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Effect of Prey Heterogeneity on the Response of a Model Food Chain to Resource Enrichment

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ABSTRACT: We demonstrated that the presence of invulnerable prey can result in a shift in the balance between top-down and bottom-up control of a model food chain. Our model food chain consisted of the bacterium *Escherichia coli* and the bacteriophage T4 (a virus that feeds on *E. coli*) in chemostats supplied with different concentrations of glucose. The *E. coli* population consisted of individuals that were susceptible to predation by T4 ("edible" *E. coli*) and individuals that were resistant to predation by T4 ("inedible" *E. coli*). The equilibrium density of a heterogeneous prey population (consisting of edible and inedible *E. coli*) increased strongly in response to an enrichment of its resources. This response consisted of an increase in the inedible fraction of the prey population but no change in the edible fraction. In contrast, a homogeneous prey population (edible *E. coli* only) increased only marginally. The equilibrium density of the predator population (bacteriophage T4) did not significantly increase in response to enrichment when its prey were heterogeneous, but it increased strongly when its prey were homogeneous.

Keywords: bacteria, bacteriophage, edibility, predation, ratio-dependent models, resource enrichment.

Ecologists have long debated the relative importance of population regulation by resources (bottom-up control) and by predators (top-down control) (Hairston et al. 1960; Murdoch 1966; Ehrlich and Birch 1967; Slobodkin et al. 1967). This debate has focused recently on what factors may determine the relative importance of these two types of population control (Power 1992). The presence of heterogeneity in prey edibility has been recognized by a number of ecologists as a factor that could shift the balance between top-down and bottom-up control (McCauley et al. 1988; Leibold 1989; Abrams 1993; Kretzschmar et al. 1993; Sarnelle 1994; Abrams and Walters 1996; Leibold 1996; Polis and Strong 1996). The presence of inedible (or less edible) individuals in a prey population has been shown theoretically to result in the damping of top-down forces and an increase in the relative importance of bottom-up control (Leibold 1989; Abrams 1993; Leibold 1996). This damping occurs because the lowered edibility of the less edible prey can act as a refuge from predation, allowing the prey population to respond numerically to resource increases.

The presence of this refuge can have a profound effect on how a prey population is regulated. For example, compare the response of the following two prey populations to an enrichment of their resources: a homogeneous and highly edible population and a population that consists of both inedible and highly edible individuals. The equilibrium density of the homogeneous population would be unaffected by enrichment, because the prey population is highly edible and thus regulated completely by predators. The homogeneous prey population would grow faster in response to enrichment, but at equilibrium this additional growth would be converted into predator biomass (Rosenzweig 1977; Abrams 1993). In contrast, the equilibrium density of the heterogeneous prey population would increase in response to enrichment. This would occur because the inedible component of the population would increase (because it is not limited by predators and thus can respond to enrichment), whereas the edible component would remain unchanged (because it is limited by predators), with the net effect being an increase in the prey population in response to resource enrichment (Phillips 1974; Abrams 1993; Leibold 1996). Adding heterogeneity in edibility to the prey population has thus shifted the balance of forces regulating the prey population from predominantly top-down to predominantly bottom-up.

Heterogeneity in prey edibility also can have a profound effect on how a predator population is regulated. Less edible prey can essentially "siphon off" resources that oth-

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erwise would be converted eventually into predator biomass, resulting in a decline in the degree of bottom-up control of the predator population (Leibold 1989). If truly inedible prey are present, they can siphon off such a large proportion of resources that the predator population no longer responds to bottom-up control. In such a situation, the equilibrium density of the heterogeneous prey population would increase in response to enrichment but the predator population would not (Abrams 1993; Leibold 1996). The addition of heterogeneity in prey edibility has thus drastically shifted the forces regulating the predator population.

Several assumptions are made by theorists when they make these predictions. A trade-off between competitive ability and edibility is assumed (i.e., the more resistant an individual is to predation, the less able it is to compete for resources); there is evidence that such trade-offs exist (Lenski 1988*a*; Simms 1992; Grover 1995; Kraaijeveld and Godfray 1997). If this assumption is not made, then the least edible prey individuals would always exclude those of higher edibility. It is also assumed that the predator has a constant death rate, although the effect of changing the death rate has been explored by some theorists (McCauley et al. 1988; Leibold 1989, 1996). Another assumption is that the prey's resource level is such that it will support a heterogeneous prey population. If the resource level is too low, then the less edible prey may not be able to coexist with the more edible prey, because of the trade-off between edibility and competitiveness (Grover 1995; Leibold 1996). If the resource level is too high, then the less edible prey may be able to support a large enough predator population that it can drive the more edible prey to extinction ("apparent competition" sensu Holt 1977). However, this cannot occur if the less edible prey is completely inedible (Levin et al. 1977; Grover 1995). A "chemostat-like" environment (e.g., constant volume, continuous input of resources, and so forth) is also assumed.

Field observations of *Daphnia* and algae provide empirical support for some of these predictions (McCauley et al. 1988; Leibold 1989; Watson et al. 1992). In some cases, populations of algae appear to be primarily top-down-regulated when they are relatively homogeneous in edibility but bottom-up-regulated when their edibilities are heterogeneous (McCauley et al. 1988). Furthermore, in populations with heterogeneous edibilities, the ratio of edible to inedible individuals has been observed to decline as the algae's resources are enriched, as theory would predict (McCauley et al. 1988; Watson et al. 1992). However, it has been suggested that these patterns are not actually due to the presence of heterogeneity in the edibility of algae. The strong damping of top-down regulation of the algae population is observed consistently only when higher trophic levels (i.e., predators of *Daphnia* and their respective predators) are present, which suggests that these responses may not be due to interactions between *Daphnia* and a heterogeneous population of algae but, instead, to cascading effects of higher trophic levels (Sarnelle 1994). There is also debate over whether *Daphnia* may feed on "inedible" algae and if so, to what degree (Leibold 1989). It has also been suggested that equilibrium may not have been reached in these studies, especially if higher trophic levels (i.e., zooplanktivorous and piscivorous fish) are present (Sarnelle 1994).

In this report, we demonstrate that heterogeneity in prey edibility can alter the balance between bottom-up and top-down forces. We describe the responses of model predator and prey populations to an enrichment of the prey's resources and contrast the responses of systems with and without heterogeneity in prey edibility. Our experimental system consisted of populations of the bacterium *Escherichia coli* and the bacteriophage T4 (a virus that feeds on *E. coli*) interacting in chemostats. We added heterogeneity in edibility to the prey population by inoculating the chemostats with two strains of *E. coli*, one strain that is susceptible to predation by T4 and one strain that is resistant. Bacteria and bacteriophages have been proposed as ideal experimental systems for studying predator-prey dynamics (Campbell 1961; Lenski and Levin 1985) and have been used successfully as such by a number of researchers (Paynter and Bungay 1969, 1971; Horne 1970; Chao et al. 1977; Levin and Lenski 1983; Lenski and Levin 1985; Lenski 1988*b*). The results we present here are entirely consistent with a model of bacteria-bacteriophage interactions proposed 20 yr ago by Levin et al. (1977), although our study is the first to demonstrate experimentally the effect of heterogeneity in edibility on the response of bacteria and bacteriophage to resource enrichment.

This study builds on our previous study of the effects of resource enrichment on chemostat communities of bacteria and bacteriophage (Bohannan and Lenski 1997). In our previous study, heterogeneity in edibility arose in our experimental communities through spontaneous evolution. However, previously we were unable to determine the effect of heterogeneity in edibility on the response of the community to enrichment because our experiments were terminated before equilibrium was reached by all the populations and because we were unable to track independently the edible and inedible components of the prey population. In our current study, we circumvented this problem by directly adding heterogeneity in edibility early in the experiment and by using edible and inedible strains that could be distinguished by the use of a genetic marker.

The use of laboratory model communities such as ours has several advantages. We are able to use organisms with short generation times, so that steady state responses to enrichment are achieved relatively quickly. We are able to

quantify unambiguously trophic-level population densities, and experimental variables such as resource input are simple to manipulate. Variables other than resource input can be controlled, and the experiments can be replicated with relative ease as well. It is also feasible to measure population parameters such as prey edibility and the tradeoff between edibility and competitiveness in laboratory model communities. Ecological experiments with model laboratory systems bridge the gap between ecological theory and natural communities. Such studies allow theoretical predictions to be examined rigorously in a biological system that is easily manipulated, replicated, controlled, and monitored in ways that would be difficult or impossible with natural communities (Lawton 1995).

Methods

Experimental System

Our experimental system consisted of *Escherichia coli* B strain REL607 (Lenski et al. 1991), *E. coli* B strain REL6584, and the virulent bacteriophage T4 (kindly provided by L. Snyder) in glucose-limited chemostats. The strain REL6584 is identical to REL607, with the exceptions that it is resistant to predation by bacteriophage T4 and it cannot utilize the sugar arabinose. The ability to utilize arabinose has been shown previously to confer neither a competitive advantage nor a disadvantage in a glucose-limited environment (Lenski 1988*b*; Lenski et al. 1991). We used this trait as a neutral marker to distinguish the two *E. coli* strains. Mutants of *E. coli* resistant to T4 have been shown to be completely invulnerable to predation by T4 (Lenski and Levin 1985; Lenski 1988*b*). Virtually all T4-resistant mutants of *E. coli* achieve this resistance through the loss of the cell surface receptor to which T4 initially, and reversibly, binds (Lenski 1988*b*). Resistant cells are not "handled" by T4 nor do they otherwise interfere with the consumption of sensitive cells. Resistance to predation by T4 has been shown to result in a competitive disadvantage in a glucose-limited environment when phage are not present (Lenski and Levin 1985; Lenski 1988*b*).

We measured the competitive disadvantage associated with T4 resistance by coinoculating REL6584 and REL607 into phage-free, glucose-limited chemostats and tracking their respective population densities. We calculated the competitive disadvantage as described by Lenski and Levin (1985). The disadvantage was approximately 35% for REL6584. We also checked the neutrality of the arabinose-utilization marker by coinoculating REL607 and REL606 into glucose-limited chemostats. Strain REL606 is the T4-sensitive progenitor of REL6584; it is identical to REL607 with the exception of its inability to utilize arabinose. We detected no effect of the arabinose-utilization marker on competitive ability.

Our chemostat vessels are similar to those described by Chao et al. (1977). The media consisted of Davis minimal broth (Carlton and Brown 1981) supplemented with 2×10^{-3} μg thiamine hydrochloride per milliliter and either 0.1 or 0.5 μg/mL glucose. These glucose concentrations were chosen so that our results could be compared to previous experiments at these concentrations (Bohannan and Lenski 1997). The volume of the chemostats was maintained at approximately 30 mL, the flow rate at approximately 0.2 turnovers/h, and the temperature at 37°C. Three replicate chemostats at each glucose concentration were maintained simultaneously. The chemostats were inoculated with the edible prey (*E. coli* strain REL607) and the predator (bacteriophage T4) approximately 75 h before inoculation with the inedible prey (*E. coli* strain REL6584), to ensure that the edible *E. coli* and the predator were coexisting prior to the introduction of the inedible *E. coli*. Control chemostats, containing only edible *E. coli* and predator, were established at each glucose concentration and maintained simultaneously with the treatment chemostats.

The population densities of the *E. coli* strains and phage T4 were estimated twice daily by dilution and plating. The REL607 cells were plated on Davis minimal agar supplemented with 2×10^{-3} μg thiamine hydrochloride per milliliter and 4×10^{3} μg/mL arabinose (this medium allows growth of REL607 but not REL6584, since REL6584 cannot utilize arabinose). Heat-killed REL607 cells were mixed with each sample to inactivate free phage prior to plating, as described by Carlson and Miller (1994). Bacteriophage T4 was plated on a lawn of REL607 with Davis minimal agar and the plate count technique described by Carlson and Miller (1994). The REL6584 cells were plated on Davis minimal agar supplemented with 2×10^{-3} μg thiamine hydrochloride per milliliter and 4×10^{3} μg/mL glucose. A concentrated phage T4 lysate was mixed with each sample to kill REL607 cells prior to plating.

To estimate the population stability and equilibrium population densities of T4 and the *E. coli* strains, we treated each chemostat as a single observational unit. We first calculated the mean and standard deviation of the T4 and *E. coli* population densities over time for each chemostat. We then estimated the equilibrium density of each population as the grand arithmetic mean of population density across replicate chemostats. We had previously determined that the arithmetic mean is superior to the geometric mean as an estimator of equilibria in our system (Bohannan and Lenski 1997). We estimated stability as the mean coefficient of variation of population densities across replicate chemostats (the higher the coefficient of variation, the lower the stability). We excluded the first six time points after

inoculation of REL6584 from our calculations of equilibria and stability, to allow time for the populations to reach equilibria.

We compared population equilibria and stability between the resource treatments with *t*-tests. One-tailed comparisons were performed because the models made directional predictions. Prior to comparison we tested for homogeneity of variances. The data were log-transformed prior to comparison whenever the variances were found to be significantly different. If heterogeneity of variances was not eliminated by transformation, Welch's approximate *t* (Zar 1984) was used instead of Student's *t* to make comparisons.

We tracked population density over time rather than biomass because density is much easier to estimate accurately in our system. We do not expect biomass per cell to vary between treatments. Biomass per bacterial cell can increase at higher growth rates (Bremer and Dennis 1987; Mongold and Lenski 1996); however, no difference in growth rates between treatments is predicted by the mathematical model described below.

Mathematical Model

We modeled our experimental system using the models first developed by Levin et al. (1977). The model consisted of four differential equations:

$$dC/dt = (C_0 - C)\omega - [\varepsilon N\psi C/(K + C)] - [\varepsilon_R R\psi_R C/(K_R + C)],$$

$$dN/dt = N\psi C/(K + C) - \alpha NP - \omega N,$$

$$dP/dt = \beta e^{-\tau\omega}(\alpha N'P') - \alpha NP - \omega P,$$

$$dR/dt = R\psi_R C/(K_R + C) - \omega R,$$

where C_0 = concentration of glucose in the reservoir, C = concentration of glucose in the chemostat, ω = flow rate, ε = reciprocal of the yield of the edible *E. coli*, N = population size of uninfected edible *E. coli*, ψ = maximum specific growth rate of edible *E. coli*, K = resource concentration at which the edible *E. coli* grow at one-half ψ, ε_R = reciprocal of the yield of the inedible *E. coli*, R = population size of inedible *E. coli*, ψ_R = maximum specific growth rate of inedible *E. coli*, K_R = resource concentration at which inedible *E. coli* grow at one-half ψ_R, α = attack (i.e., adsorption) rate of T4, β = number of T4 progeny per edible *E. coli* cell, τ = time lag between infection and release of T4 progeny, $e^{-\tau\omega}$ = fraction of edible *E. coli* infected at time $t - \tau$ that has not washed out of the chemostat before releasing T4 progeny, N' = population size of uninfected edible *E. coli* at time $t - \tau$, and P' = population size of T4 at time $t - \tau$.

We used the following parameter values for this model: C_0 = either 0.1 or 0.5 μg/mL, ω = 0.2/h, $\varepsilon = 2 \times 10^{-6}$ μg (Lenski 1988*a*), ψ = 0.7726/h (Vasi et al. 1994), K = 0.0727 μg/mL (Vasi et al. 1994), $\alpha = 3 \times 10^{-7}$ mL/h (Lenski and Levin 1985), β = 80 viruses per bacterial cell infected (Lenski and Levin 1985), τ = 0.6 h (Lenski and Levin 1985), $\varepsilon_R = 2 \times 10^{-6}$ μg (Bohannan and Lenski 1997), ψ_R = 0.7027/h (Bohannan and Lenski 1997), and K_R = 0.123 μg/mL (Bohannan and Lenski 1997).

It should be noted that we chose to use a linear (Type I) rather than a saturating (Type II) functional response in our model, as did Levin et al. (1977). It is actually more precise to describe phage-bacteria interactions with a Type II functional response, because the functional response does saturate at high densities of prey bacteria. However, nearly all modeling of phage-bacteria interactions assumes a Type I response because saturation occurs at very high prey densities, densities that are seldom reached in laboratory experiments or, for that matter, in nature (Stent and Wollman 1952; Lenski 1988*a*). These prey densities were never reached in our experiment, and thus a Type I functional response is appropriate.

We solved this model analytically for equilibria and examined the behavior of the model numerically using STELLA II simulation software (High Performance Systems 1994). A time step of 0.05 h was used in the simulations. However, we "sampled" the output of each simulation every 12 h (the approximate sampling interval of our experiments) to produce the dynamical predictions shown in figure 1.

Results

Model Predictions

The model predicts that the equilibrium density of the inedible *Escherichia coli* will increase in response to enrichment, while the equilibrium density of the edible *E. coli* remains unchanged, resulting in an increase in total *E. coli* (edible and inedible combined) in response to enrichment (fig. 1*A*, *B*). It also predicts that the equilibrium density of the predator will be unaffected by resource enrichment. The edible prey and predator populations are predicted to be destabilized by enrichment (i.e., the amplitude of oscillations will increase relative to the mean population density; fig. 1*A*, *B*). This is the classic "paradox of enrichment" described by Rosenzweig (1977). The inedible prey population is predicted to be stabilized by enrichment (i.e., the amplitude of oscillations will decrease relative to the mean population density; fig. 1*A*, *B*). This stabilization is predicted to occur because the fluctuations

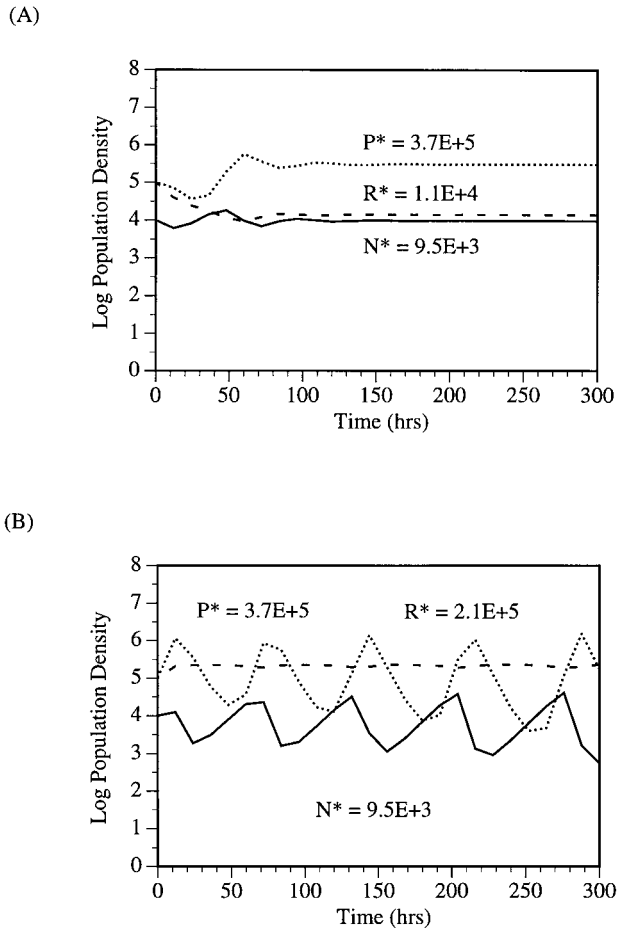

Figure 1: Population equilibria and dynamics predicted by the mathematical model. Equilibria are from analytical solutions of the model; dynamics are from numerical simulations of the model, "sampled" at 12-h intervals. The population densities (viruses per milliliter or bacteria per milliliter) have been log-transformed. *A*, Glucose input concentration of 0.1 μg/mL. *B*, Glucose input concentration of 0.5 μg/mL. R^* = inedible *Escherichia coli* equilibrium density; N^* = edible *E. coli* equilibrium density; P^* = T4 equilibrium density; *solid line* = edible *E. coli* dynamics; *dashed line* = inedible *E. coli* dynamics; *dotted line* = T4 dynamics.

in glucose concentration that are caused by population cycling of the edible prey and predator (and which cause the inedible prey population to oscillate) represent proportionately less of the inedible prey's glucose budget at the higher input concentration than at the lower.

Empirical Observations

The dynamics of the T4 and *E. coli* populations are shown in figure 2 for representative chemostats with two different input concentrations of glucose. The populations persisted in all chemostats with apparent population cycles. There was a large and highly significant increase in the average density of the inedible *E. coli* in response to enrichment ($t = 15.238$, df = 4, one-tailed $P < .0001$; fig. 3*A*). The average density of the edible *E. coli* did not change significantly in response to enrichment (Welch's approximate $t = 0.4976$, df = 2, one-tailed $P = .3340$; fig. 3*A*), nor did the predator population ($t = 1.2421$, df = 4, one-tailed $P = .1410$; fig. 3*A*). We did not observe T4-resistant mutants of the edible *E. coli* strain (REL607) in any of the treatment chemostats, although they did appear in the control chemostats (fig. 4).

Enrichment significantly destabilized the edible *E. coli* ($t = 2.4503$, df = 4, one-tailed $P = .0352$; fig. 3*B*), and the predator population ($t = 2.7991$, df = 4, one-tailed

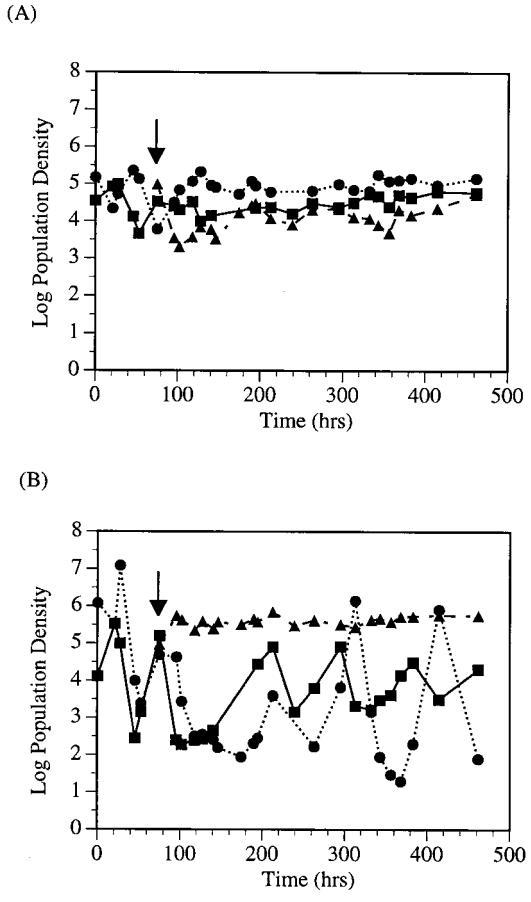

Figure 2: Dynamics of edible *Escherichia coli* (*squares*), inedible *E. coli* (*triangles*), and bacteriophage T4 (*circles*) in chemostats supplied with media containing different amounts of glucose. Arrows indicate when the chemostats were inoculated with inedible *E. coli*. The population densities (viruses per milliliter or bacteria per milliliter) have been log-transformed. *A*, Glucose input concentration of 0.1 μg/mL. *B*, Glucose input concentration of 0.5 μg/mL.

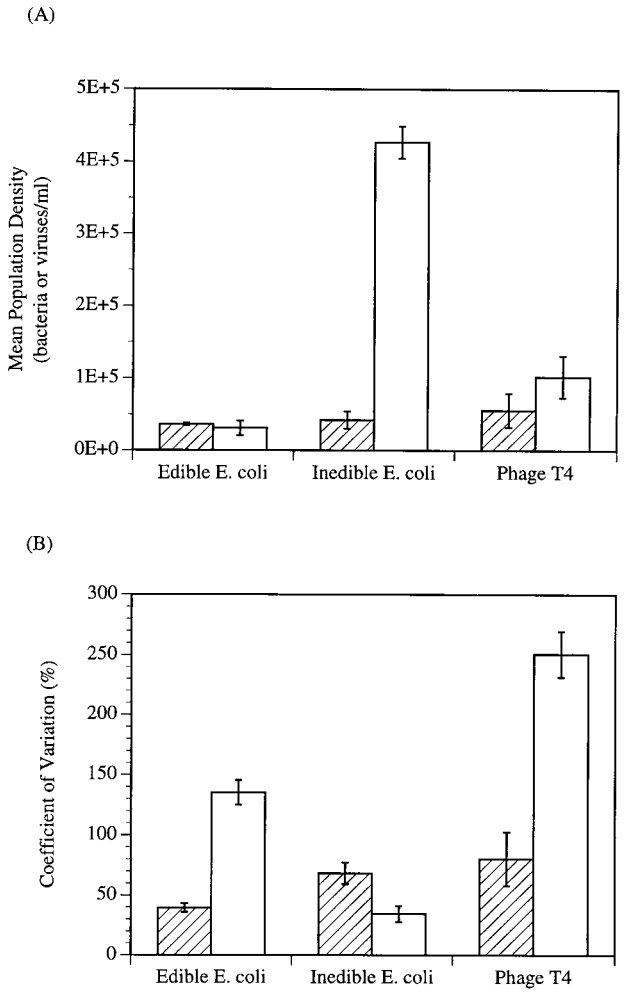

Figure 3: Effect of glucose input concentration on equilibrium population densities and instability of edible *Escherichia coli*, inedible *E. coli*, and bacteriophage T4 interacting in a chemostat. Equilibrium population density is estimated as the grand mean of the mean population densities in three replicate chemostats. Population instability is estimated as the mean of the coefficient of variation in three replicate chemostats. Error bars are standard error of the mean. *A*, Equilibrium density. *B*, Instability.

$P = .0244$; fig. 3*B*). The inedible *E. coli* became more stable with enrichment ($t = 7.0875$, df = 4, one-tailed $P = .0010$; fig. 3*B*). The population oscillations of the edible *E. coli* in the higher-glucose treatment appeared to be damped in figure 2*B* and possibly could have converged on a stable equilibrium; however, this apparent damping did not occur in the other replicate chemostats at this glucose concentration.

The dynamics of the control chemostats (inoculated with edible *E. coli* and T4 only) are shown in figure 4. They were essentially identical to those reported earlier for edible *E. coli* and T4 (Bohannan and Lenski 1997). Enrichment appeared to destabilize both the *E. coli* and T4 populations in the control chemostats. Enrichment also resulted in a large increase in the average population density of T4 (approximately 30-fold) and a very small increase in *E. coli* (approximately 1.5-fold). *Escherichia coli* and T4 persisted in the control chemostats in apparent population cycles until the appearance of, and subsequent invasion by, T4-resistant mutants of *E. coli*. The T4-resistant *E. coli* mutants appeared much sooner in the higher-glucose control (after 127 h; fig. 4) than in the lower

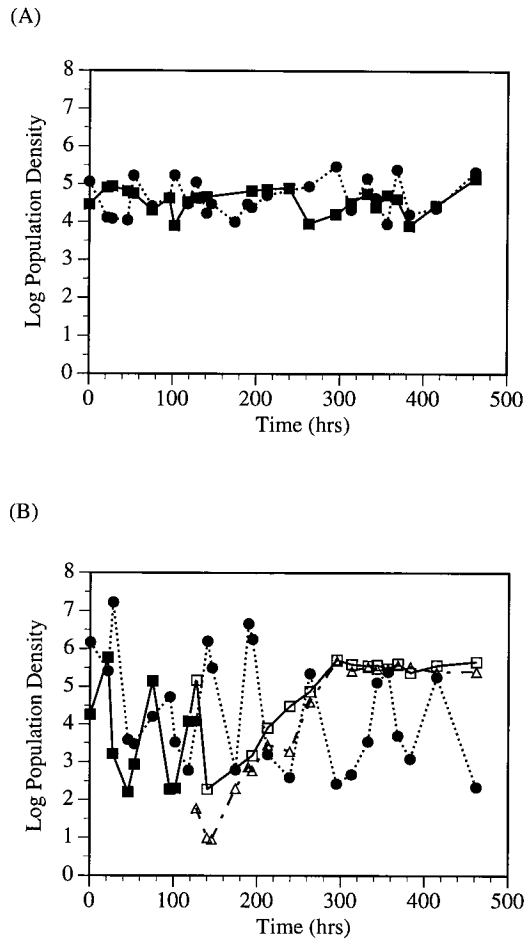

Figure 4: Dynamics of edible *Escherichia coli* (*solid squares*) and bacteriophage T4 (*circles*) in control chemostats supplied with media containing different amounts of glucose. Inedible mutants of the edible *E. coli* (*open triangles*) arose through spontaneous evolution. Once these mutants invaded the chemostats, we were no longer able to track the edible *E. coli* directly; only the total *E. coli* (*open squares*) could be quantified. The population densities (viruses per milliliter or bacteria per milliliter) have been log-transformed. *A*, Glucose input concentration of 0.1 μg/mL. *B*, Glucose input concentration of 0.5 μg/mL.

glucose control (after 631 h; data not shown), as reported previously (Bohannan and Lenski 1997). Following invasion by the T4-resistant *E. coli* mutants, the T4 population continued to persist in cycles.

Discussion

The relative importance of population regulation by resources (bottom-up control) and by predators (top-down control) has long fascinated ecologists. The central question in this debate has evolved from "Do resources or predators regulate this particular population?" to "What factors may modulate resource limitation and predation in this system, determining when and where predators or resources will dominate in regulating populations?" (Power 1992). Many potential factors have been recognized, including heterogeneity in prey edibility. Several theorists have shown that heterogeneity in prey edibility can have a profound effect on the balance between top-down and bottom-up control (Leibold 1989, 1996; Abrams 1993). Patterns predicted by this theory have been observed in natural systems (McCauley et al. 1988; Watson et al. 1992), but it is unclear if the process underlying these patterns is actually heterogeneity in edibility (Leibold 1989; Sarnelle 1994).

One of the advantages of laboratory model systems is that patterns can be linked clearly to processes. In this study, we have demonstrated clearly that heterogeneity in edibility (in its most extreme form, the presence of inedible individuals in a prey population) can result in a shift in the relative importance of top-down and bottom-up control. The average density of our model heterogeneous prey population (edible and inedible *Escherichia coli*) increased strongly in response to an enrichment of its resources (fig. 3*A*). In contrast, the model prey population without heterogeneity (edible *E. coli* only) increased only marginally, if at all (Bohannan and Lenski 1997). The model predator population (bacteriophage T4) did not significantly increase when its prey were heterogeneous (fig. 3*A*), even though it increased strongly in response to enrichment when its prey were homogeneous (Bohannan and Lenski 1997). The mechanism underlying this change in regulation can be seen vividly in figure 3*A*. The inedible fraction of the prey population increased dramatically in response to enrichment, while the edible fraction remained unchanged. This pattern is consistent with the hypothesis that the inedible fraction is "siphoning off" resources from the edible prey and predator and thus preventing the edible prey and predator from responding to enrichment.

Our observations are consistent not only with the models of Levin et al. (1977) but also with other models of predator-prey-resource interactions, such as those of Phillips (1974), Grover (1995, 1997), and Leibold (1996). These models are similar in that they are highly detailed, mechanistic models that require detailed knowledge of predator, prey, and resource interactions. Such detailed knowledge is not obtained readily for most ecological systems. Several approaches to modeling predator-prey interactions have been proposed that require less detailed knowledge of the interactions. For example, it has been suggested that the dynamics of predators and heterogeneous prey can be described much more simply using ratio-dependent models (see appendix). This approach involves assuming that the predator's functional response is a function of the ratio of prey to predator densities rather than a function of the prey's density alone, as assumed in the traditional models described above. This ratio is assumed to incorporate the "net effect" of heterogeneity on population dynamics. We found that a ratio-dependent model accurately predicted that the average density of *E. coli* (edible and inedible individuals combined) would increase with productivity, but it did not predict that the average phage density would not change with productivity, nor did it predict the destabilizing effect of productivity (see appendix).

Another approach to modeling food chains with heterogeneous prey has been described by Kretzschmar et al. (1993). This approach is intermediate in mathematical complexity and mechanistic realism between the two approaches above. It consists of modeling the interactions between the predator and edible prey through the use of traditional predator-prey equations and modeling the interactions between inedible prey and edible prey with Lotka-Volterra competition equations. The dynamics of the resource are not explicitly included in these models. These models make qualitative predictions consistent with many of our observations. Enrichment is predicted to destabilize edible prey and predators, and the equilibrium density of inedible prey is predicted to increase with enrichment, while the edible prey equilibrium is predicted to remain unchanged. However, these models also predict that the predator population will increase in density at equilibrium in response to enrichment, a prediction inconsistent with our observations.

The mathematical modeling of "modules" (i.e., small numbers of interacting populations) has formed the theoretical backbone of community ecology (Holt 1995). In our research we have applied this modular approach to experimental community ecology, using modules of increasing complexity. In previous work, we analyzed the response of a simple food chain module to enrichment (Bohannan and Lenski 1997). Our present study builds on this previous work by adding complexity, in the form of inedible individuals, to this module. In future work, we plan to study increasingly more complex modules (e.g., modules with prey that differ quantitatively in edibility),

as well as to examine the effects of linking two or more modules involving multiple resources and/or predators.

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APPENDIX

Ratio-Dependent Model

The mathematical model described in the "Methods" section requires detailed knowledge of the interactions between predator and prey. It is rare to have such understanding outside of well-studied laboratory systems. It has been suggested that the dynamics of predators and heterogeneous prey can be described much more simply by using ratio-dependent models (Arditi et al. 1991; Sarnelle 1994). In these models, the attack rate of predators is assumed to depend on the ratio of prey to predator density. Proponents of this approach have suggested that this ratio incorporates the "net effect" of heterogeneity on population dynamics (Arditi and Ginzburg 1989). The ratio-dependent approach is much simpler mathematically than the traditional approach; however, this simplicity comes at the cost of mechanistic realism. In an attempt to explore the costs and benefits of this approach, we also modeled our experimental system using a ratio-dependent model.

The ratio-dependent model consisted of three differential equations:

$$dC/dt = (C_0 - C)\omega - \varepsilon B\psi C/(K + C),$$

$$dB/dt = B\psi C/(K + C) - g(B/P)P - \omega B,$$

$$dP/dt = \beta[g(B/P)P] - g(B/P)P - \omega P,$$

where, in addition to the parameters defined in the "Methods" section, $g(B/P)$ = the ratio-dependent functional response and B = the total population size of uninfected *Escherichia coli* (edible and inedible combined). This model therefore combines the T4-sensitive and T4-resistant *E. coli* into one population that is heterogeneous in edibility. We solved for g as a function of the equilibrium densities of predator and prey, and calculated g using estimates of equilibria from previously published observations (Lenski and Levin 1985) of coexisting populations

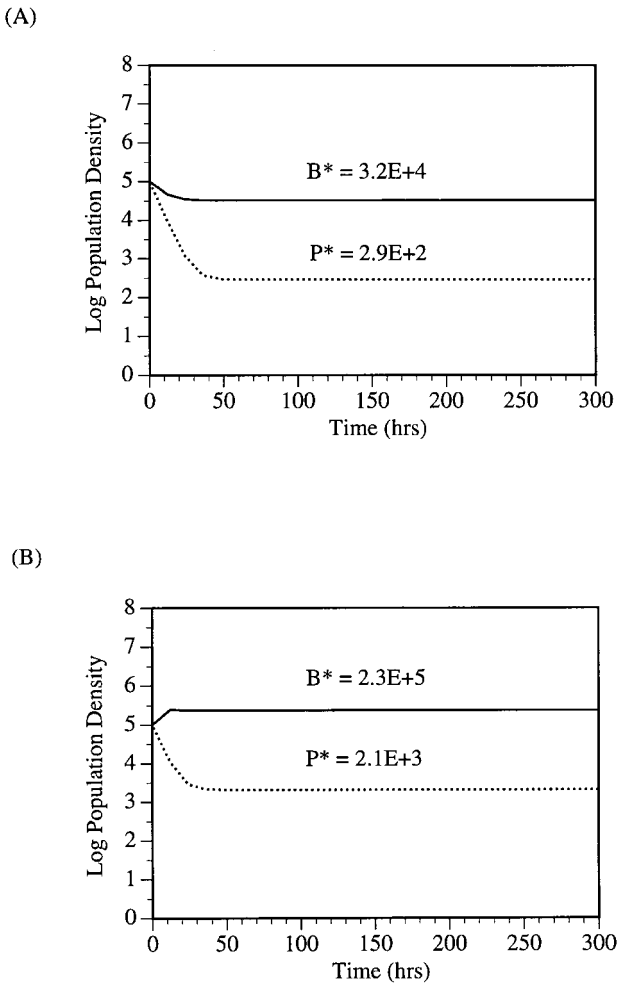

Figure A1: Population equilibria and dynamics predicted by the ratio-dependent model. Equilibria are from analytical solutions of the model; dynamics are from numerical simulations of the model, "sampled" at 12-h intervals. The population densities (viruses per milliliter or bacteria per milliliter) have been log-transformed. *A*, Glucose input concentration of 0.1 μg/mL. *B*, Glucose input concentration of 0.5 μg/mL. B^* = total *Escherichia coli* equilibrium density; P^* = T4 equilibrium density; *solid line* = *E. coli* dynamics; *dotted line* = T4 dynamics.

of T4-sensitive *E. coli*, T4-resistant *E. coli*, and T4. These observations were made in chemostats with glucose input concentrations of 300 μg/mL, and they gave an estimated value for g of 2.28×10^{-5}/h. We used the average of ψ and ψ_R (0.7376/h) as the value for ψ in the ratio-dependent model and the average of K and K_R (0.0978 μg/mL) as the value of K. Proponents of ratio-dependent models have suggested that the ratio-dependent functional response incorporates the effect of temporal heterogeneity on population dynamics of predators and prey; therefore, a time lag is not explicitly included in the ratio-dependent model

(Arditi and Ginzburg 1989; Oksanen et al. 1992). All other parameters were the same as in the detailed model. We solved this model analytically for equilibria and examined the behavior of the model numerically as described for the detailed model.

The ratio-dependent model predicts that equilibrium densities of both the predator population and the total *E. coli* population (edible and inedible combined) will increase in response to enrichment (fig. A1*A*, *B*). It does not predict any changes in population stability in response to enrichment (fig. A1*A*, *B*).

We found that the ratio-dependent model accurately predicted that the average density of *E. coli* (edible and inedible individuals combined) would increase with productivity, but it did not predict that the average phage density would not change with productivity, nor did it predict the destabilizing effect of productivity. We believe that these discrepancies occurred for two reasons. First, the prey population in our system had completely inedible individuals. An increase in both predator and prey could occur if less edible (but not inedible) prey are present (Abrams 1993; Leibold 1996). Ratio-dependent models might adequately capture the effects of this type of heterogeneity (edible and less edible prey) while failing to capture the effects of the type of heterogeneity present in our system (edible and inedible prey). Second, we have shown previously that ratio-dependent models tend to overestimate the stability of simple systems (Bohannan and Lenski 1997). This may explain the inability of these models to predict the changes in population stability that we observed in our current study.

However, ratio-dependent models may still be useful for modeling other, more complex, systems. Trophic complexities (e.g., omnivory, nutrient subsidies, prey refuges, and so forth) may lead to strong bottom-up control and possibly greater population stability (Polis and Strong 1996), and these might be approximated by ratio-dependent models. However, trade-offs between simplicity and mechanistic realism need to be considered carefully before using the ratio-dependent approach (Diehl et al. 1993).

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