specificity by impacting foraging time [27]. Generalism is predicted to be favored at low prey densities when foraging time is high, while specialism is favored at high prey densities; this prediction has been validated in a microbial system [27]. Optimal foraging theory also emphasizes the importance of relative prey abundances, such that predators should experience selection to exploit the most abundant prey types, even if those prey are low-quality or intraspecific competition between predators is strong [22-23, 28-29]. However, even when relative abundances are considered, most studies on diet breadth assume a static ratio of prey types over time, omitting a critical dimension of natural communities.

Interactions between prey complicate the assumption of static ratios by generating correlations between prey abundances [30-32]. Nutrient competition between bacteria tends to generate anti-correlated abundances between species, while positive interactions such as obligate mutualism generate positively correlated abundances [29-32]. When predators are consuming prey species with anti-correlated abundances, a generalist strategy is likely to be favored, because predation on one species should lead to an increase in the abundance of the alternative prey through competitive release (Figure 1B). The expectation that competing prey should favor predator generalism is consistent with R^* theory, which suggests that the outcome of competition on a shared limiting resource is determined by which species can survive on the lowest levels of the resource [33]. Given that a generalist predator has alternative resources available to it, it should always be able to survive on the lower levels of a resource shared with a specialist. Additional theoretical work provides support for the notion that there are situations in

which competing prey should favor generalist predators [29, 34]. When prey compete, mathematical modeling suggests that competitive dominance by a novel prey type is generally required for the evolution of broadened predator diet breadth when fitness trade-offs for generalism are present [29]. Comparatively little work has been done investigating the impact of prey engaged in direct positive interactions on predator diet breadth. Positively correlated prey abundances are likely to favor specialist predators because predation by a specialist would also lead to a reduction in abundance of the alternative prey (Figure 1B). The interdependence between prey species may also increase the likelihood of overexploitation by predators [35-37]. However, the hypothesis that different types of interactions between prey should alter predator specificity has not been fully validated theoretically or empirically.

Here we use a mathematical model and an *in vitro* system to investigate how interactions between prey species govern ecological selection on predator diet breadth. We found that, in our model, obligately mutualistic interactions between microbial prey were more likely to favor a specialist phage predator, while competition between prey was more likely to favor a generalist phage predator. These findings were in accordance with our initial hypotheses and are relevant to systems where interactions between prey drive correlations in their abundance. We tested these findings in an experimental system (Figure 1C) and reproduced our ecological modeling results despite differences in the mechanism of fitness trade-off experienced by the generalist phage. Our work provides insight into how interactions between two microbial prey species alter ecological selection on phage specificity in well-mixed environments and provides a foundation for predicting the evolution and maintenance of specificity in bacteriophage, with implications for designing and managing microbial communities.

Results

In a phenomenological model, phage relative abundance depends on prey interactions and fitness trade-offs for phage generalism

We used a phenomenological model (Figure 1D; Materials and Methods) to predict how communities of two interacting prey species respond to attack by predatory lytic phage during chemostatic growth. We predicted that competition between prey was likely to favor predator generalism by increasing temporal heterogeneity in resource availability [38-39], while obligate mutualism between prey species would result in less temporal heterogeneity, as bacterial species would either occur together or not at all, likely favoring specialization [38].

We first investigated the behavior of the model with a single parameter set. Using our default parameters (Table 1), we examined cases in which phage were not present, or when only one phage type was present. When phage were not modeled in our system, prey species reached a 50:50 ratio at equilibrium (Figure 2A, left panel). The introduction of a specialist resulted in competitive release of *E. coli* when prey competed and correlated reductions in bacterial abundances when prey were mutualistic (Figure 2A, middle panel). This behavior was consistent with our conceptual model (Figure 1B). In comparison, the introduction of a generalist phage predator reduced the density of both prey species at equilibrium, keeping their abundances at a 50:50 ratio (Figure 2A, right panel). We also considered the behavior of the model when phage phenotypes competed against one another. When phage were parametrically identical, the generalist was more prevalent regardless of prey interaction type (Figure 2B and C, top panels), though interaction type altered the degree of dominance. This finding suggested the importance of the “jack of all trades, master of none” hypothesis for the predominance of specialization, which asserts that, all other things equal, a cost of generalism is required to favor a specialist [40-46]. Following the incorporation of a cost of generalism by increasing the specialist’s burst

size to five times greater than that of the generalist, we found that our modeling results were consistent with our conceptual model such that the generalist dominated when prey competed (Figure 2B), while the specialist dominated when prey were mutualistic (Figure 2C).

To further understand the behavior of the model when phage phenotypes competed against one another, we then sought to simplify the system, applying R^* theory to Eqs. 3-4. R^* theory [33] suggests that, when two species compete for the same resource, the species that requires fewer resources for zero net population growth will be able to competitively exclude the other species. This is formalized by computing the resource needed for zero net population growth, R^* , for each species independently and comparing those values. To apply this to our system, we treated the shared prey, *S. enterica*, as a resource, and identified domains in which the amount of *S. enterica* required by the specialist to hold its net population growth at zero, S_p^* , would be expected to be less than the amount of *S. enterica* required for zero net growth of the generalist population, S_G^* [33]. To do so, we assumed that the generalist phage had the same burst size and attachment rate on both prey types and that the intrinsic rate for mortality due to dilution or death was not species-specific, such that γ_G described the burst size of the generalist on both prey, ζ_G described the attachment rate of the generalist on both prey, γ_P described the burst size of the specialist on *S. enterica*, ζ_P described the attachment rate of the specialist on *S. enterica*, and δ described the intrinsic mortality rate of all four species. S_G^* and S_P^* were obtained by setting the left-hand side of Eqs. 3-4 equal to 0 and solving for S . Using these equations, in accordance with R^* theory, requiring that

$$S_P^* < S_G^* \quad \text{Equation (5)}$$

leads to:

$$\frac{\gamma_G * \varsigma_G}{\gamma_P * \varsigma_P} < 1 - \frac{\gamma_G * \varsigma_G * E}{\delta} \quad \text{Equation (6)}$$

This analysis therefore suggested that the specialist phage could dominate (i.e. have the lower S^*) when the alternative prey source *E. coli* was sufficiently rare (E sufficiently small), or when the generalist suffered a fitness trade-off for expanded specificity in the form of reduced burst size and/or attachment rate ($\gamma_G * \varsigma_G$ sufficiently small). More generally, a cost of generalism is required for the specialist to dominate, as Eq. 5 can only be fulfilled if $\gamma_G * \varsigma_G < \gamma_P * \varsigma_P$. While interactions between prey alone can alter the magnitude of selection, a cost of generalism is required to alter the direction of selection between interaction types. This inequality supports the relevance of the “jack of all trades, master of none” hypothesis [40-46].

To verify the intuition of our S^* inequality, we used our default parameters (Table 1) to consider various domains in which the specialist phage predator was favored. To do so, we systematically changed the burst size of the specialist phage on the shared prey *S. enterica* ($\gamma_{S,P}$) and considered the relative abundance of each phage type at equilibrium (Figure 3; for changing attachment rate $\varsigma_{S,P}$ instead of burst size $\gamma_{S,P}$, see Supplemental Figure 1). We found that even as the cost of phage generalism increased, the generalist phage was always favored when prey competed, although there were no stable fixed points using our default parameters (Figure 3A, left panel; Supplemental File 1). Conversely, the specialist was favored on mutualistic prey (Figure 2C; Figure 3A, right panel) given a minimum cost of generalism. If $\varsigma_P * \gamma_P > 2.83\varsigma_G * \gamma_G$, i.e. the product of the specialist’s burst size and attachment rate was 2.83 times as large as that of the generalist, then the only stable fixed point was that of the two bacterial species coexisting with the specialist. As such, given a threshold cost of generalism, only the specialist could coexist with mutualistic bacterial species, regardless of initial conditions.

To visualize the next prediction of our S^* inequality - that the relative availability of the alternative prey source *E. coli* mattered for the competitive outcome of phage specificity strategies - we loosened the previous assumption that bacterial species should reach a 50:50 ratio without predators. In addition to varying the ratio of prey growth rates (in both competition and mutualism) or the ratio of interaction coefficients ($\alpha_{E,S}$ for mutualism, $\beta_{E,S}$ for competition), we systematically altered the cost of generalism (either through burst size, in Figure 3, or attachment rate, in Supplemental Figure 1). Our analyses demonstrated that when prey were competing, relative growth rates and competition coefficients mediated the abundance of the specialist such that it had a fitness equivalent to or greater than that of the generalist only in those cases where the alternative prey *E. coli* was competitively excluded by the shared prey *S. enterica* (Figure 3B). In contrast, when prey were mutualistic, neither relative growth rates nor relative mutualistic benefit coefficients altered phage relative abundance greatly. Instead, ecological dominance of the specialist depended mainly on the cost of generalism, such that the specialist tended to proliferate given a sufficient cost regardless of biased bacterial abundances driven by unequal mutualistic benefit (Figure 3C).

Finally, to ensure that our tested parameters captured the fundamental behavior of the model, we performed two sensitivity analyses - a Morris screening and a Sobol variance analysis - to determine which parameters had the largest impact on the final biomass of each phage type. In the case of both obligate mutualism and competition, Morris screening methods suggested that the death/dilution rate, burst size and attachment rates of both phage, and the interaction parameters for the microbial species were of greatest impact (Supplemental Table 1). The variance-based Sobol method reinforced the importance of dilution rate (Supplemental Table 2). These results were consistent both with the parameters identified by fixed point analysis, our S^*

inequality, and the basic construction of the model, which requires that both phage types have reproductive parameters sufficient to offset the chemostat-induced mortality rate.

Phage relative abundance *in vitro* aligns with modeling results in co-culture

Using our wet-lab experimental cross-feeding system, we tested the mathematical prediction that generalist predators would be favored on competing prey and specialist predators would be favored on mutualistic prey. We first verified that over 48 hours our specialist phage (P22*vir*) could only replicate on *S. enterica* and that our generalist phage (EH7) could replicate on both prey species (Figure 4A). Each phage alone also grew well on competitive and mutualistic bacterial co-cultures. The final density of EH7 appeared similar when replicating on both interaction types (Figure 4A, $p = 0.999$; Supplemental Table 3). This was true for P22*vir* as well, such that type of interaction did not result in significantly different final titers (Figure 4A, $p = 0.909$; Supplemental Table 3). However, in those experiments, EH7 reached a higher titer than P22*vir* on both competitive (Figure 4A, $p < 0.0001$; Supplemental Table 3) and mutualistic co-culture (Figure 4A, $p < 0.0001$; Supplemental Table 3). Consistent with our model, P22*vir* increased *E. coli* frequency relative to the no-phage control in competitive co-culture (Figure 4C, $p < 0.0001$; Supplemental Table 3). Applying the specialist phage suppressed, but did not eliminate, both bacterial species in mutualism (Figure 4D). In contrast, the generalist phage more effectively suppressed *E. coli* in competitive co-culture, resulting in a higher relative density of *S. enterica* compared to the no-phage control (Figure 4C, $p = 0.0035$; Supplemental Table 3). Unlike P22*vir*, the application of EH7 to mutualistic co-culture did not suppress co-culture growth for the duration of the experiment (Figure 4D).

We also evaluated population dynamics when the two phages competed against one another (Figure 4B, 4C and 4D). When both phage were present in competitive co-culture, the

generalist reached a higher final density than the specialist (Figure 4B, $p < 0.0001$; Supplemental Table 3), as predicted by our modeling results. Bacterial dynamics were also consistent with our model, with *E. coli* dominating through competitive release (Figure 4C, $p < 0.0001$; Supplemental Table 3). When both phage were present in mutualistic co-culture, the specialist reached a higher final titer (Figure 4B, $p < 0.0001$; Supplemental Table 3) and co-culture densities were suppressed for the duration of the experiment (Figure 4D). Curiously, however, the presence of the specialist phage reduced EH7 below the limit of detection when prey were mutualistic (Figure 4B), a result that we interrogated further.

In vitro, cost of generalism manifests as increased rate of degradation and reduced infectivity of starved cells

To understand our inability to detect the generalist phage in mutualistic co-culture at the end of our experimental window, we investigated the ability of each phage to reproduce on starved cells by adding phage to monocultures in lactose minimal media as described in Materials and Methods. When placed in wells without bacterial cells or with starved *E. coli*, P22_{vir} titer remained unchanged over the 48-hour growth period (Figure 5A). When placed in wells with starved *S. enterica*, which were also expected to be physiologically inaccessible, P22_{vir} titer increased relative to the condition without cells (Figure 5A, $p = 0.001$; Supplemental Table 4) or with only *E. coli* (Figure 5A, $p = 0.0001$; Supplemental Table 4).

In comparison, the generalist phage EH7 decreased in abundance in all conditions after the 48-hour growth period. There were no detectable infectious phage particles in wells without cells or with starved *E. coli*. In wells with only starved *S. enterica*, some infectious phages were still detectable, although phage titer was greatly reduced, suggesting that *S. enterica* may be more physiologically accessible to EH7 than *E. coli* in a starved state (Figure 5A, $p = 0.012$;

Supplemental Table 4). Taken together, these results indicate that the generalist phage suffers a cost that manifests in two ways: first, a rapid rate of degradation in minimal media, and, second, a reduced capacity to infect and reproduce inside starved cells relative to the specialist phage P22_{vir}. We therefore expect that, when competing with P22_{vir} on mutualistic co-culture, the starved physiology of the interdependent cells reduces the generalist's infective ability relative to the specialist. This reduction, in combination with the generalist's higher degradation rate, provides an explanation for our inability to detect it at the end of previous experiments.

Interestingly, these results are specific to minimal media, as EH7 does not degrade in LB (Supplemental Analysis; Supplemental Figure 2, Supplemental Table 5). We were not able to identify which component of our minimal media was responsible for the degradation of the phage, though it does not appear to be related to the presence of metals or the result of osmolarity (Supplemental Analysis; Supplemental Figure 2).

The generalist is favored in competition even when a cost is imposed *in vitro*

The *in vitro* experiments replicated our modeling results when bacterial prey were mutualistic or competing. However, the modeling results assumed a cost of generalism we did not observe in the *in vitro* system when both phage types were competing for actively growing *S. enterica*, with each phage reaching titers that did not differ significantly from one another (Figure 5B, 'no cost', $p = 0.999$; Supplemental Table 4). A clear cost of generalism may thus only manifest in the mutualistic treatment where the generalist phage EH7 degrades rapidly due to bacterial growth delayed by P22_{vir} predation on *S. enterica*. Phage durability is well-established as a critical component of phage fitness and has been shown in some cases to trade-off with fecundity [47]. This potentially explains our results on the shared prey: while EH7 reproduction on actively growing *S. enterica* matches or exceeds that of P22_{vir}, its reproductive

capacity may trade off with its degradation rate, given its poorer environmental durability in minimal media.

Therefore, to impose a cost of generalism across all treatments that more closely matched that observed in the mutualistic co-culture, we repeated our phage competition assay experiments by incubating the phage for 24 hours prior to the addition of cells, anticipating that some degradation of the generalist EH7 would occur, while the titer of P22*vir* would remain unchanged. Previous preliminary experiments had indicated that, starting with 10^3 phage particles, EH7 titer generally drops below the limit of detection after 24 hours (Supplemental Figure 2). However, the phage can be recovered following the addition of cells, suggesting that infectious phage particles remain. We expected that the reduced titer of EH7 when cells were applied would mirror the conditions of mutualism when phage competed. Given that the presence of P22*vir* in competitive co-culture increases *E. coli* frequency (Figure 4C), we hypothesized that a lower titer of EH7 when cells are added may still result in a higher final titer of the generalist phage relative to the specialist, because any remaining EH7 would be able to utilize *E. coli*.

When this cost was imposed and phage were competed on *S. enterica* monoculture, the generalist disappeared below the limit of detection, while P22*vir* final titer was not significantly different than in the condition without cost (Figure 5B, $p = 0.982$; Supplemental Table 4). In contrast, we found that when bacteria competed, final titers of both EH7 (Figure 5B, $p = 0.723$; Supplemental Table 4) and P22*vir* (Figure 5B, $p = 0.999$; Supplemental Table 4) were comparable to the no cost condition, as we had expected. These results are consistent with our modeling results, suggesting that, even with a substantial cost of generalism, a generalist predator should be favored on competing prey.

In a phenomenological model, prey interactions determine the intrinsic death rate needed to favor
specialism

Finally, we amended our model to see if we could replicate the results of our initial *in vitro* experiment, given the known constraints of higher rates of degradation of the generalist phage when cultured in minimal media. To do so, we imposed a cost of generalism not as burst size (or attachment rate), but instead as an increased intrinsic mortality rate for the generalist phage, while keeping the mortality rate the same for the three other species.

We observed that, when fitness cost was modeled as increased mortality, our qualitative results matched those when fitness cost was measured as burst size or attachment rate (compare Figure 6 with Figure 3A and Supplemental Figure 1A, respectively). The generalist phage could be maintained on competing prey even as its mortality rate increased significantly; competition with the specialist phage decreased the abundance of the generalist, but competitive release of *E. coli* ensured that the generalist had access to sufficient prey for positive population growth across a wide range of parameters (Figure 6). In contrast, the same intrinsic mortality rate at which the generalist persisted on competing hosts (Figure 6) resulted in the loss of the generalist when prey were mutualistic due to the specialist phage driving correlated reductions of both *S. enterica* and *E. coli* (Figure 6). It is worth noting that our model is not currently designed to capture changes in the relative phage attachment rates or burst sizes as a function of physiological state.

Therefore, our model assumes that the relative reproductive abilities of the two phage are static and that the relationship between phage reproductive rate and intrinsic mortality is driven solely by the relative abundance of prey, assumptions which do not capture the full biological reality of our *in vitro* system. Regardless, in its current form, it reinforces our previous qualitative modeling predictions and our *in vitro* findings that suggest that, when a cost of generalism is present, regardless of its mechanism, competition between prey favors generalist predators

across even severe fitness trade-offs, while a minimal fitness cost favors specialist predators when prey are engaged in mutualism.

Discussion

We aimed to determine whether ecological interactions between bacterial prey species impacted the abundance of phage with different specificities. We developed a simple four-species phenomenological model composed of two interacting bacterial species, a specialist phage, and a generalist phage. Using this chemostatic model, given a cost of generalism, we found that specialist phage were favored when prey are mutualistic, while generalist phage were favored when prey compete. These qualitative results were largely robust to initial conditions, suggesting that they may be both ecologically and evolutionarily informative. We found that our modeling predictions were well-matched by the outcome of batch culture experimental phage competition assays. The alignment between outcomes in our model and our system was observed despite differences in the mechanisms driving the cost of generalism. In our model, a cost of generalism was imposed as either a lower burst size or worse attachment rate and was assumed to be static over time. *In vitro*, the cost of generalism was dynamic over time, driven by differences in phage durability and ability to reproduce on bacteria in variable physiological states. Our results highlight that ecological interactions between prey can alter ecological selection on predator specificity in predictable ways when a cost of generalism exists, regardless of the exact mechanism of cost.

Our modeling results suggest that interactions between bacterial prey impact the prevalence of phage specificity phenotypes when a cost of generalism exists. Experimental evolution has previously shown that the presence of different types of resources can select for generalism [6, 19, 40]. Both absolute and relative prey densities are relevant predictors of phage

specificity [22-23, 27-28]. However, while much of the previous work done on diet breadth has assumed a constant relative abundance of available prey, our model upended that assumption by allowing relative prey abundances to vary as a function of prey ecology. Previous theoretical modeling has demonstrated that resource competition between prey species can select for expanded predator diet breadth even when trade-offs for generalism exist, although this result generally required the competitive dominance of the novel prey source [29, 34]. Our results align with these findings, underscoring that resource competition should favor a generalist strategy in most cases, even when a severe fitness trade-off is present. Additionally, we expanded previous findings to include mutualistic interactions between prey, showing that a specialist predator strategy dominated assuming even a minimal trade-off for generalism. Our model demonstrates that ecological interactions between prey species favor different predator diet strategies when there is a cost of generalism because switching from competition to mutualism changes relative prey abundances from being anti-correlated to being positively correlated. We anticipate that our modeling result will apply to systems when interactions between prey generate correlations in their abundance and a cost of generalism is present.

The experimental results of our study align with our modeling predictions, although they also highlight two important aspects of our microbial system. First, *in vitro*, we did not observe a reproductive fitness cost of generalism on the shared prey species *S. enterica* under optimal growth conditions, a cost we had anticipated in our model. Instead, we found that the trade-off manifested as an interplay between the generalist's increased degradation in minimal media and its reduced replication rates on starved cells. Our results contribute to the body of work suggesting that pleiotropic costs are often context dependent [18, 36, 40-46]. They also reinforce the observed importance of durability as an important component of phage fitness [47].

370 Additionally, our experiments emphasize that in addition to altering population dynamics,
371 interactions between bacteria can impact phage specificity by altering prey physiology. Bacterial
372 sensitivity to phage is not a binary trait and can change between physiological states due to
373 differences in growth rate, metabolism, transcription and translational activity, and the
374 availability of intracellular components [48-50]. Phage reproduction on slow-growing or
375 stationary phase cells is often more difficult due to reduced cell size and lower densities of the
376 receptors phage use to adsorb [28, 51]. Because microbial physiology is driven by resource
377 availability and interactions between bacterial species [48], our work suggests that ecological
378 selection on phage specificity is impacted by how interactions between bacterial prey shift prey
379 abundances and physiological states over time.

380 There are limitations to the study we performed that may impact the generality of the
381 results. First of all, our study is limited by its focus on the types of interactions that we chose to
382 examine: namely, obligate cross-feeding and resource competition. Other interactions or even
383 other types of mutualistic or competitive interactions - for example, defensive mutualisms or
384 interference competition - could result in different selective patterns on phage diet breadth.
385 Additionally, we do not consider bacterial resistance to phage, which would create
386 subpopulations within interacting species and complicate correlations in bacterial abundance.

387 We also note that the two phage types tested in these experiments differ in ways
388 unrelated to specificity. P22_{vir} attaches to the O-antigen of *S. enterica*'s lipopolysaccharides
389 (LPS), while EH7 uses BtuB, a vitamin B12 uptake receptor. Previous work has demonstrated
390 that BtuB expression is context dependent, while the LPS is constitutively expressed [52-60].
391 Our phages also have different genome sizes, with EH7 consisting of 110kb and 154 putative
392 proteins, while P22_{vir} is much smaller at 41kb and 72 proteins [61]. We expect that the poor

infective capacity of EH7 on starved cells is a result of both receptor type and genome size. When cells are starved, reduced BtuB receptor density may depress EH7 attachment rate, while P22_{vir} attachment rate remains unchanged. Likewise, differences in phage genome size and protein content mean that intracellular demands for producing EH7 will be higher than P22_{vir}, so its burst size may be reduced when cells are starved. Critically, EH7 is also less durable than P22_{vir} in minimal media. However, these differences, while notable, are present whether phage are grown on competitive or mutualistic co-cultures; bacterial interactions determine the consequences of these differences for phage fitness. Fundamentally, the differences between the two phage types ensure that there is a cost for the generalist phage in our *in vitro* system – though the cost may not be directly due to generalism itself – and our work suggests that, if a cost of generalism exists in some form, bacterial interactions will have consequences for the direction of ecological selection on phage host range. Future work should test the competitive ability of phages that use the same or similar receptors and with greater stoichiometric similarity, though we expect that, in cases where a cost of generalism exists, our results will be applicable.

Our results suggest numerous directions for future study. It would be interesting to select EH7 for increased durability in minimal media to examine whether the improvement is sufficient to offset reproductive costs on slow-growing cells, or if a trade-off in fecundity is observed [47]. In the context of phage therapy, the performance of EH7 in minimal media emphasizes the necessity of testing how different environments affect phages and whether phage characteristics such as specificity tend to correlate with susceptibility to degradation [62]. These data also suggest that the ways bacteria modify their environments through alteration to local pH or metabolite concentrations will have consequences for their viral predators. For example, human gut microbes often compete with hosts for vitamin B12 [63]; the resultant availability of B12 in

the human gut may alter the efficacy of BtuB-specific phages in phage therapy applications. Continued characterization of phage-bacteria interactions in the complex communities in which they are found will improve our ability to use phage for engineering and biomedical purposes. We also expect that increasing the number of bacterial species or incorporating the evolution of resistance will complicate our findings by allowing for the emergence of phage with intermediate specificities. Finally, we note that the spatial structure of interacting bacterial species, as in a biofilm, will alter local prey availability in natural environments such that our results may not be applicable [38].

We took a simple modeling approach, paired with an ecological experiment, to gain insight into the role of prey ecology on the competitive ability of bacteriophage with different specificities. We found that, in both our model and *in vitro* experiments, prey interactions shaped the prevalence of phage specificity phenotypes, though the focal mechanisms differed between our modeling approach and synthetic community. Management and design of microbial communities is contingent upon our ability to predict the evolutionary outcomes and higher order ecological effects of multitrophic interactions. Understanding the complex biotic factors driving ecological and evolutionary outcomes for bacteriophage is a critical step for harnessing microbes in industrial and biomedical applications. We suggest that microbial interactions should be studied across a diversity of systems to understand the generality of their impact on phage host range.

Materials and Methods

Model description

We constructed a model of the concentrations of two interacting bacterial species, a generalist phage, and a specialist phage. Bacteria (dimensionless biomass denoted by E or S) can

either compete for resources or engage in obligate mutualism [adapted from Hoek et al., 2016; 64]. Biomass of prey changes through growth (with Lotka-Volterra-like dynamics depending on the interaction with other prey species) and decreases due to predation and death or dilution:

$$\frac{dE}{dt} = \mu_E * E * \frac{\alpha_{E,S} * S}{(\alpha_{E,S} * S) + \kappa_E} * (R - E - (\beta_{E,S} * S)) - (\zeta_{E,G} * G * E) - (\delta_E * E) \quad \text{Equation (1)}$$

$$\frac{dS}{dt} = \mu_S * S * \frac{\alpha_{S,E} * E}{(\alpha_{S,E} * E) + \kappa_S} * (R - S - (\beta_{S,E} * E)) - (\zeta_{S,P} * P * S) - (\zeta_{S,G} * G * S) - (\delta_S * S) \quad \text{Equation (2)}$$

Biomass of predators increases through predation and decreases through death or dilution:

$$\frac{dG}{dt} = (\gamma_{S,G} * \zeta_{S,G} * G * S) + (\gamma_{E,G} * \zeta_{E,G} * G * E) - (\delta_G * G) \quad \text{Equation (3)}$$

$$\frac{dP}{dt} = (\gamma_{S,P} * \zeta_{S,P} * P * S) - (\delta_P * P) \quad \text{Equation (4)}$$

Our model was constructed such that prey had an intrinsic maximum growth rate μ_i , carrying capacity R , and three parameters determined prey interactions: κ_i , $\alpha_{j,i}$, and $\beta_{j,i}$. Mutualism is determined by the saturation constant κ_i and the mutualism coefficient $\alpha_{j,i}$, which reflects the beneficial effect of prey species i on the per capita growth of prey species j . If all κ_i and $\alpha_{j,i}$ values are positive, bacterial species grow faster together and cannot grow alone. Competition is driven by the coefficient $\beta_{j,i}$, where $\beta_{j,i}$ determines the competitive effect of prey species i on the per capita growth rate of prey species j . Phage reproduction is modeled via adsorption with attachment rate on species i by phage X as $\zeta_{i,X}$ which directly leads to lysis by phage X on species i with burst size $\gamma_{i,X}$. The default natural death rate δ_i was initially identical for all four species, as in a chemostat (Table 1), unless noted otherwise.

Using these equations, we investigated the extremes of pure mutualism (κ_i and $\alpha_{j,i} > 0$, $\beta_{j,i} = 0$), and pure competition ($\kappa_i = 0$, $\alpha_{j,i} = 1$, $\beta_{j,i} > 0$) (Table 1). Carrying capacity was increased when prey competed relative to the value used when prey were mutualistic in order to

standardize the total final biomass in each condition. This is required by a structural limitation of the Lotka-Volterra style model, where obligate mutualism inflates the carrying capacity over that set by the value of $\square$.

Model analyses

To predict the biomass of both prey and predator over time, we numerically solved differential equations 1-4 in R v. 4.2.1 with the DeSolve package v. 1.32, using the LSODA solver. To investigate the equilibrium or steady state dynamics of the system of equations, we integrated Eqs. 1-4 until species abundances no longer changed between timepoints. These results were verified by fixed point stability analysis in Mathematica 13.2.1. We evaluated equilibrium abundance of the phage predators under three different scenarios: 1) imposing a trade-off for expanded specificity by penalizing the burst size (or attachment rate) of the generalist phage, 2) altering the intrinsic growth rates or interaction coefficients of the bacterial prey, or 3) some combination of scenarios 1 and 2 (Table 2). To quantify phage coexistence, we used the equilibrium abundance of both phage. Relative abundance was calculated as the equilibrium density of the specialist divided by the sum of the equilibrium density of the specialist plus the equilibrium density of the generalist. Values greater than 0.5 indicated that the specialist was more abundant. Initial densities were the same across numerical simulations; all four species were always initialized at a density of 0.1. To confirm the significance of the parameters tested, we conducted two types of sensitivity analyses on our ODE system: the Morris screening method and the variance-based Sobol' test [65-67]. Morris screening and Sobol sensitivity analyses were performed in R with the ODEsensitivity package v. 1.1.2 using the same parameter distribution ranges for each test type (Supplemental Table 6).

Finally, following the *in vitro* finding of high rates of degradation of the generalist phage, we amended our model to impose a cost of generalism by increasing the death rate of the generalist relative to the three other species.

Bacterial co-culture system and phage strains

The bacterial strains have been previously described [68]. Strains are listed in Supplemental Table 7. The *Salmonella enterica* serovar Typhimurium LT2 strain secretes methionine due to mutations in *metA* and *metJ* [69]. The *Escherichia coli* is a methionine auxotroph due to a deletion of *metB* [68]. To track bacterial abundances and relative ratios during growth, *E. coli* was tagged with a cyan fluorescent protein and *S. enterica* was tagged with a yellow fluorescent protein [70].

The specialist phage used was P22*vir*. It is an obligately lytic version of the lysogenic *S. enterica*-specific phage P22, created through several point mutations in its prophage repressor gene (Supplemental Table 8). P22*vir* was provided by I.J. Molineux. A generalist phage strain, EH7, was isolated and provided by E. Hansen and S. Bowden. EH7 is an obligately lytic T5-like siphovirus that uses BtuB, a differentially expressed outer membrane protein for vitamin B12 uptake, as a receptor. It is similar to T5-like coliphages described in Kim and Ryu (2011) [16] and Switt et al (2015) [61].

Two additional bacterial strains were used for plaque assays (Supplemental Table 7). They were chosen so that, in mixed cultures of phage, phage types could be quantified independently of each other. The *E. coli* K-12 BW25113 $\Delta trxA$ from the Keio collection was used to quantify EH7 densities as no plaques of P22*vir* form on that host [71]. An *S. enterica* serovar Typhimurium NCTC 74 strain with *btuB* knocked out through a transposon insertion

(EZ-Tn5 <Kan-2>, Lucigen) was used to quantify P22_{vir} as no plaques of EH7 form on that host. The $\Delta btuB$ *S. enterica* strain was provided by S. Bowden.

Media

Minimal hypho liquid media for experiments was prepared as previously described, with each component sterilized prior to mixing [72] (Supplemental Table 9). In addition to the appropriate carbon source, solutions containing sulfur, nitrogen, phosphorus, and metals were supplemented into each media type (Supplemental Table 9). Routine culturing of all bacterial strains was carried out on Miller Lysogeny Broth (LB) unless otherwise indicated. Working stocks of both phage types were grown on log-phase *S. enterica* LT2 cultures in LB and stored at 4°C. Stock titer was determined by plaque overlay assay on the appropriate strains.

Phage competition assays

Phage competition assays were performed in 96-well flat bottom plates on a Tecan Infinite Pro200 plate reader for 48 hours at 37°C with shaking at 432 rotations per minute. Experiment duration was chosen to allow batch culture experiments to reach a final state (stationary phase, phage densities unchanging), thus allowing us to compare to our chemostatic model. Overnight stationary phase cultures in LB started from single colonies were washed three times in saline, adjusted to a density of 10^7 cells per mL, and used to inoculate 200 μ L of appropriate medium with 2.0×10^5 total cells per well (i.e. 2.0×10^5 total *S. enterica* cells in monoculture, 1.0×10^5 total *S. enterica* cells and 1.0×10^5 total *E. coli* cells in co-cultures). Phage stocks were diluted in saline to 10^5 plaque-forming units per mL and inoculated into the appropriate wells to an MOI between 0.005 and 0.01 for a given phage, depending on the fraction of infectable cells for each phage type. Phage strains were added either in isolation (10^3 total phage particles of either P22_{vir} or EH7) or in a one-to-one ratio (10^3 total phage particles of

P22_{vir} and 10³ total phage particles of EH7 for a final density of 2 x 10³ total phage particles). Phage densities were confirmed by plaque overlay assay at the start of the experiment on the appropriate strains.

To quantify bacterial abundances throughout the duration of the experiment, OD₆₀₀, *E. coli*-specific CFP (Ex: 430 nm; Em: 480 nm), and *S. enterica*-specific YFP (Ex: 500 nm; Em: 530 nm) fluorescence were read every 20 minutes. Fluorescent protein signals were used to infer species-specific OD equivalents based on an experimentally determined conversion factor as previously described [73]. To determine phage density at the end of the 48-hour growth period, we plated for PFUs for each replicate from half of the total 200μL volume using plaque overlay assays on LB plates with 0.7% LB top agar. All replicates were quantified using plaque assays on both $\Delta trxA$ *E. coli* and $\Delta btuB$ *S. enterica*. $\Delta trxA$ *E. coli* and $\Delta btuB$ *S. enterica* were prepared for use in plaque assays through overnight culture growth in LB, prior to being diluted 1:10 ($\Delta btuB$ *S. enterica*) or 1:5 ($\Delta trxA$ *E. coli*) and allowed to grow for 30 minutes. Plaque assays were otherwise performed as previously described using 2μL of phage spot dilutions from 10⁰ to 10⁻⁷ with three technical replicates per dilution per sample [51, 74]. The lower limit of detection was 500 PFU/mL. Change in phage titer was represented as the natural log of the final phage density divided by the starting phage density (ln(final PFU/mL / initial PFU/mL)). All plates were incubated overnight at 37°C.

A single initial experiment was completed to confirm the reproductive ability of each phage on *S. enterica* monoculture or *E. coli* monoculture. Full factorial experiments testing all three phage conditions (P22_{vir}, EH7 or EH7 + P22_{vir}) on either *S. enterica* monoculture, mutualistic co-culture, or competitive co-culture were then completed with four biological replicates per condition, plus three biological replicates for no-phage controls per condition.

Three independent experiments were set up and completed during different weeks to protect against batch effects and confirm the repeatability of the results. One representative experimental run was chosen for display in this paper.

To impose a cost of generalism in our system, we repeated the phage competition assays, incubating the phage in minimal media at 37°C with shaking for 24 hours prior to the addition of cells in either *S. enterica* monoculture or competitive co-culture. Mutualistic co-culture was not tested. Once cells were added, cultures were grown for an additional 24 hours. Phage densities were quantified at the beginning and end of the 48-hour experiment. The experiment was completed once following preliminary trials to confirm that EH7 did not degrade below the limit of recovery after 24 hours.

Phage degradation assays

We examined the impact of cell starvation on the formation of new EH7 particles using a full factorial design of both phage types and *E. coli* or *S. enterica* monoculture in lactose hypho minimal media. Neither bacterial strain could grow, as each was starved of essential nutrients. Bacteria were inoculated in lactose hypho monoculture at a density of 10^5 cells per 200 μ L of medium in a 96-well plate and treated with either EH7 or P22 $_{vir}$ at a total density of 10^3 PFU (MOI = 0.01) or incubated in a no-phage control. Additionally, we tested each phage in isolation in lactose minimal media without cells to determine phage decay rates. Each condition consisted of three technical replicates. Both experiments were completed once at 37°C with shaking at 432 rotations per minute. Phage density in each well was determined by plaque overlay assay at the beginning and end of the 48-hour experiment. Change in phage titer was again represented as the natural log of the final phage density divided by the starting phage density ($\ln(\text{final PFU/mL} / \text{initial PFU/mL})$).

Phage sequencing and genomic analysis

Phage samples were sequenced following DNA extraction. To isolate DNA, 450 μ L of each phage stock was combined in a microcentrifuge tube with 50 μ L DNase I 10x Buffer (Invitrogen), 5 μ L DNase I (Invitrogen), and 1 μ L RNase A (Qiagen). The solution was incubated at 37°C without shaking for 1.5 hours, followed by inactivation of DNase I and RNase A through the application of 20 μ L of 0.5M EDTA and incubation at 75°C for 10 minutes. 1.25 μ L Proteinase K (Invitrogen) was then added to the tube and the solution was incubated for an additional 1.5 hours at 56°C without shaking. DNA was then purified using the Qiagen DNeasy Blood and Tissue kit and quantified on a Nanodrop. Samples were sent to Seq Center, LLC (<https://www.seqcenter.com/>) for sequencing.

Once returned, reads were assembled and evaluated. Point mutations knocking out lysogeny in our lab strain of P22*vir* (reads available at SRA accession SRX22993822) were identified using *breseq* v. 0.28 [75] to assemble and analyze reads relative to the ancestral, lysogenic version of P22 (GenBank accession NC_002371.2). A complete EH7 annotation was created by E. Hansen and S. Bowden using Unicycler v. 0.5.0 [76] (GenBank accession OR413347.1).

Scripts and data availability

Numerical simulations, sensitivity analyses, data analysis, statistics, and figure generation were performed using R v. 4.2.1 using custom scripts available at <https://github.com/bisesi/Host-Ecology-and-Host-Range>. Raw experimental data and Mathematica notebooks for fixed point analysis are available at the same link.

Acknowledgements

The authors thank X. Xiong, J.N.V. Martinson, J. Chacón, A.K. Shaw, M. Torstenson, C. Wojan, N. Narayanan, and D. Kim for helpful comments on the manuscript. The authors would also like to thank members of the Möbius and Nadell labs for input throughout the conceptualization and design of this project, as well as I.J. Molineux for providing our lytic P22_{vir} strain. This work was supported by BBSRC via BBSRC-NSF/BIO grant BB/V011464/1 to W.M., and the National Science Foundation IOS-2017879 to C.D.N. and IOS-2019304 to W.R.H.

Competing Interests

The authors declare no conflicts of interest.

References

- [1] Friman VP, Teppo H, Jouni L, Veijo K. Availability of Prey Resources Drives Evolution of Predator–Prey Interaction. *Proceedings of the Royal Society B: Biological Sciences*. 2008; 1643(275): 1625–33.
- [2] Suttle C. Viruses in the sea. *Nature*. 2005; 437(1): 356–36.
- [3] Bohannan BJM, Lenski RE. Effect of Prey Heterogeneity on the Response of a Model Food Chain to Resource Enrichment. *The American Naturalist*. 1999; 153(1): 73-82.
- [4] Forister ML, Jenkins SH. A Neutral Model for the Evolution of Diet Breadth. *The American Naturalist*. 2017; 190(2): E40–54.
- [5] Loxdale HD, Lushai G, Harvey JA. The Evolutionary Improbability of ‘Generalism’ in Nature, with Special Reference to Insects. *Biological Journal of the Linnean Society*. 2011; 103(1):1–18.
- [6] McLeish M, Sacristán S, Fraile A, García-Arenal F. Scale Dependencies and Generalism in Host Use Shape Virus Prevalence. *Proceedings of the Royal Society B: Biological Sciences*. 2017; 284(1869): 20172066.
- [7] Dennis RLH, Dapporto L, Fattorini S, Cook LM. The Generalism–Specialism Debate: The Role of Generalists in the Life and Death of Species. *Biological Journal of the Linnean Society*. 2011; 104(4):725–37.
- [8] Kawecki TJ. Red Queen meets Santa Rosalia: Arms races and the evolution of host specialization in organisms with parasitic lifestyles. *The American Naturalist*. 1998; 152: 635–651.
- [9] Abrams PA, Holt RD, Roth JD. Apparent Competition or Apparent Mutualism? Shared Predation When Populations Cycle. *Ecology*. 1998; 79(1): 201–12.

- [10] Hanski I, Hansson L, Henttonen H. Specialist Predators, Generalist Predators, and the Microtine Rodent Cycle. *Journal of Animal Ecology*. 1991; 60(1): 353–67.
- [11] May RM. (1981). Models for two interacting populations. *Theoretical Ecology* (Ed. by R.M. May), pp. 78-104. Blackwell Scientific Publications, Oxford.
- [12] Schreiber SJ. Generalist and Specialist Predators That Mediate Permanence in Ecological Communities. *Journal of Mathematical Biology*. 1997; 36(2): 133–48.
- [13] Schreiber SJ, Křivan V. Holt (1977) and Apparent Competition. *Theoretical Population Biology*. 2020; 133: 17–18.
- [14] Abrams PA, Matsuda H. Positive Indirect Effects Between Prey Species That Share Predators. *Ecology*. 1996; 77(2): 610–16.
- [15] Kortright KE, Chaan BK, Koff JL, Turner PE. Phage Therapy: A Renewed Approach to Combat Antibiotic-Resistant Bacteria. *Cell Host & Microbe*. 2019; 25(2): 219–32.
- [16] Kim M, Ryu S. Characterization of a T5-Like Coliphage, SPC35, and Differential Development of Resistance to SPC35 in Salmonella Enterica Serovar Typhimurium and Escherichia Coli. *Applied and Environmental Microbiology*. 2011; 77(6): 2042–50.
- [17] Hyman P, Abedon ST. Bacteriophage Host Range and Bacterial Resistance. *Advances in Applied Microbiology*. 2010: 217-248.
- [18] Duffy S, Turner PE, Burch CL. Pleiotropic costs of niche expansion in the RNA bacteriophage $\phi 6$. *Genetics*. 2006; 172:751–757.
- [19] Sant DG, Woods LC, Barr JJ, McDonald MJ. Host Diversity Slows Bacteriophage Adaptation by Selecting Generalists over Specialists. *Nature Ecology & Evolution*. 2021; 5: 350-359.
- [20] Woolhouse ME, Taylor LH, Haydon DT. Population biology of multihost pathogens. *Science*. 2001; 292: 1109–1112.
- [21] Duffy S, Turner PE. (2009). Phage evolutionary biology. In S. T. Abedon (Ed.), *Bacteriophage Ecology* (pp. 147–176). Cambridge, UK: Cambridge University Press.
- [22] Bono LM, Gensel CL, Pfennig DW, Burch CL. Evolutionary Rescue and the Coexistence of Generalist and Specialist Competitors: An Experimental Test. *Proceedings of the Royal Society B: Biological Sciences*. 2015; 282(1821).
- [23] Bono LM, Gensel CL, Pfennig DW, Burch CL. Competition and the Origins of Novelty: Experimental Evolution of Niche-Width Expansion in a Virus. *Biology Letters*. 2013; 9(1): 20120616.

- [24] Benmayor R, Hodgson DJ, Perron GG, Buckling A. Host Mixing and Disease Emergence. *Current Biology*. 2009; 19(9):764–67.
- [25] Chappell TM, Rausher, MD. Evolution of Host Range in *Coleosporium Ipomoeae*, a Plant Pathogen with Multiple Hosts. *Proceedings of the National Academy of Sciences*. 2016; 113(19): 5346–51.
- [26] Betts A, Rafaluk C, King, KC. Host and parasite evolution in a tangled bank. *Trends Parasitol*. 2016; 32: 863–873.
- [27] Guyader S, Burch CL. Optimal Foraging Predicts the Ecology but Not the Evolution of Host Specialization in Bacteriophages. *PLoS ONE*. 2008; 3(4): e1946.
- [28] Heineman RH, Springman R, Bull JJ. Optimal Foraging by Bacteriophages through Host Avoidance. *The American Naturalist*, 2008; 171(4): E149–57.
- [29] Okamoto KW, Amarasekare P, Post DP, Vasseur DA, Turner PE. The Interplay between Host Community Structure and Pathogen Life-history Constraints in Driving the Evolution of Host-range Shifts. *Functional Ecology* 2019; 33(12): 2338–53.
- [30] Mizrahi P, Sivan AG, Gore J. Community Interactions Drive the Evolution of Antibiotic Tolerance in Bacteria. *Proceedings of the National Academy of Sciences*. 2023; 120(3): e2209043119.
- [31] Adamowicz EM, Muza M, Chacón JM, Harcombe WR. Cross-feeding modulates the rate and mechanism of antibiotic resistance evolution in a model microbial community of *Escherichia coli* and *salmonella enterica*. *PLoS Pathogens*. 2020; 16: e1008700.
- [32] Pauli B, Oña L, Hermann M, Kost C. Obligate mutualistic cooperation limits evolvability. *Nature Communications*. 2022; 13: 1–11.
- [33] Tilman D. Resource Competition and Community Structure. *Monographs in Population Biology*. December 1982.
- [34] Velzen E, Etienne RS. The evolution and coexistence of generalist and specialist herbivores under between-plant competition. *Theoretical Ecology*. 2012; 6: 87–98.
- [35] Pels B, Sabelis MW. Local Dynamics, Overexploitation and Predator Dispersal in an Acarine Predator-Prey System. *Oikos*. 1999; 86(3) 573–83.
- [36] Murdoch WW. Switching in General Predators: Experiments on Predator Specificity and Stability of Prey Populations. *Ecological Monographs*. 1969; 39(4): 335–54.
- [37] Knight TM, Chase JM, Hillebrand H, Holt RD. Predation on Mutualists Can Reduce the Strength of Trophic Cascades. *Ecology Letters* 2006; 9(11): 1173–78.

- [38] Kassen, R. The Experimental Evolution of Specialists, Generalists, and the Maintenance of Diversity: Experimental Evolution in Variable Environments. *Journal of Evolutionary Biology*. 2002; 15(2): 173–90.
- [39] Buckling A, Brockhurst MA, Travisano M, Rainey PB. Experimental Adaptation to High and Low Quality Environments under Different Scales of Temporal Variation. *Journal of Evolutionary Biology*. 2007; 8(1): 296–300.
- [40] Wilson DS, Yoshimura J. On the coexistence of specialists and generalists. *The American Naturalist*. 1994; 144:692–707.
- [41] Visser E, Boots M. The Problem of Mediocre Generalists: Population Genetics and Evolutionary Perspectives on Host Breadth Evolution in Pathogens.” *Proceedings of the Royal Society B: Biological Sciences*. 2020; 287(1933): 20201230.
- [42] Remold SK. Understanding specialism when the jack of all trades can be the master of all. *Proceedings of the Royal Society B: Biological Sciences*. 2012; 279: 4861-4869.
- [43] Satterwhite RS, Cooper TF. Constraints on adaptation of *Escherichia coli* to mixed-resource environments increase over time. *Evolution*. 2015; 69: 2067-2078.
- [44] Kawecki TJ. Accumulation of deleterious mutations and the evolutionary cost of being a generalist. *The American Naturalist*. 1994; 144: 833-838.
- [45] Abrams PA. The Prerequisites for and Likelihood of Generalist-Specialist Coexistence. *The American Naturalist*. 2006; 167(3): 329–42.
- [46] Agrawal AA. Host-range evolution: adaptation and tradeoffs in fitness of mites on alternative hosts. *Ecology*. 2000; 81:500–508.
- [47] Heineman, RH, Brown SP. Experimental Evolution of a Bacteriophage Virus Reveals the Trajectory of Adaptation across a Fecundity/Longevity Trade-off. *PLoS One*. 2012; 7(10): e46322.
- [48] Zimmerman AE, Howard-Varona C, Needham DM, John SG, Worden AZ, Sullivan MB, Waldbauer JR, Coleman ML. Metabolic and Biogeochemical Consequences of Viral Infection in Aquatic Ecosystems. *Nature Reviews Microbiology*. 2020; 18(1): 21–34.
- [49] Voigt E, Rall BC, Chatzinotas A, Brose U, Rosenbaum B. Phage Strategies Facilitate Bacterial Coexistence under Environmental Variability. *PeerJ*. 2021; 9: e12194.
- [50] Netter Z, Dunham DT, Seed K. Adaptation to Bile and Anaerobicity Limits *Vibrio Cholerae* Phage Adsorption. *bioRxiv*. 2023. <https://doi.org/10.1101/2023.04.22.537938>.
- [51] Adams MH. 1959. Bacteriophages. Interscience, New York.

- [52] Aufrere R, Tempete M, Bohin JP. Regulation of Expression of the Gene for Vitamin B12 Receptor Cloned on a Multicopy Plasmid in Escherichia Cob". *Molecular Genetics and Genomics*. 1986; 205:358-365.
- [53] Bradbeer C, Woodrow ML, Khalifah LI. Transport of vitamin B12 in Escherichia coli: common receptor system for vitamin B12 and bacteriophage BF23 on the outer membrane of the cell envelope. *Journal of Bacteriology*. 1976; 125:1032-1039.
- [54] Lundrigan, MD, Köster W, Kadner RJ. Transcribed Sequences of the Escherichia coli BtuB Gene Control Its Expression and Regulation by Vitamin B12. *Proceedings of the National Academy of Sciences* 1991; 88(4): 1479–83.
- [55] Ravnum S, Andersson DI. Vitamin B12 Repression of the BtuB Gene in Salmonella Typhimurium Is Mediated via a Translational Control Which Requires Leader and Coding Sequences. *Molecular Microbiology*. 1997; 23(1): 35–42.
- [56] Wang J, Jiang Y, Vincent M, Sun Y, Yu H, Wang J, Bao Q, Kong H, Hu S. Complete genome sequence of bacteriophage T5. *Virology*. 2005; 332:45–65.
- [57] Niu YD, Stanford K, Kropinski AM, Ackermann HW, Johnson RP, She YM, Ahmed R, Villegas A, McAllister TA. Genomic, proteomic and physiological characterization of a T5-like bacteriophage for control of Shiga toxin-producing Escherichia coli O157:H7. *PLoS One*. 2012; 7:e34585.
- [58] Hong J, Kim KP, Heu S, Lee SJ, Adhya S, Ryu S. Identification of host receptor and receptor-binding module of a newly sequenced T5-like phage EPS7. *FEMS Microbiology Letters*. 2008; 289:202–209.
- [59] Venza Colon CJ, Vasquez Leon AY, Villafane RJ. Initial interaction of the P22 phage with the Salmonella typhimurium surface. *Puerto Rico Health Sciences Journal*. 23:95–101.
- [60] Steinbacher S, Miller S, Baxa U, Weintraub A, Seckler R. Interaction of Salmonella phage P22 with its O-antigen receptor studied by X-ray crystallography. *Biological Chemistry*. 1997; 378:337–34.
- [61] Switt AIM, Sulakvelidze A, Wiedmann M, Kropinski AM, Wishart DS, Poppe C, Liang Y. Salmonella Phages and Prophages: Genomics, Taxonomy, and Applied Aspects. In *Salmonella: Methods and Protocols*, edited by Heide Schatten and Abraham Eisenstark, 237–87. Methods in Molecular Biology. New York, NY: Springer, 2015.
- [62] Blazanin M, Lam WT, Vasen E, Chan BK, Turner PE. Decay and Damage of Therapeutic Phage OMKO1 by Environmental Stressors. *PLoS One*. 2022; 17(2): e0263887.
- [63] Degnan PH, Taga ME, Goodman AL. Vitamin B12 as a Modulator of Gut Microbial Ecology. *Cell Metabolism*. 2014; 20(5)769–78.

- [64] Hoek TA, Axelrod K, Biancalani T, Yurtsev EA, Liu J, Gore J. Resource Availability Modulates the Cooperative and Competitive Nature of a Microbial Cross-Feeding Mutualism. *PLoS Biology*. 2016; 14(8): e1002540.
- [65] Renardy M, Joslyn LR, Millar JA, Kirschner DE. To Sobol or Not to Sobol? The Effects of Sampling Schemes in Systems Biology Applications. *Mathematical Biosciences*. 2021; 337:108593.
- [66] Qian G, Mahdi A. Sensitivity Analysis Methods in the Biomedical Sciences. *Mathematical Biosciences*. 2020; 323:108306.
- [67] Weber F, Theers S, Surmann D, Ligges U, Weihs C. Sensitivity Analysis of Ordinary Differential Equation Models: Methods by Morris and Sobol' and Application in R. (2018)
- [68] Harcombe W. Novel Cooperation Experimentally Evolved Between Species. *Evolution*. 2010; 64(7): 2166–72.
- [69] Douglas SM, Chubiz LM, Harcombe WR, Marx CJ. Identification of the Potentiating Mutations and Synergistic Epistasis That Enabled the Evolution of Inter-Species Cooperation. *PLoS One*. 2017; 12(5): e0174345.
- [70] Harcombe WR, Riehl WJ, Dukovski, I Granger DR, Betts A, Lang AH, Bonilla G, Amrita K, Leiby N, Mehta P, Marx CJ, Segre D. Metabolic Resource Allocation in Individual Microbes Determines Ecosystem Interactions and Spatial Dynamics. *Cell Reports*. 2014; 7(4): 1104–15.
- [71] Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita M, Wanner BL, Mori H. Construction of Escherichia Coli K-12 in-Frame, Single-Gene Knockout Mutants: The Keio Collection. *Molecular Systems Biology*. 2006; 2: 2006.0008.
- [72] Delaney NF, Kaczmarek ME, Ward LM, Swanson PK, Lee MC, Marx CJ. Development of an Optimized Medium, Strain and High-Throughput Culturing Methods for *Methylobacterium Exorquens*. *PLoS One*. 2013; 8(4): e62957.
- [73] Adamowicz EM, Flynn J, Hunter RC, Harcombe WR. Cross-Feeding Modulates Antibiotic Tolerance in Bacterial Communities. *The ISME Journal*. 2018; 12(11): 2723–35.
- [74] Kutter E. Phage Host Range and Efficiency of Plating. In *Bacteriophages: Methods and Protocols, Volume 1: Isolation, Characterization, and Interactions*, edited by Martha R.J. Clokie and Andrew M. Kropinski, 141–49. Methods in Molecular Biology. Totowa, NJ: Humana Press, 2009.
- [75] Deatherage DE, Barrick JE. Identification of mutations in laboratory-evolved microbes from next-generation sequencing data using *breseq*. *Methods in Molecular Biology* 2014; 1151: 165–188.

860 [76] Wick, RR, Judd, LM, Gorrie, CL, Holt, KE. Unicycler: Resolving bacterial genome
861 assemblies from short and long sequencing reads. *PLoS Computational Biology*. 2017; 13(6):
862 e1005595.

863 Tables

Parameter	Competition Default Value	Mutualism Default Value	Description
$\alpha_{\square,\square}$	1	1	Mutualistic coefficient, benefit of prey species S to prey species E
$\beta_{\square,\square}$	1	0	Competition coefficient, effect of prey species S on prey species E
$\alpha_{\square,\square}$	1	1	Mutualistic coefficient, benefit of prey species E to prey species S
$\beta_{\square,\square}$	1	0	Competition coefficient, effect of prey species E on prey species S
$\mu_{\square}$	0.5	0.5	Maximum intrinsic growth rate of prey species E
$\mu_{\square}$	0.5	0.5	Maximum intrinsic growth rate of prey species S
$\gamma_{\square,\square}$	20	20	Burst size of generalist phage on prey species E
$\gamma_{\square,\square}$	20	20	Burst size of specialist phage on prey species E
$\gamma_{\square,\square}$	20	20	Burst size of specialist phage on prey species S
$\varsigma_{\square,\square}$	0.001	0.001	Attachment rate of generalist phage on prey species E
$\varsigma_{\square,\square}$	0.001	0.001	Attachment rate of specialist phage on prey species E
$\varsigma_{\square,\square}$	0.001	0.001	Attachment rate of specialist phage on prey species E

$\delta_{\square}$	0.03	0.03	Intrinsic death rate of prey species E
$\delta_{\square}$	0.03	0.03	Intrinsic death rate of prey species S
$\delta_{\square}$	0.03	0.03	Intrinsic death rate of generalist phage
$\delta_{\square}$	0.03	0.03	Intrinsic death rate of specialist phage
$\kappa_{\square}$	0	1	Half-saturation constant of species E
$\kappa_{\square}$	0	1	Half-saturation constant of species S
$\square$	2	1	System carrying capacity

864 **Table 1. Dimensionless phenomenological model parameters, default values, and**
865 **descriptions.**

866

Trade-off	Parameter Combinations	Significance
None	$\gamma_{\square,\square} = \square_{\square,\square} = 20$ and $\varsigma_{\square,\square} = \square_{\square,\square} = 0.001$	Generalist and specialist phage are parametrically identical
Cost of generalism (burst size)	$\gamma_{\square,\square} = \gamma_{E,\square} = 20$ and $\gamma_{\square,\square} < \square_{\square,\square}$	Generalist and specialist phage differ in their abilities to kill prey due to differences in burst size
Cost of generalism (attachment rate)	$\varsigma_{\square,\square} = \varsigma_{E,\square} = 0.001$ and $\varsigma_{\square,\square} < \square_{\square,\square}$	Generalist and specialist phage differ in their abilities to kill prey due to differences in attachment rate
Interaction outcome (growth rate)	$\mu_{\square} = 0.5$ and $\mu_{\square} \neq \square_{\square}$	Prey species coexistence in the absence of phage is biased or impossible due to differences in growth rate
Interaction outcome (interaction coefficient, competition)	$\beta_{\square,\square} = 1$ and $\beta_{\square,\square} \neq \square_{\square,\square}$	Prey species coexistence when competing in the absence of phage is biased or impossible due to differences in interaction coefficients

Interaction outcome (interaction coefficient, mutualism)	$\alpha_{\square,\square} = 1$ and $\alpha_{\square,\square} \neq \square_{\square,\square}$	Prey species coexistence when mutualistic in the absence of phage is biased or impossible due to differences in interaction coefficients
Cost of generalism and interaction outcome (growth rate)	$(\gamma_{\square,\square} = 20 \text{ and } \gamma_{\square,\square} < \square_{\square,\square} \text{ and } \mu_{\square} = 0.5 \text{ and } \mu_{\square} \neq \square_{\square})$ or $(\varsigma_{\square,\square} = 0.001 \text{ and } \varsigma_{\square,\square} < \square_{\square,\square} \text{ and } \mu_{\square} = 0.5 \text{ and } \mu_{\square} \neq \square_{\square})$	Generalist and specialist phage differ in their ability to kill prey and prey species coexistence in the absence of phage is biased or impossible due to differences in growth rate
Cost of generalism and interaction outcome (interaction coefficient, competition)	$(\gamma_{\square,\square} = 20 \text{ and } \gamma_{\square,\square} < \square_{\square,\square} \text{ and } \beta_{\square,\square} = 1 \text{ and } \beta_{\square,\square} \neq \square_{\square,\square})$ or $(\varsigma_{\square,\square} = 0.001 \text{ and } \varsigma_{\square,\square} < \square_{\square,\square} \text{ and } \beta_{\square,\square} = 1 \text{ and } \beta_{\square,\square} \neq \square_{\square,\square})$	Generalist and specialist phage differ in their ability to kill prey and prey species coexistence when competing in the absence of phage is biased or impossible due to differences in interaction coefficients
Cost of generalism and interaction outcome (interaction coefficient, mutualism)	$(\gamma_{\square,\square} = 20 \text{ and } \gamma_{\square,\square} < \square_{\square,\square} \text{ and } \alpha_{\square,\square} = 1 \text{ and } \alpha_{\square,\square} \neq \square_{\square,\square})$ or $(\varsigma_{\square,\square} = 0.001 \text{ and } \varsigma_{\square,\square} < \square_{\square,\square} \text{ and } \alpha_{\square,\square} = 1 \text{ and } \alpha_{\square,\square} \neq \square_{\square,\square})$	Generalist and specialist phage differ in their ability to kill prey and prey species coexistence when mutualistic in the absence of phage is biased or impossible due to differences in interaction coefficients

867 **Table 2. Parameter trade-offs tested in our phenomenological model and their biological**
868 **significance.** Parameters not listed here were set to default values in Table 1.

869 Figures

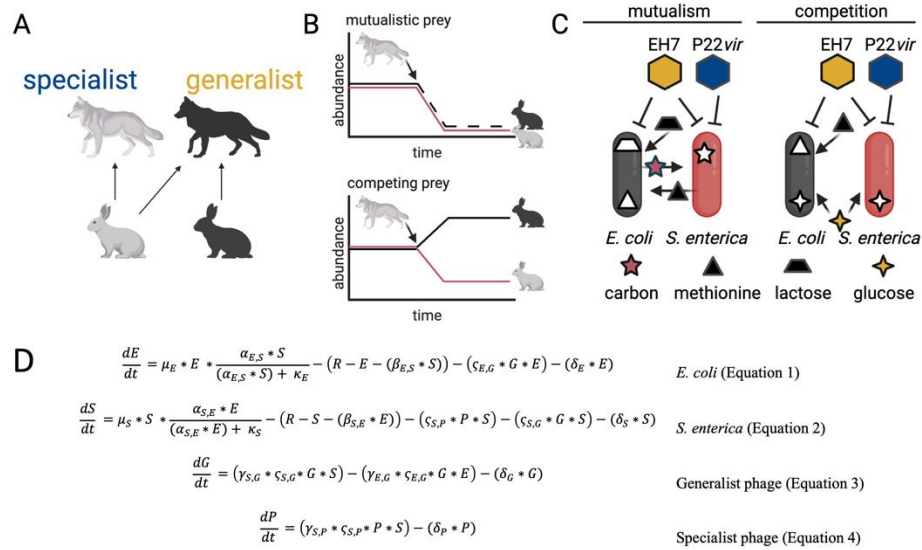
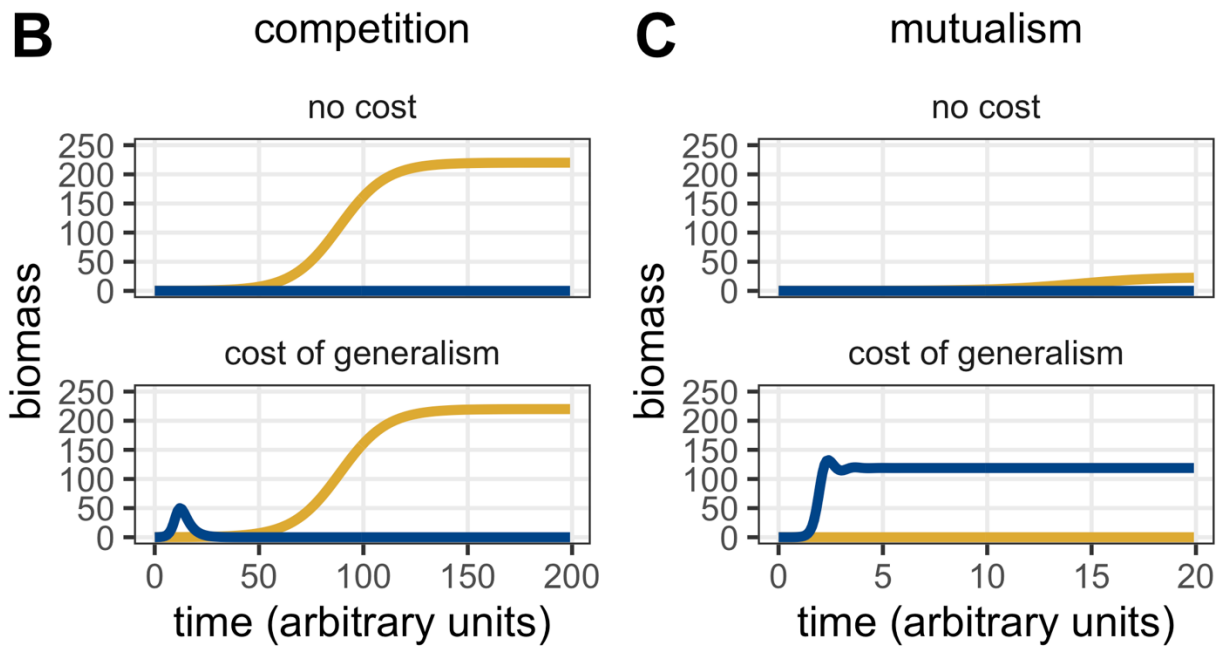
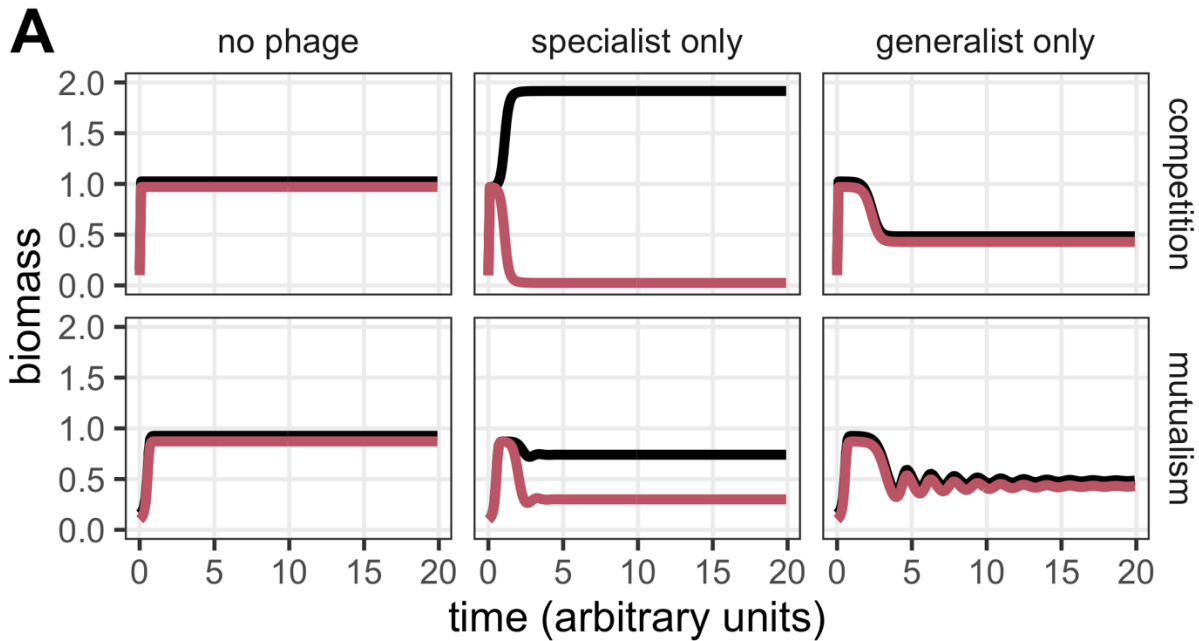


Figure 1. Specificity in a microbial synthetic community. **A:** Schematic of diet breadth in macropredators. Species with generalist diets have wide diet breadths spanning multiple resources, while specialists have narrower diet breadths, sometimes specific to a single resource. **B:** Expected community dynamics when mutualistic or competing prey species are challenged by a specialist predator. When prey are mutualistic, predation will reduce abundances of both species. When prey compete, predation will reduce the abundance of one species and result in an increase in the abundance of the other species through competitive release. **C:** Schematic of cross-feeding system consisting of an *E. coli* methionine auxotroph and *S. enterica* methionine secreter. In lactose minimal media, *E. coli* provides carbon byproducts to *S. enterica* and *S. enterica* provides methionine to *E. coli*. In glucose minimal media with methionine, the two bacteria compete. The phage P22vir is a specialist on *S. enterica*, while the phage EH7 is a generalist that can attack both bacterial species. **D:** Lotka-Volterra style modeling equations. For additional details, see Materials and Methods. **For A, B, and C:** Figure created with BioRender.



species ■ *E. coli* ■ *S. enterica* ■ Generalist(EH7) ■ Specialist(P22vir)

Figure 2. Numerically-simulated bacterial dynamics demonstrate that competing prey provide a different selective environment for phage than mutualistic prey. A: In the absence of phage, both prey species reach an equilibrium ratio of 50:50. In the presence of only a specialist phage, the prey attacked by the specialist (pink line, *S. enterica*) decreases. The prey

889 that is not attacked (black line, *E. coli*) reaches a higher equilibrium frequency if the prey are
890 competing and a lower equilibrium frequency if the prey are mutualists. When only the
891 generalist predator is present, regardless of prey interactions, prey remain at a 50:50 ratio
892 throughout the simulated growth period. Note that in panels where abundances are identical,
893 biomass has been deliberately offset for effective visualization. **B:** When prey compete and both
894 phage types are present and parametrically identical (no cost), the generalist (yellow line, EH7)
895 dominates over the specialist (blue line, P22*vir*). When prey compete and both phage types are
896 present but the specialist's burst size increased to five times that of the generalist (cost of
897 generalism), the generalist (yellow line, EH7) still dominates over the specialist (blue line,
898 P22*vir*). **C:** When prey are mutualistic and both phage types are present and parametrically
899 identical (no cost), the generalist (yellow line, EH7) dominates over the specialist (blue line,
900 P22*vir*). When prey are mutualistic and both phage types are present but the specialist's burst
901 size increased to five times that of the generalist (cost of generalism), the specialist (blue line,
902 P22*vir*) dominates over the generalist (yellow line, EH7).

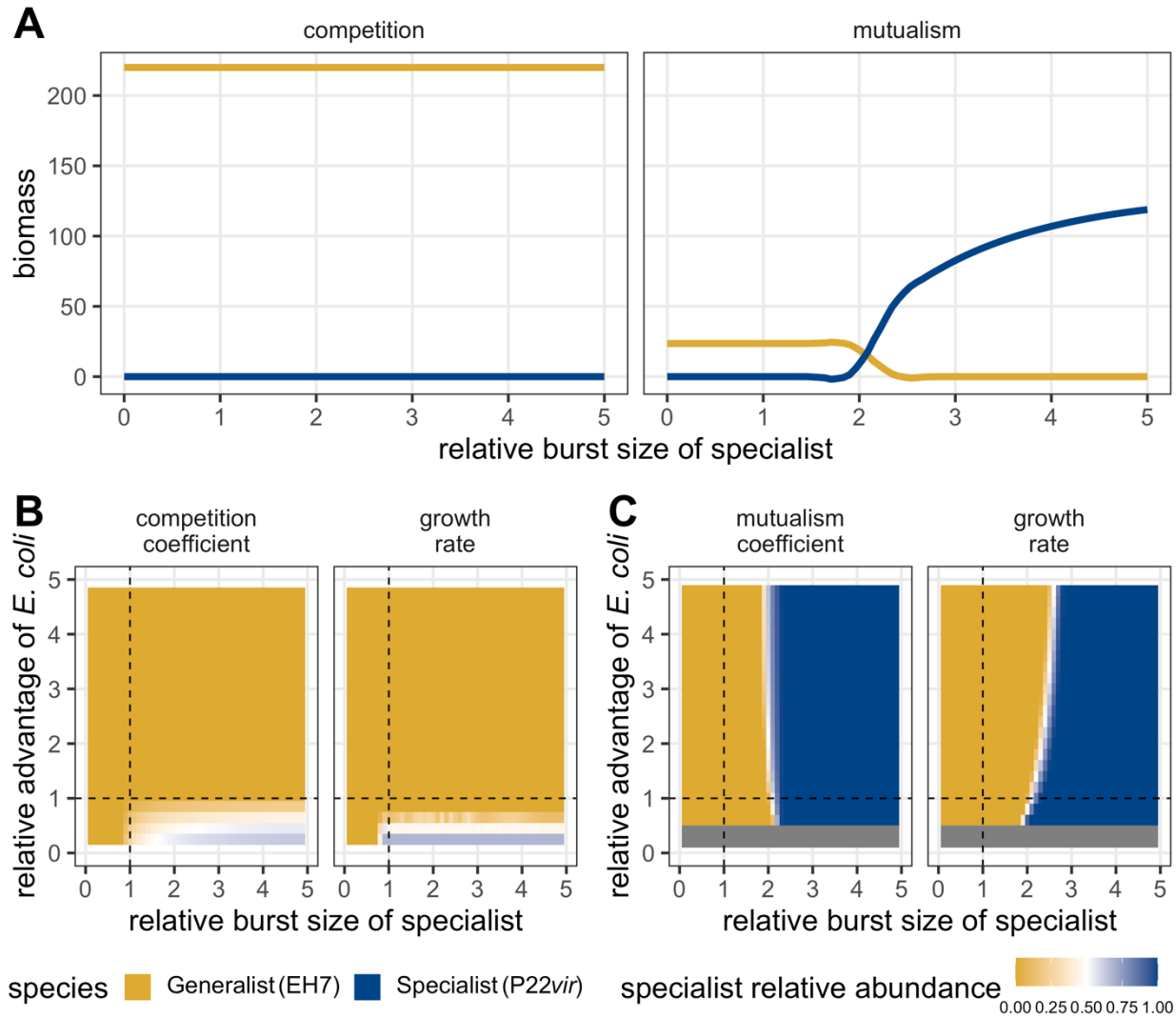


Figure 3. End points of numerically-simulated phage dynamics given a variety of parameter trade-offs demonstrate that prey interactions result in different patterns of predator abundance. A: The final density of each phage type as a function of bacterial interactions and increasing cost of generalism modeled as increasing specialist burst size. When prey are mutualistic, a relative burst size above 2.1 favors specialist phage (blue line, P22vir) over generalists (yellow line, EH7). When prey compete, there is no relative burst size that favors the specialist phage; this is true even as the specialist's burst size increases well beyond the values displayed here. For these analyses, the generalist's burst size is set to 20, with the

specialist's burst size increased relative to that value. All other default parameter values can be found in Table 1. **B:** The relative abundance of the specialist phage on competing prey as a function of increasing cost of generalism and relative growth advantage of the alternative prey *E. coli*. Whether prey growth advantage is modeled through growth rate (μ) or competitive coefficients (β), the generalist is favored (yellow, EH7) except in a small subset of cases where the alternative prey is competitively excluded. For these analyses, the generalist's burst size is set to 20, with the specialist's burst size increased relative to that value. For the competition coefficient panel, the competition coefficient of *S. enterica* is 1, and the competition coefficient of *E. coli* is set relative to that value. For the growth rate panel, the growth rate of *S. enterica* is 0.5, and the growth rate of *E. coli* is set relative to that value. All other default parameter values can be found in Table 1. **C:** The relative abundance of the specialist phage on mutualistic prey *E. coli*. Whether prey growth advantage is modeled through growth rate (μ) or mutualistic benefit (α), a cost of generalism exists above which specialism is favored (blue, P22_{vir}). Note that there are benefit and growth rate values for *E. coli* below which the mutualistic system cannot be supported, indicated by the grey bar. For these analyses, the generalist's burst size is set to 20, with the specialist's burst size increased relative to that value. For the mutualism coefficient panel, the mutualism coefficient of *S. enterica* is 1, and the competition coefficient of *E. coli* is set relative to that value. For the growth rate panel, the growth rate of *S. enterica* is 0.5, and the growth rate of *E. coli* is set relative to that value. All other default parameter values can be found in Table 1.

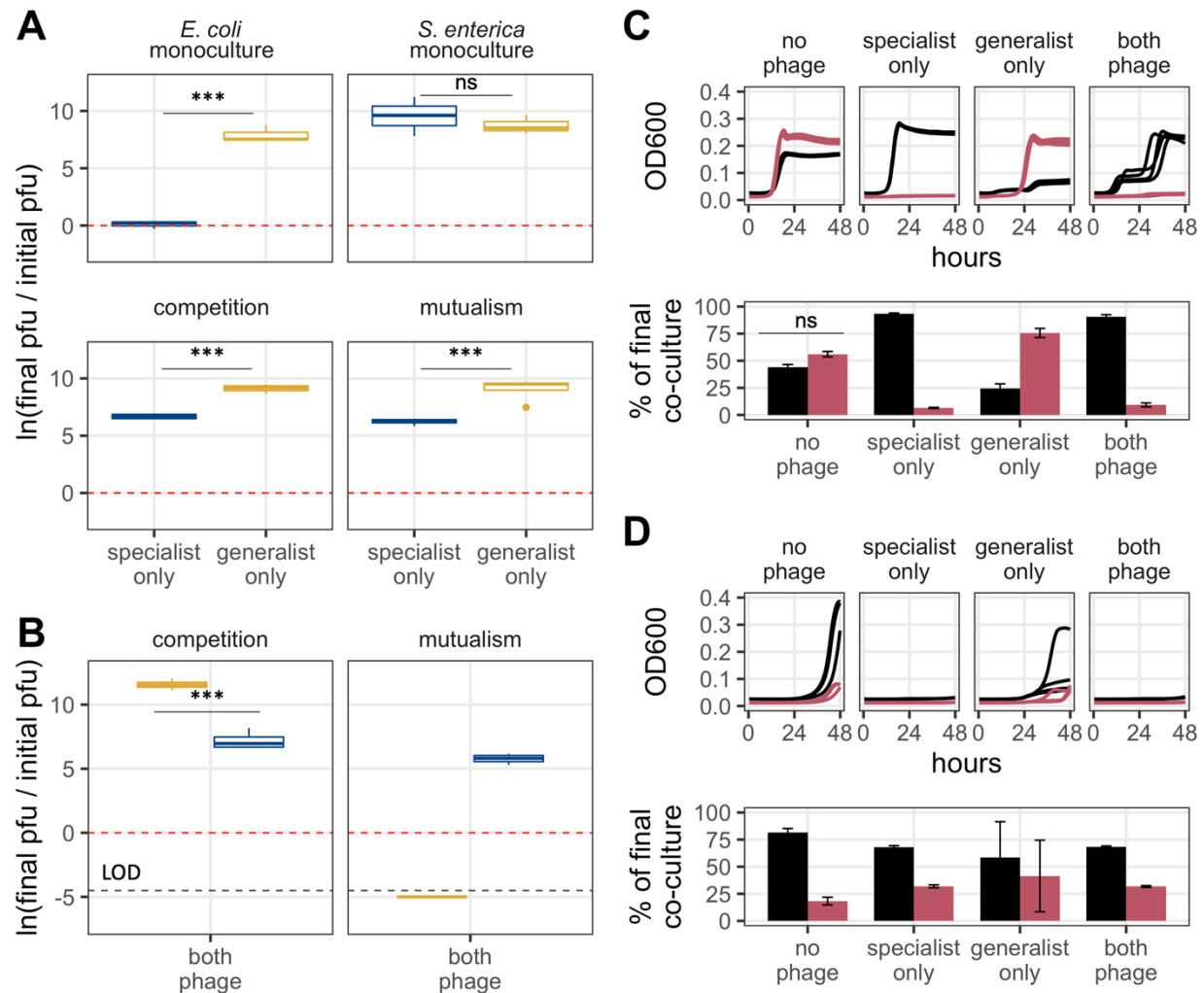


Figure 4. Phage and bacterial dynamics *in vitro* align with modeling expectations. A:

Change in individual phage titer on bacterial monocultures and co-cultures over a 48-hour time-period. EH7 grows on both bacterial strains, while P22vir cannot replicate on *E. coli* but does effectively replicate on *S. enterica*. EH7 reaches approximately equivalent densities mixed prey with both interaction types ($p = 0.999$), as does P22vir ($p = 0.909$), although its final density is reduced compared to EH7 (competition: $p < 0.0001$, mutualism: $p < 0.0001$). **B:** Change in phage titer when phage compete on different bacterial interaction types over a 48-hour time-period. When competing against the specialist, EH7 dominates when prey compete ($p < 0.0001$). P22vir dominates when prey are mutualistic ($p < 0.0001$), while EH7 disappears below the limit

944 of detection (LOD). **For A and B:** The dotted red line indicates no change in titer from the start
945 of the experiment to the end. Values greater than zero indicate an increase in titer, while values
946 below zero indicate a decrease in titer. Statistical significance was determined using a two-way
947 ANOVA with Tukey's HSD multiple comparison test (Supplemental Table 3). The black dotted
948 line indicates the limit of detection. **C:** Inferred species-specific ODs over time across treatment
949 conditions (top) and final fraction of bacterial co-culture composed of each strain across
950 treatment conditions (bottom) when bacteria compete. The fraction of *E. coli* increases in those
951 cases in which *S. enterica* is predominantly suppressed by phage (specialist only and both phage
952 treatments). *E. coli* frequency is reduced relative to no-phage controls when only EH7 is applied
953 ($p = 0.0035$). **D:** Inferred species-specific ODs over time across treatment conditions (top) and
954 final fraction of bacterial population composed of each strain across treatment conditions
955 (bottom) when bacteria are mutualistic. *E. coli* dominates at similar levels in all treatment
956 conditions, even in cases where overall growth is suppressed. **For C and D:** Statistical
957 significance for bar graphs was determined using a two-way ANOVA with Tukey's HSD
958 multiple comparison test (Supplemental Table 3). OD600 traces represent 4 biological replicates
959 for conditions with phage and 3 biological replicates for conditions without phage.

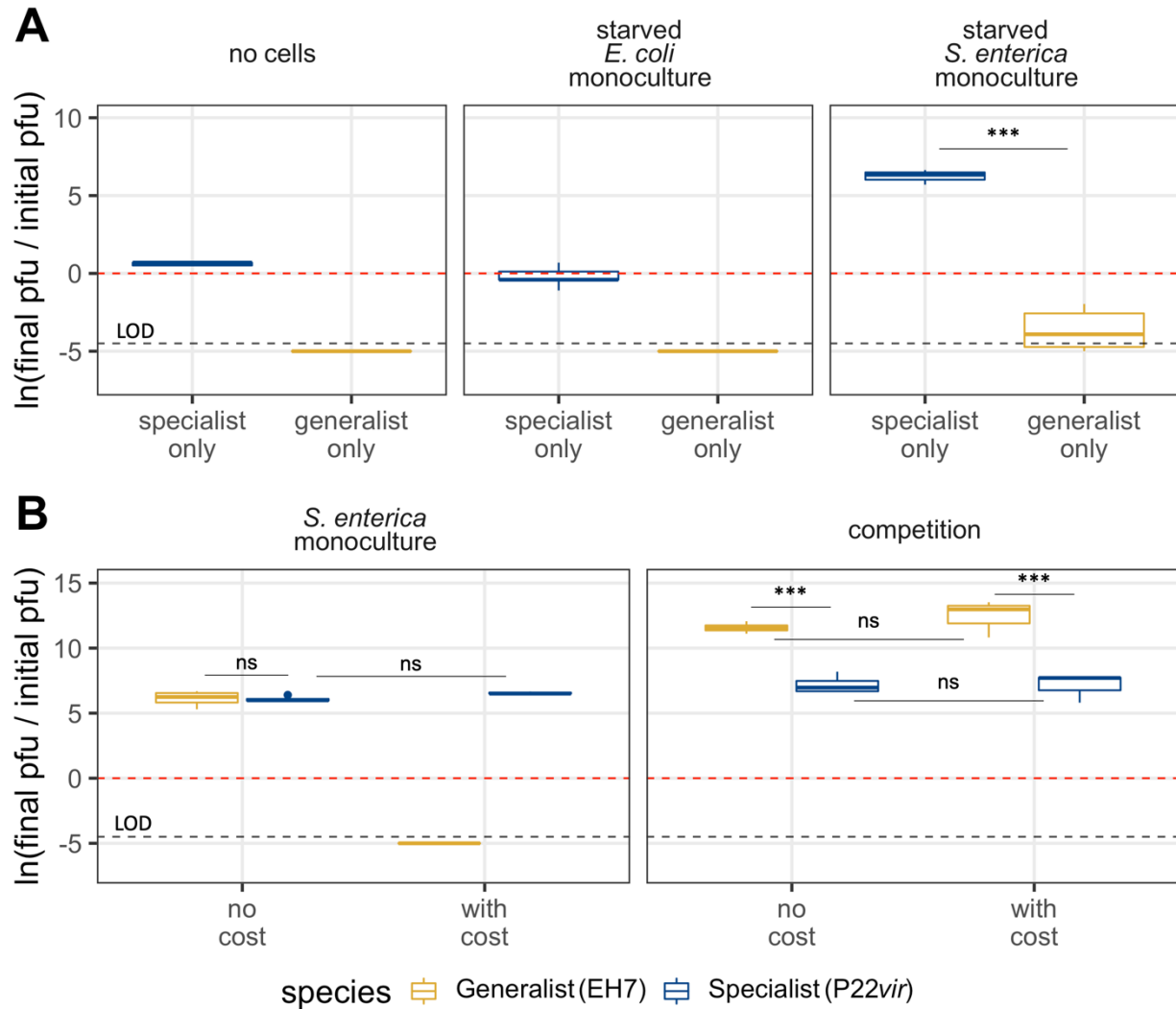


Figure 5. Imposing a cost of generalism *in vitro* as phage intrinsic mortality reproduces modeling results on *S. enterica* monoculture. A: Change in phage titer across interaction types and phage treatments when cells are starved or not present in cultures. P22vir does not degrade in minimal media over the 48-hour growth period, even in cases when there are no cells present that it can productively infect. Additionally, it can reproduce even on starved *S. enterica*. EH7 degrades below the limit of detection in all conditions but does not disappear completely when *S. enterica* is present. **B:** Change in phage titer across interaction types when cells are either added to minimal media at the same time as both phage (no cost) or 24 hours later (with cost). In the no

969 cost condition, EH7 reaches comparable titer to P22_{vir} on *S. enterica* monoculture ($p = 0.999$)
970 and wins when prey compete ($p < 0.0001$). In the condition where cells are added after a period
971 of phage incubation, EH7 degrades below the limit of detection on *S. enterica* monoculture but
972 dominates when prey are competing ($p < 0.0001$). **For A and B:** The dotted red line indicates no
973 change in titer from the start of the experiment to the end. Values greater than zero indicate an
974 increase in titer, while values below zero indicate a decrease in titer. Statistical significance was
975 determined using a two-way ANOVA with Tukey's HSD multiple comparison test
976 (Supplemental Table 4). The black dotted line indicates the limit of detection (LOD).
977

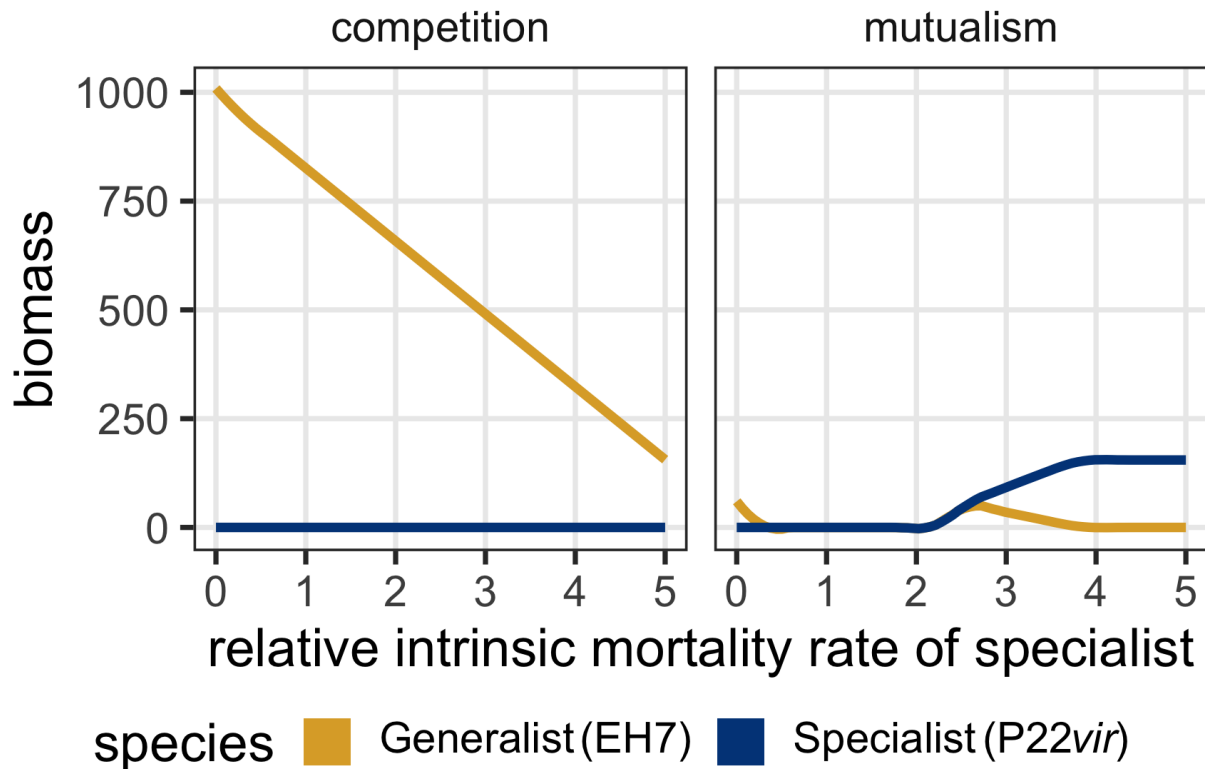
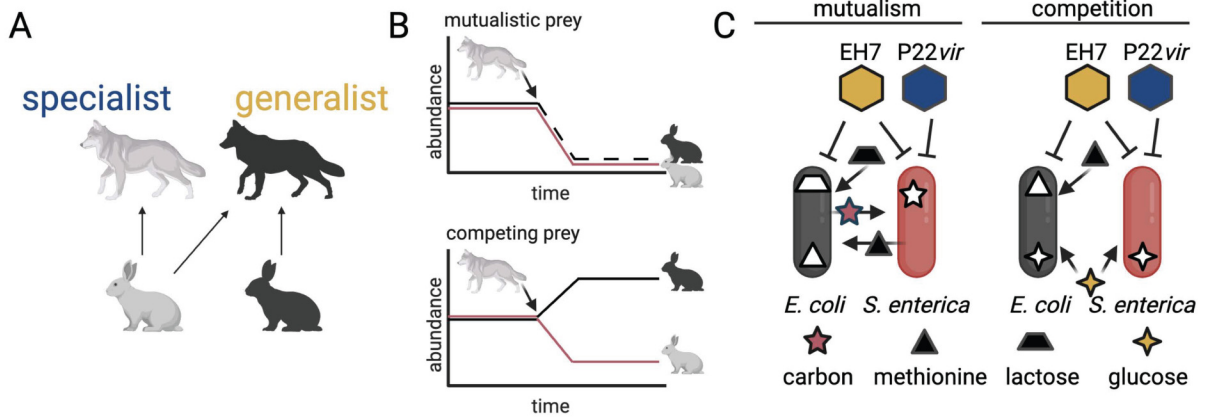


Figure 6. When a cost of generalism is modeled as intrinsic mortality, qualitative patterns of ecological selection on predator specificity match findings when cost of generalism is modeled as burst size (Figure 3) or attachment rate (Supplemental Figure 1). As the intrinsic mortality rate of the generalist phage increases, it maintains its advantage longer when prey compete and is driven extinct at a minimal cost when prey are mutualistic. These qualitative results align with previous modeling findings when cost of generalism is imposed as burst size or attachment rate. For these analyses, the intrinsic mortality rate of the specialist was set to 0.0067, with the generalist's mortality rate increased relative to that value. All other default parameter values can be found in Table 1.



D

$$\frac{dE}{dt} = \mu_E * E * \frac{\alpha_{E,S} * S}{(\alpha_{E,S} * S) + \kappa_E} - (R - E - (\beta_{E,S} * S)) - (\zeta_{E,G} * G * E) - (\delta_E * E)$$

E. coli (Equation 1)

$$\frac{dS}{dt} = \mu_S * S * \frac{\alpha_{S,E} * E}{(\alpha_{S,E} * E) + \kappa_S} - (R - S - (\beta_{S,E} * E)) - (\zeta_{S,P} * P * S) - (\zeta_{S,G} * G * S) - (\delta_S * S)$$

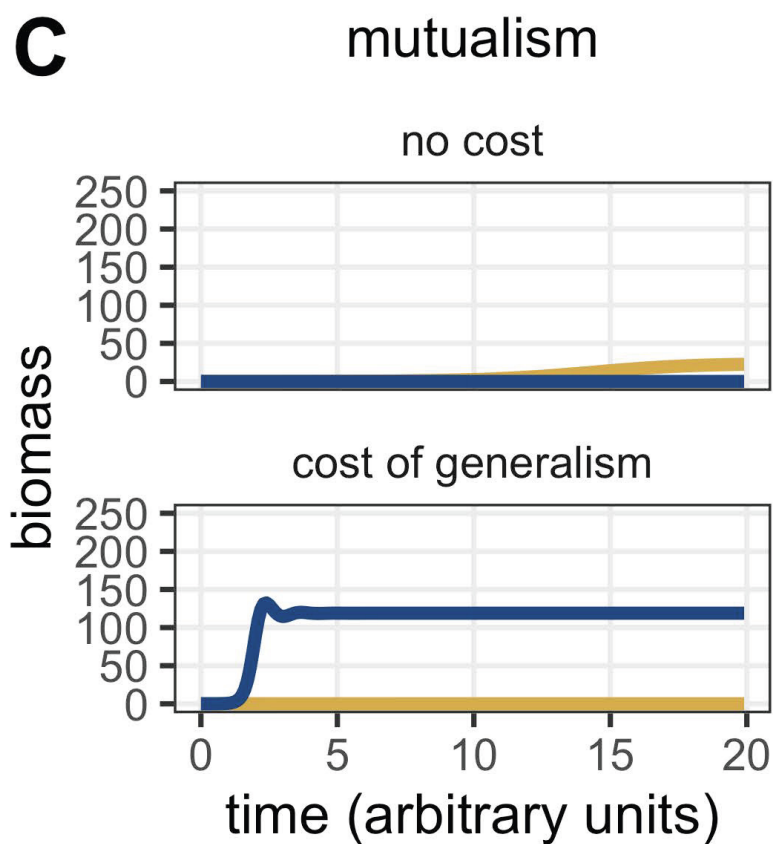
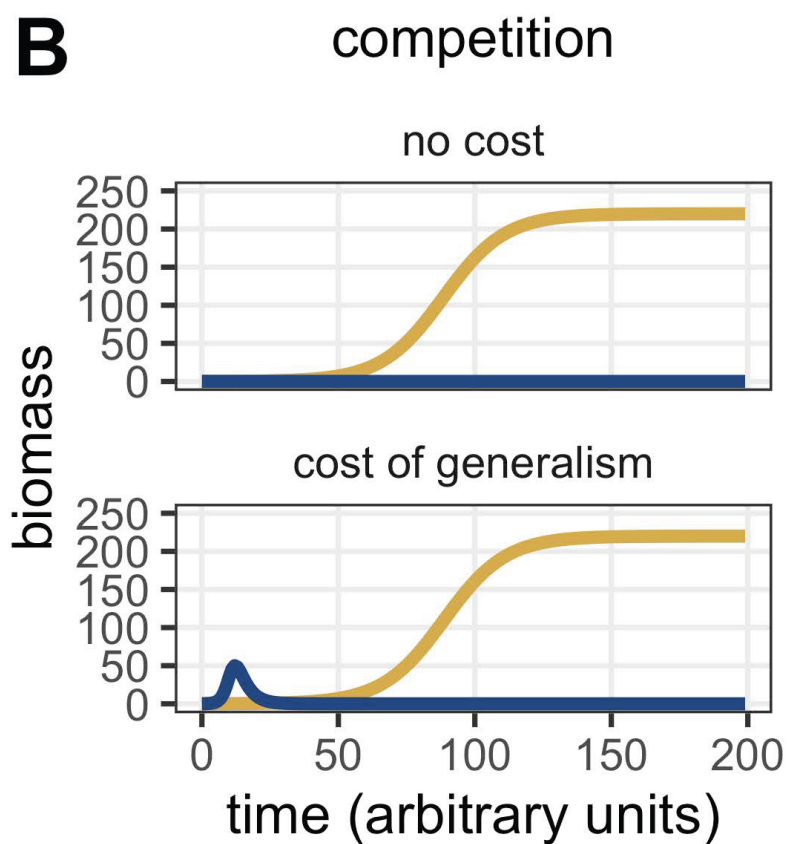
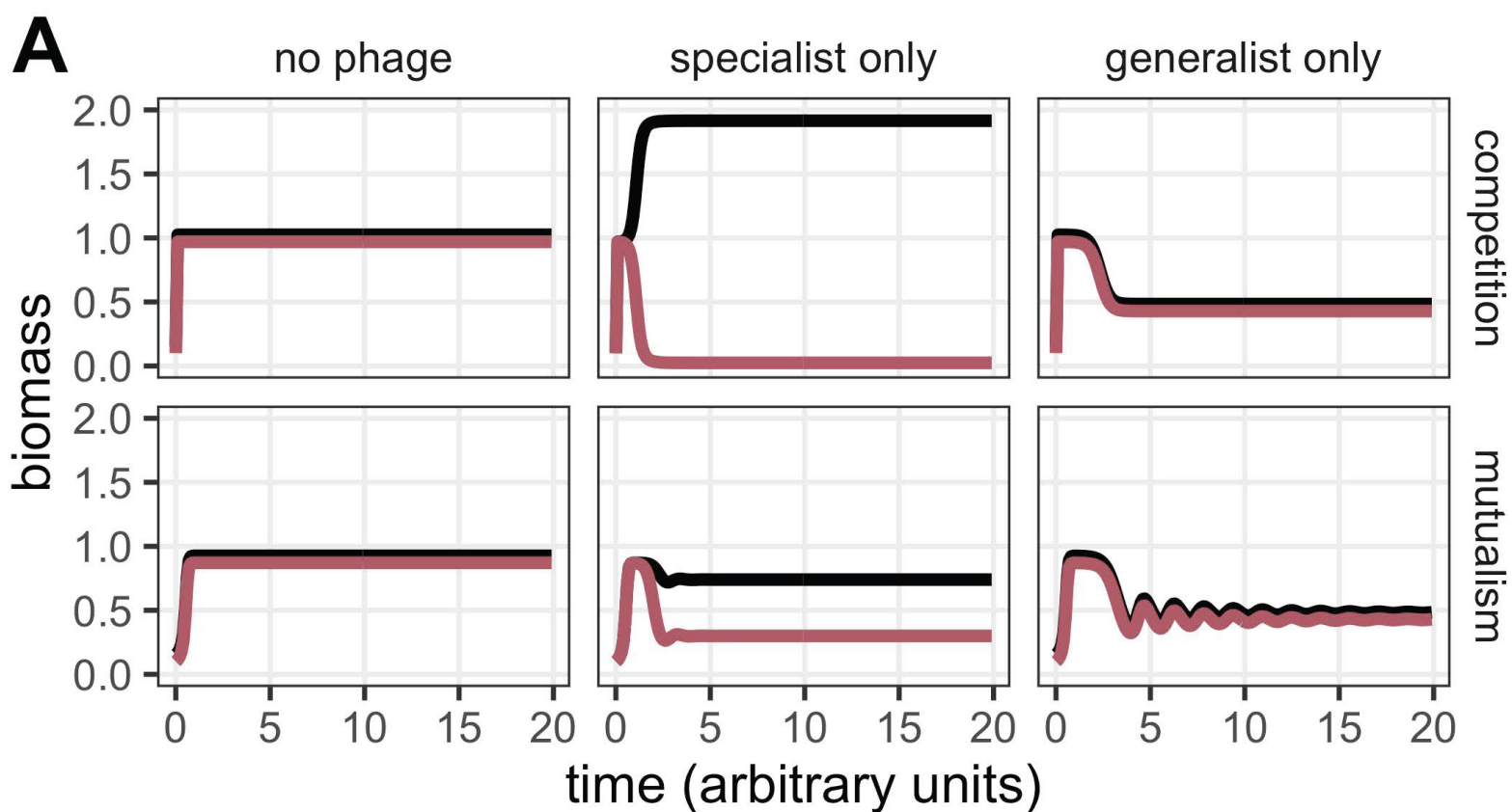
S. enterica (Equation 2)

$$\frac{dG}{dt} = (\gamma_{S,G} * \zeta_{S,G} * G * S) - (\gamma_{E,G} * \zeta_{E,G} * G * E) - (\delta_G * G)$$

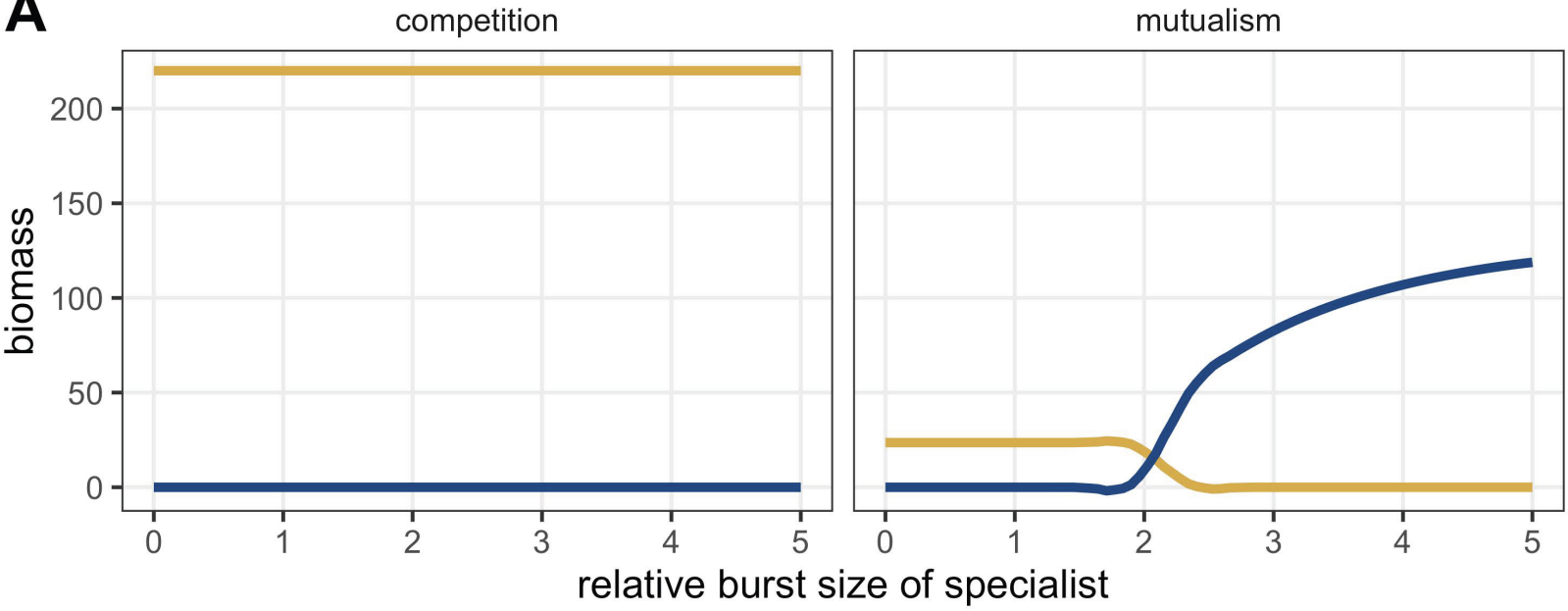
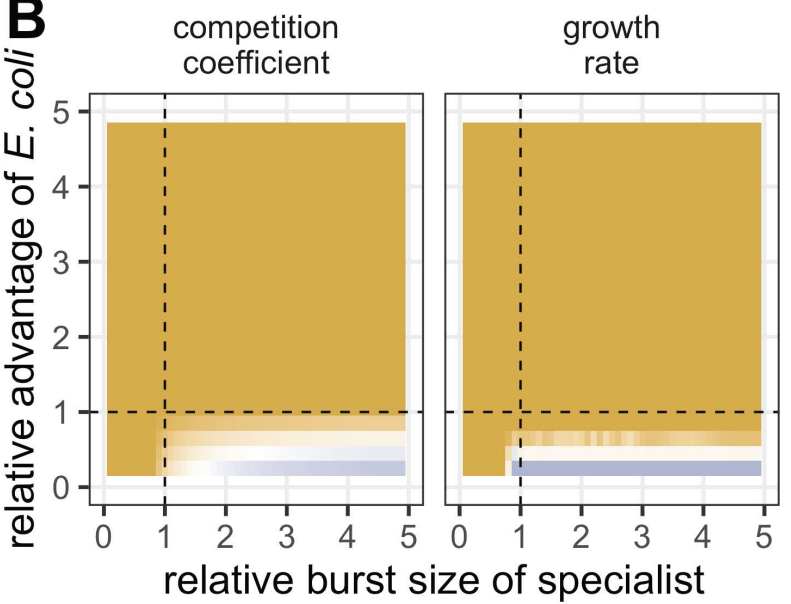
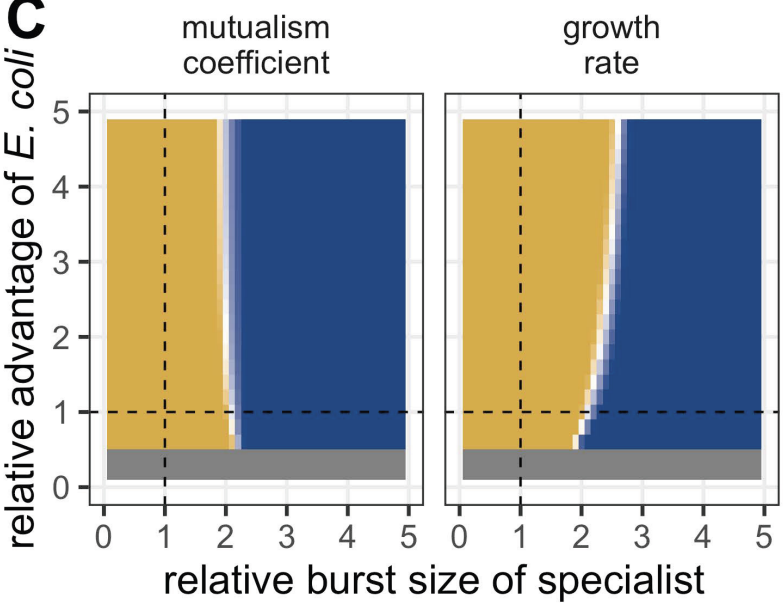
Generalist phage (Equation 3)

$$\frac{dP}{dt} = (\gamma_{S,P} * \zeta_{S,P} * P * S) - (\delta_P * P)$$

Specialist phage (Equation 4)

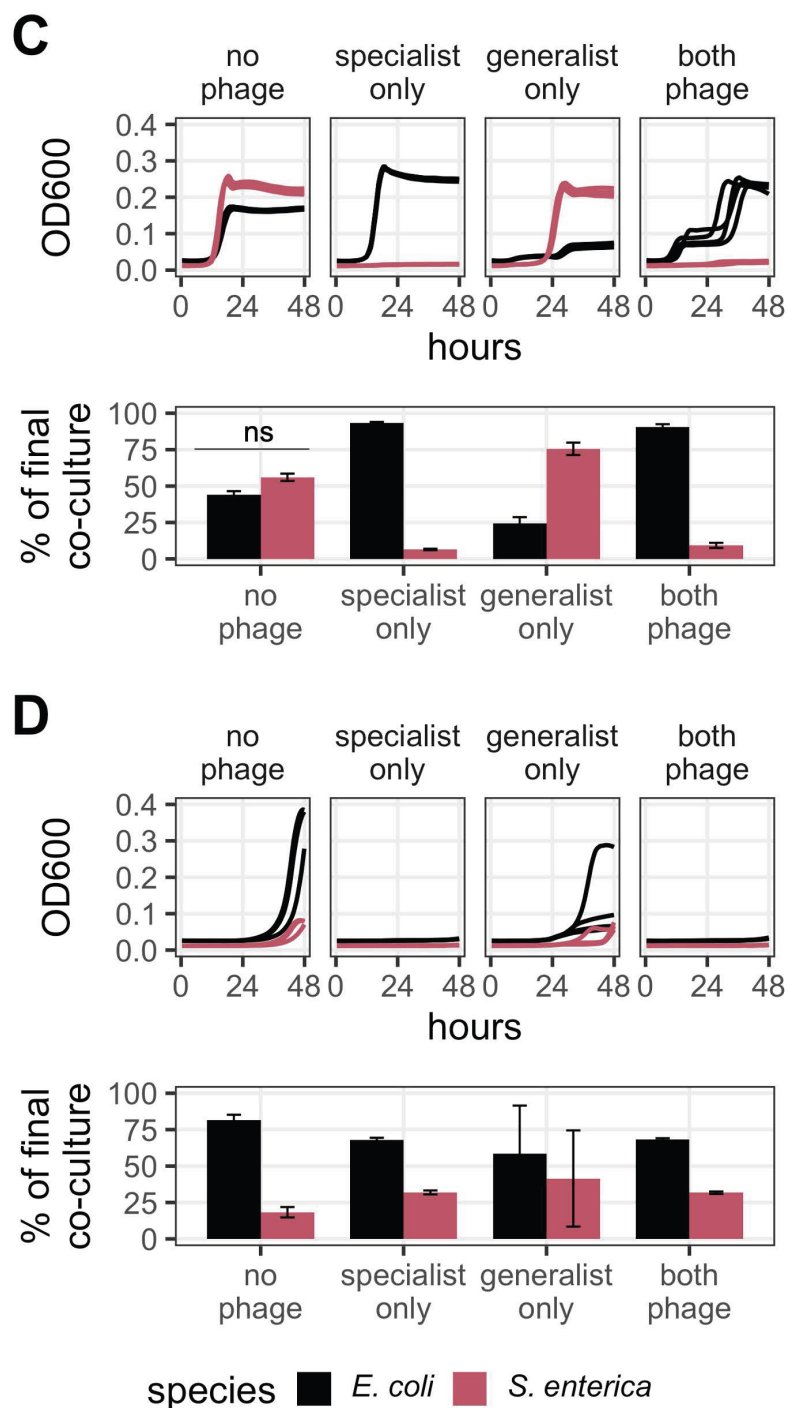
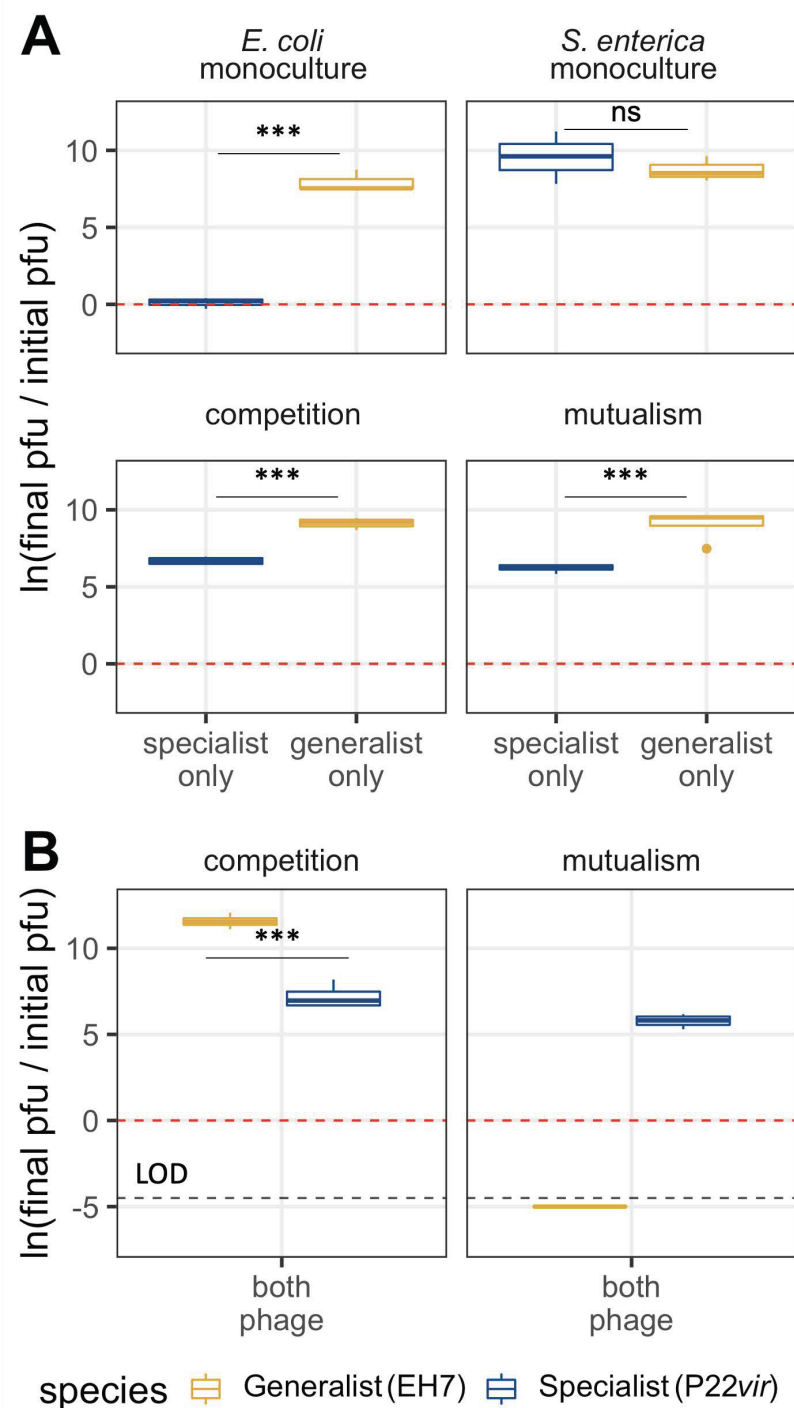


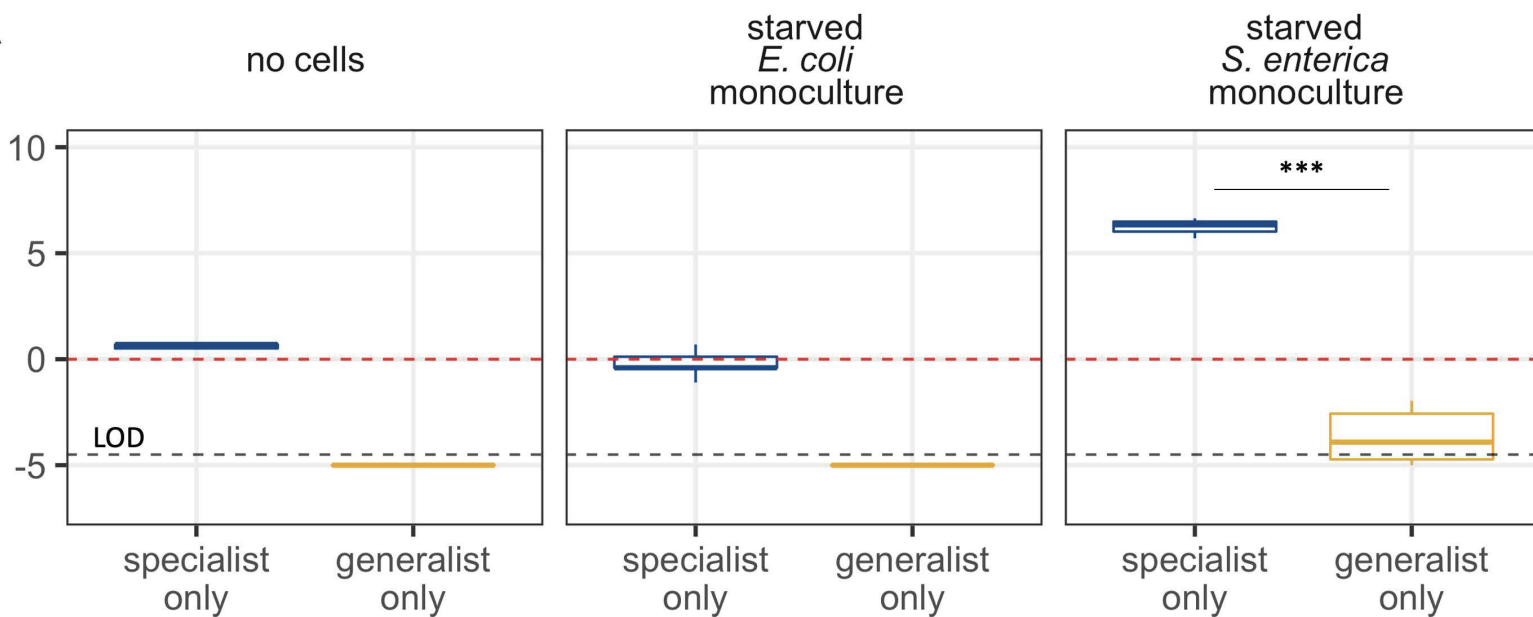
species *E. coli* *S. enterica* Generalist(EH7) Specialist(P22vir)

A**B****C**

species ■ Generalist (EH7) ■ Specialist (P22vir)

specialist relative abundance 0.00 0.25 0.50 0.75 1.00



$$\ln(\text{final pfu} / \text{initial pfu})$$

$$\ln(\text{final pfu} / \text{initial pfu})$$
