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RESPONSE OF MODEL MICROBIAL COMMUNITIES TO INCREASED PRODUCTIVITY

By

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ABSTRACT

RESPONSE OF MODEL MICROBIAL COMMUNITIES TO INCREASED PRODUCTIVITY

By

Brendan James Marc Bohannan

I studied the effect of increased productivity on three microbial model communities that differed in complexity. All three communities were maintained in glucose-limited chemostats. Productivity was manipulated in these model communities by altering the concentration of glucose in the incoming media.

In a simple food chain consisting of bacteriophage T4 and T4-sensitive *E. coli*, the bacteriophage population responded to increased productivity with a large and highly significant increase in equilibrium density. In contrast, the *E. coli* population responded with a small but significant increase in equilibrium density. Both populations had a significant decrease in stability in response to increased productivity. T4-resistant *E. coli* mutants were detected in both higher and lower productivity treatments. These mutants appeared significantly sooner, and invaded at a faster rate, in the higher productivity treatment than in the lower productivity treatment.

In a food web consisting of bacteriophage T4, T4-sensitive *E. coli*, and T4-resistant *E. coli*, neither the bacteriophage nor the T4-sensitive *E. coli* population changed in equilibrium density in response to increased productivity. Only the T4-resistant *E. coli* responded to increased productivity with an increase in equilibrium density. However, both the bacteriophage and the T4-sensitive *E. coli* populations decreased in stability in response to increased productivity, although to a lesser degree than the populations in the simple food chain. The T4-

resistant *E. coli* population increased in stability in response to increased productivity.

In a food web consisting of bacteriophage T2, T2-sensitive *E. coli*, and partially T2-resistant *E. coli*, increased productivity resulted in the exclusion of the sensitive *E. coli* due to apparent competition. T2-resistant *E. coli* mutants were not detected in the lower productivity treatments; however, they were detected in the higher glucose treatment. Invasion of the higher glucose treatment by T2-resistant mutants temporarily halted the exclusion of the sensitive *E. coli*.

The responses of all three different microbial model communities to increased productivity were better predicted by prey-dependent mathematical models than by ratio-dependent mathematical models.

To my father, Jim, who taught me to cherish books and learning; and to my son, Colin, who carries on the tradition.

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MICROBES, MODELS AND MODULES:
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BRIDGING THE GAP BETWEEN MICROBIOLOGY AND ECOLOGY

One way that science confronts the complexity of the natural world is by dividing the study of nature among a variety of different disciplines, each with its own perspective, traditions and history. This can be a very powerful approach, providing multiple, overlapping windows on the natural world. However, with time disciplines can become isolated from one another. This separation can act as an impediment to understanding as barriers to communication grow between disciplines. Because of these barriers, and the opportunity to surmount them, doing research at the interface between disciplines can be very exciting. The clash of perspective, approach, and history, and the resolution of this conflict, can lead to new insights, novel approaches and great leaps in understanding. Recent research at the interface of the disciplines of microbiology and ecology is an excellent example of this process (Andrews 1991, Atlas and Bartha 1993). These two disciplines have natural complementary strengths, but these strengths have been overshadowed by the separation of these two disciplines over time.

Microbiology and ecology have been isolated from one another since early in their respective histories. The science of ecology developed initially as a subdiscipline of botany and zoology, heavily influenced by the naturalist tradition

in these disciplines (Kingsland 1995). As such, it was a science in which the field observation of organisms played a central role. Microbiology, on the other hand, developed initially from interests in industry and medicine, with the principle approach being laboratory experimentation on pure cultures (Atlas and Bartha 1993). Ecological research with microbes had to overcome tremendous methodological obstacles (such as small size and lack of morphological detail) that ecological research with plants and animals did not. This distinction served to separate ecology from microbiology early on in their development as disciplines.

Communication between ecologists and microbiologists in the early twentieth century was minimal; the two disciplines had no common language or approach (Atlas and Bartha 1993). However, this is not to say that they did not influence one another. Mathematical modeling of the interactions between pathogenic microorganisms and their hosts had a major influence on the development of mathematical theory in ecology (e.g., Ross 1911), and research with microbial microcosms played a major role in unifying theory and experiment in ecology (e.g., Gause 1934). Microbiologists were certainly aware of the work of ecologists; the Dutch microbiologist Martinus Beijerinck described his approach to microbiology as "the study of microbial ecology" as early as 1905 (Van Iterson Jr. et al. 1940). Despite these examples, however, the disciplines of microbiology and ecology became increasingly isolated from one another during the first half of this century (Atlas and Bartha 1993). During this time, microbial ecology developed as a distinct subdiscipline of microbiology, isolated from general ecology, and with a different approach and perspective. The ultimate result of this separation is that microbial ecology today lacks a solid theoretical foundation, and general ecology has been largely prevented from studying the ecology of the dominant form of life on earth, microorganisms.

There remains a significant communication barrier between these disciplines even today. For example, a recent review by ecologists of the consequences of shared predators (Holt and Lawton 1994) failed to cite early theoretical and experimental work by microbiologists (Levin et al. 1977). Similarly, a recent review by a general ecologist of ratio-dependent predator-prey theory (Berryman 1992) failed to mention that such theory was developed, and experimentally tested, by microbiologists decades before ecologists rediscovered such theory (Contois 1959). Microbiologists are equally guilty of such nearsightedness. A recent journal article describing an experimental test of top-down versus bottom-up controls of bacterial populations did not cite a single article from the extensive literature on this topic in general ecology (Shiah and Ducklow 1995). In another recent article, several microbiologists proposed a conceptual model of the control of microbial diversity that was essentially a reinvention of classical theory from general ecology, including the exploitation ecosystem hypothesis, the role of keystone predators, and specialist versus generalist predators (Thingstad et al. 1997). Not a single one of the classical papers describing these theories was cited. Such myopic views of ecology and microbiology retard the growth of both disciplines.

In the last twenty-five years, however, there has been a growing interest by both general ecologists and microbial ecologists in bridging the gap between these disciplines (Andrews 1991, Atlas and Bartha 1993). There is an increasing awareness of the complementary strengths of microbiology and ecology. Ecology has to offer microbiology a wealth of ecological theory, theory that could be used to form the theoretical foundation for microbial ecology that has been historically lacking. Microbiology has to offer ecology powerful model systems, systems with short generation times, that are easy to manipulate, and that can be used to test ecological ideas.

THE USE OF MICROBIAL MODEL SYSTEMS TO TEST ECOLOGICAL THEORY

The use of microbial model systems to test ecological ideas has a long history in ecology, dating at least to Woodruff's (1912) exploration of succession in hay infusions and Gause's (1934) famous studies on protozoan competition and predation. However, the use of laboratory model systems has always been controversial (Kingsland 1995). Mertz and McCauley (1982) have argued that the controversy surrounding laboratory studies in general ecology has increased steadily since the 1960's. This claim is supported by the observation that the number of laboratory studies published in major ecological journals has declined (as a percentage of the total papers published in these journals) since 1960 (Ives et al. 1996). Mertz and McCauley (1982) attributed the decline in the popularity of laboratory studies to the influence of Robert MacArthur on the development of general ecology during this period. MacArthur was gifted as both a theoretical ecologist and a field ecologist, but he was highly critical of laboratory studies as being generally uninteresting and unimportant, referring to them condescendingly as "bottle experiments" (MacArthur 1972). MacArthur was not the only eminent ecologist to weigh in against laboratory studies during this period; G. Evelyn Hutchinson criticized laboratory model systems as being highly artificial and essentially "a rather inaccurate analogue computer... using organisms as its moving parts." (Hutchinson 1978).

Recent critics of laboratory studies have expanded on these earlier critiques, raising concerns about the validity of laboratory microcosm research in general, and microbial microcosms in particular (Peters 1991, Carpenter 1996,

Lawton 1996). Laboratory microcosms have been criticized for being too simple, too artificial, and too small in spatial and temporal scale to be useful for most ecological questions. In addition, microbial microcosms have been criticized in particular for using model organisms that are not representative of most other organisms. As I will demonstrate below, these critiques suffer from confusion about the purpose of a model system, misconceptions about laboratory experiments, and a general ignorance of microbial biology.

Much of the criticism that has been directed at laboratory model systems is centered on their simplicity. Laboratory model systems have been criticized as lacking in realism and/or generality because of their simplicity (Diamond 1986, Carpenter 1996, Lawton 1996), for not incorporating complexities such as seasonality, disturbance or immigration (Lawton 1996), and for being "analogies" (i.e., overly simplistic representations of natural systems) (Peters 1991). But these critiques suffer from confusion about the purpose of laboratory model systems. Laboratory model systems are not intended to be miniature versions of field systems. Laboratory ecologists do not intend to reproduce nature in a laboratory model system any more than theoreticians intend to reproduce nature with a mathematical model; rather, the purpose is to simplify nature so that aspects of it can be better understood (Lawton 1995, Drake et al. 1996, Lawton 1996). Like mathematical models, laboratory model systems are necessary because we do not have full access, in time or space, to phenomena in nature (Oreskes et al. 1994). Thus, simplicity is a strength of laboratory model systems, not a weakness (Drake et al. 1996).

There is, however, no reason that complexities such as immigration, disturbance, or seasonality cannot be included in a laboratory model system, if the research question being addressed warrants the inclusion of these complexities. One of the advantages of laboratory systems is that the decision

whether to include particular complexities is up to the experimenter, not imposed upon the experiment by the vagaries of nature (Lawton 1995, Drake et al. 1996). It is possible with a laboratory model system, for example, for complexity to be first reduced and then increased in a controlled fashion.

It is a misconception that laboratory experiments lack realism or that laboratory systems behave like computers; neither the experimental organisms nor their interactions are creations of the experimenter, nor are they under the direct control of the experimenter (Mertz and McCauley 1982). It is also a misconception that laboratory systems lack generality. Laboratory experiments usually address fundamental ecological questions using simple systems and because of this they potentially have more generality than studies of more complex and thus more idiosyncratic field systems (Drake et al. 1996).

Laboratory studies have been criticized for using artificial communities and for being too small in spatial and temporal scale (Lawton 1996). However, few laboratory studies use truly artificial communities. Most studies use species that co-occur in a particular habitat and in that sense they are no more artificial than exclosure experiments in the field (Lawton 1996). It is a misconception that laboratory experiments usually occur on small temporal scales; based on a literature search, Ives et al. (1996) concluded that microcosm studies may actually have longer average duration, in terms of generations of the organisms involved, than field studies. In addition, one of the advantages of using microorganisms in laboratory studies is that large relative temporal and spatial scales are possible.

The criticism that the use of microorganisms in laboratory studies is suspect because of their unique biology (Diamond 1986, Carpenter 1996) reflects an ignorance of microbial biology. Considering that microorganisms are the dominant form of life on earth, this criticism is more valid in reverse - What can

we learn about a natural world dominated by microorganisms by studying plants and animals? More to the point, while there are unique aspects to being of small size (e.g., living in a world primarily governed by intermolecular forces rather than by gravity) and being prokaryotic (e.g., parasexuality), prokaryotic and eukaryotic microorganisms share the fundamental properties of larger organisms (Andrews 1991). Microorganisms are valid model organisms for questions that are concerned with these fundamental properties. Certainly there are questions that are not easily addressed using microorganisms, such as studies of life span, age-related phenomena, gene flow or behavioral ecology (Andrews 1991). But this is true of all experimental systems; the major challenge all experimenters face is matching research questions with appropriate experimental systems.

There is considerable debate about the role laboratory model systems play in ecology, and the relationship of laboratory studies to ecological theory and field experiments. Carpenter (1996) and Diamond (1986) have argued that laboratory studies should be primarily supportive of field studies, i.e., that the purpose of laboratory studies is to provide supporting information for field studies that is impossible or impractical to gather in the field. Others have argued that laboratory studies are primarily supportive of ecological theory; i.e., that a major role laboratory experiments play is to provide "clean tests" of ecological theory (Daehler and Strong 1996, Drake et al. 1996). Still others have argued that rather than being in the service of theoretical ecology or field ecology, laboratory ecology should act as a bridge between theory and the field (Lawton 1995). Yet another view is expressed by Slobodkin (1961):

> In one sense, the distinction between theoretician, laboratory worker and field worker is that the theoretician deals with all conceivable worlds while the laboratory worker deals with all

> possible worlds and the field worker is confined to the real world. The laboratory ecologist must ask the theoretician if his possible world is an interesting one and must ask the field worker if it is at all related to the real one.

Another way to state this is that the role of theory is to define what is logically *possible* (given a set of assumptions), the role of laboratory experiment is to determine what is biologically *plausible* and the role of field study is to delineate what is ecologically *relevant*.

There are at least two points that both proponents and critics of laboratory model systems appear to agree on (Diamond 1986, Lawton 1995, Carpenter 1996, Drake et al. 1996). First, all agree that laboratory model systems have the definite advantages of replicability, reproducibility, mastery of environmental variables and ease of manipulation. While there is debate over how important these advantages are, the existence of these advantages is not in question. Second, all parties agree that laboratory model systems are just one of many tools available to ecologists. Or as Lawton (Lawton 1995) describes it, model systems are "one part of a rich, interrelated web of approaches to understanding and predicting the behavior of populations and systems." Just as there are trade-offs between different approaches to ecological modeling (Levins 1966), there are trade-offs between different approaches to ecological experimentation (Diamond 1986). Many ecologists have suggested that the ideal strategy is to address ecological questions by utilizing multiple approaches, because conclusions tested by different methodologies become more robust (Diamond 1986, Lawton 1995, Drake et al. 1996).

Like all tools, laboratory model systems do some things well, some things poorly and some things not at all (Lawton 1995). One area of ecology where

laboratory model systems could perform well is community ecology. Lawton (1995) and Drake (1996) suggest that the need for microcosm work is especially critical at the community level, where complexity becomes particularly unwieldy in the field (but see Carpenter 1996 for a differing opinion). One way in particular that laboratory model systems could make a significant contribution to community ecology is by testing community module theories.

THE MODULE APPROACH TO COMMUNITY ECOLOGY THEORY

Ecological communities are among the most complex entities studied by scientists. Because of this, a number of approaches have been used to study communities. One approach that has been particularly fruitful is the community module approach (Holt 1995). This approach consists of mathematically modeling abstract communities made up of small numbers of species (2 to 6) that are linked in a specified structure of interactions (Figure 1). This approach involves taking the wealth of theory available to describe pairwise interactions and extending this theory to multiple populations. This approach combines the explanatory power of population biology with the exciting questions and complexity of community ecology.

A number of fundamental ecological questions have been addressed using the community module approach (Holt 1995). One question that has been addressed in depth with community modules (Abrams 1993) is: how do communities respond to increased productivity? Productivity is the rate at which energy flows in an ecological system (Rosenzweig 1995). Productivity is assumed to be proportional to the input of resources into the bottom trophic level of a community, and productivity is often manipulated by manipulating the

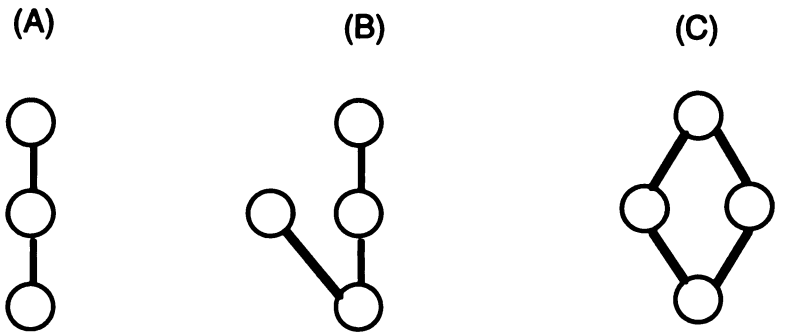


Figure 1. Community modules. (A) a simple food chain module, (B) a keystone predator module, (c) a shared predator module.

nutrient input into a community (a process called enrichment) (Abrams 1993, Rosenzweig 1995). How communities respond to increased productivity is a question of not only great theoretical interest, but also great practical importance. One of the major impacts that humans have on the environment is by increasing productivity, either intentionally (e.g., by the use of agricultural fertilizers) or unintentionally (e.g., by the introduction of pollutants into the environment) (Abrams 1993).

The response that one would predict a community module to have to increased productivity is dependent in part on what assumptions are made about the interactions between trophic levels in a community module. There are a number of different ways that trophic or predator-prey interactions can be mathematically modeled. Two approaches that have gotten a great deal of attention recently are the prey-dependent approach and the ratio-dependent approach (Berryman 1992).

PREY-DEPENDENT VERSUS RATIO-DEPENDENT PREDATOR-PREY THEORY

Predator-prey interactions are usually modeled using coupled differential equations which in their simplest form

$$dN/dt = f(N)N - g(.)P$$

$$dP/dt = eg(.)P - mP$$

consist of a production rate *f* of prey *N*, a conversion rate *e* of prey into predators *P*, a predator death rate *m*, and a per capita consumption rate *g(.)* of prey by predators (this term is also called the functional response or the trophic function). Prey-dependent models assume that the abundance of predators has no effect on the per capita consumption of prey and therefore that g = g(N) (Berryman 1992). The basic assumption underlying prey-dependent models is that predators and prey interact in a manner analogous to random encounters between particles in a homogeneously mixed gas or liquid (i.e. they obey the law of mass action).

In contrast, ratio-dependent models assume that predator abundance does affect per capita consumption of prey and that g = g(N/P) (Arditi and Ginzburg 1989) . The basic assumption underlying ratio-dependent models is that predators have increasing difficulty meeting their energy demands as their population density increases (i.e. they obey the "law of diminishing returns") (Berryman et al. 1995). Several mechanisms have been proposed to be responsible for the "diminished returns" that predators face. These include predator interference, prey refuges, intermittent prey reproduction, non-random searching behavior by predators, and other spatial or temporal heterogeneities. It has been argued that ratio-dependent trophic functions may represent the cumulative effects of heterogeneity on population dynamics (Arditi and Ginzburg 1989).

These approaches are just two of many different approaches that can be used to model predator-prey interactions. For example, models that are intermediate between prey-dependent and ratio-dependent models in the effect of predator abundance on per capita consumption of prey have been developed (Berryman 1992). However, the prey-dependent and ratio-dependent

approaches represent two extremes on a continuum and because of this it is useful to contrast them (Power 1992).

Models of community modules are essentially predator-prey models extended to multiple trophic levels. Community module models built from prey-dependent models and those built from ratio-dependent models make very different predictions concerning how a community will respond to increased productivity. For example, prey-dependent food chain models predict that increasing productivity in a three trophic level food chain (e.g., by increasing the input of limiting resource to the first trophic level) will result in an increase in the equilibrium population sizes of the primary producers and the secondary consumers, but not an increase in the equilibrium population size of the primary consumers. The mechanisms of population regulation appear to alternate between trophic levels, with the first and third trophic levels primarily bottom-up regulated (i.e., regulated by resources) and the second trophic level primarily top-down regulated (i.e., regulated by predators) (Hairston et al. 1960, Fretwell 1977, Oksanen et al. 1981). These models also predict that adding or removing a trophic level will change the primary regulatory mechanism at each trophic level (i.e. from primarily top-down to primarily bottom-up or vice-versa). Prey-dependent food chain models also predict that as productivity of a food chain is increased, the number of possible trophic levels in the food chain increases; therefore at low levels of productivity multiple trophic levels cannot exist (Oksanen et al. 1981). A third prediction of prey-dependent food chain models is that a trophic level that is heterogeneous with respect to edibility can have a different response to changes in productivity than a homogeneous trophic level (e.g. it can change from top-down regulated to bottom-up regulated as less edible variants out-reproduce the more edible) (Leibold 1989, Watson et al. 1992).

Ratio-dependent food chain models make completely different predictions. These models predict that increasing productivity (e.g., by increasing resource input) in a food chain will result in increases in the equilibrium population sizes at all trophic levels in a food chain (Power 1992). The degree of response by each trophic level is determined by the relative strength of top-down *vs.* bottom-up regulatory mechanisms, but the primary control is bottom-up. Ratio-dependent models also predict that the number of trophic levels possible in a food chain is not necessarily determined by the resource input; therefore multiple trophic levels can exist at low levels of resource input. Ratio-dependent models also predict that the response of a trophic level to resource input is not effected by heterogeneity in edibility within the trophic level.

Recently, attempts have been made to determine which of these sets of predictions better describes the response of communities to increased productivity. Most of these attempts have utilized one of the following approaches: 1. Constructing model food chains and measuring the response of the food chains to changes in resource input (Schmitz 1993, Balciunas and Lawler 1995), 2. Measuring the response of a natural community to the manipulation of the number of trophic levels and/or the resource input (O'Brien et al. 1992, Wooton and Power 1993, Stow et al. 1995), 3. Comparing trophic structure and/or trophic level biomass across natural gradients of productivity (Arditi et al. 1991a, Ginzburg and Akçakaya 1992, Hansson 1992, Persson et al. 1992). The results of these attempts have been inconclusive. In some studies prey-dependent models appeared to predict the responses of the biological communities accurately (Hansson 1992, Persson et al. 1992, Wooton and Power 1993), while in other studies the response appeared to be better described by ratio-dependent models (Arditi et al. 1991a, Ginzburg and Akçakaya 1992, O'Brien et al. 1992, Schmitz 1993, Balciunas and Lawler 1995).

Those studies that utilized the comparative approach (approach 3 above) have come under a great deal of criticism. There is considerable debate over whether the statistical analyses used in these studies have biased their interpretation (Diehl et al. 1993), whether food chains can be accurately positioned along a productivity gradient such that comparisons can be made (Power 1992), and whether the comparative approach has sufficient resolution to distinguish between the predictions of the alternative models (Diehl et al. 1993, Lundberg and Fryxell 1995). Those studies that have utilized experimental approaches (approaches 1 and 2 above) have also been criticized. A major criticism of these studies is that the experimental time scale is too short to distinguish adequately between the predictions of the alternative models (Ginzburg and Akçakaya 1992). The different predictions of the two models refer to steady-state (i.e. equilibrium) properties of food chains, not to short-term dynamic responses of the food chains to experimental manipulation. To distinguish adequately between the models, "press" perturbation experiments must be used in which the resource input level is altered and maintained in the altered state long enough for the populations to reach equilibrium, rather than "pulse" perturbations in which the perturbation is maintained for a short time period relative to the time required for the attainment of equilibrium (Bender et al. 1984). Press experiments are difficult to perform because of the long time scale usually required and the difficulty of determining when equilibria are attained.

Another criticism of the studies described above is that few attempts have been made to determine the mechanisms that could underlie a ratio-dependent food chain response when one is observed (Diehl et al. 1993, Abrams 1994a). Ratio-dependent food chain models are essentially phenomenological in nature; although underlying mechanisms have been suggested, few attempts have been made to determine if these mechanisms are actually responsible for the

response. In those studies where attempts have been made to determine underlying mechanisms(Arditi et al. 1991b, Arditi and Saiah 1992), there is considerable debate over whether the experimental results are best described by modifying mechanistic prey-dependent models or by using phenomenological ratio-dependent models (Arditi and Saiah 1992, Ruxton and Gurney 1992, Diehl et al. 1993, Gleeson 1994, Abrams 1994a, Akçakaya et al. 1995, Berryman et al. 1995). The complexity of the experimental systems used in these studies makes assigning mechanisms to the observed responses very difficult.

Finally, studies conducted on natural systems have run into a number of difficulties stemming from the complexity of the systems studied. Researchers working with field systems have had difficulty quantifying trophic level biomass (especially if omnivory is present), manipulating food chain variables experimentally, determining the appropriate temporal and spatial scales for study and even defining the boundaries of a food chain (Power 1992).

The experimental difficulties described above can be avoided by using microbial model food chains to test the ratio-dependent and prey-dependent food chain models. Most microorganisms have relatively short generation times under laboratory conditions, so the amount of time required for a press perturbation can be quite short. The manipulation of experimental variables such as resource input is relatively simple in a laboratory system, as are replication and enumeration. The interactions between many microorganisms are also well understood; therefore it is possible to determine the mechanistic basis for the dynamics of the interacting populations.

THE BIOLOGY OF BACTERIOPHAGE-BACTERIA INTERACTIONS

Bacteria and bacteriophages have been proposed as ideal model systems for studying predator-prey dynamics (Campbell 1961, Lenski and Levin 1985) and have been successfully used as such by a number of researchers (Paynter and Bungay 1969, Horne 1970, Paynter and Bungay 1971, Chao et al. 1977, Levin et al. 1977, Levin and Lenski 1983, Lenski and Levin 1985, Lenski 1988a, Schrag and Mittler 1996). There are two major groups of bacteriophages: temperate phages (i.e., phages that can lie dormant in bacterial cells after infection) and lytic phages (i.e., phages that invariably kill the host cell following infection and phage reproduction) (Lenski 1988c). Most studies that have used bacteriophage as model predators have used lytic bacteriophages. The lytic T-series bacteriophages (voracious predators of the bacterium *Escherichia coli*) are the most commonly used bacteriophage model predators (Chao et al. 1977, Levin et al. 1977, Lenski and Levin 1985, Lenski 1988a, Schrag and Mittler 1996), although virulent mutants of bacteriophage lambda have also been used (Horne 1970, Schrag and Mittler 1996).

The life cycle of a T phage begins with the adsorption of phage to the surface of a prey bacterium (Goldberg et al. 1994). Under favorable conditions the rate of adsorption can be very close to the rate of collision by Brownian motion (Delbruck 1940a, Schlesinger 1960, Schwartz 1976). This rate can be affected by the ratio of phage to bacteria; at a high ratio of phage to bacteria competition for adsorption sites is possible and the adsorption rate per phage can decrease (Lenski 1988c). If the ratio is extremely high, lysis-from-without can occur whereby a large number of adsorptions can cause the cell to prematurely burst and render the infection nonproductive (Delbruck 1940b, Abedon 1994). The rate of adsorption is also affected by the number of bacteria; at high

densities of bacteria, adsorption can reach a maximum independent of bacterial density (Stent and Wollman 1952). The adsorption rate can also be affected by the medium in which the interaction takes place, and by the physiological state of the cell (Delbruck 1940a).

Adsorption of T phage to the bacterial surface occurs through an interaction between the distal end of the phage's tail fibers and the receptor molecule (Goldberg et al. 1994). The interaction between tail fiber and receptor molecule is highly specific. For example, phage T4 tail fibers bind only to the glucosyl-α-1,3 terminus of rough lipopolysaccharide in *E. coli* B or to the OmpC outer membrane protein in *E. coli* K12, while phage T2 binds to either the outer membrane protein OmpF or to glucosyl-α-1,3 terminus of rough lipopolysaccharide in *E. coli* B, and phage T7 binds only to the heptose residues in the lipopolysaccharide core (Goldberg et al. 1994).

This initial specific binding is weak and reversible. Once this binding has occurred, the phage is thought to "walk" around the bacterial cell by making and breaking attachments to different individual receptor molecules (Goldberg et al. 1994). This walking continues until an injection site is reached. At the injection site the phage attaches irreversibly to the bacterial cell through interactions between the phage baseplate and the bacterial cell surface. The specific phage and bacterial receptors involved in irreversible binding are unknown, but there is some evidence that the injection sites are associated with Bayer's junctions, sites where the cytoplasmic membrane and the outer membrane of *E. coli* appear to merge (Nikaido and Vaara 1987). Once irreversible binding occurs, the phage DNA is ejected and taken up by the bacterial cell. Little is known of the mechanics of DNA ejection or uptake.

Infection by phage DNA blocks bacterial DNA, RNA and protein synthesis, turning E. coli into an efficient factory for the production of phage (Goldberg et al.

1994). After a latent period during which phage particles are assembled, the bacterial cell is destroyed and the phage progeny are released. A process know as lysis inhibition can occur with phages T4, T2 and T6 (Doermann 1948, Abedon 1994). With lysis inhibition, the adsorption of a second phage following an initial phage infection can prolong the latent period and increase the total burst size of the phage (the total number of phage progeny released per bacterial cell). Burst size and latent period can also be affected by growth media and physiological state of the bacterial cell (Hadas et al. 1997).

Mutant bacteria that are invulnerable to predation by phage have been reported (Lenski 1988c). Most of these phage-resistant mutants achieve resistance through the loss or modification of the receptor molecule to which the phage initially binds. This loss or modification can result in a competitive cost because these receptor molecules are also involved in bacterial metabolism (Lenski 1988c). The cost of phage resistance can vary depending on the species of phage to which resistance is directed, the environment in which the cost is measured, the genetic background and the specific mutation (Szmelcman and Hofnung 1975, Lenski 1988a, Lenski 1988b).

OVERVIEW OF DISSERTATION RESEARCH

My dissertation research involves using chemostat communities of bacteria and bacteriophage as model systems to study the effects of increased productivity on ecological communities. I assembled model communities of *E. coli* and T bacteriophages in glucose-limited chemostats and maintained them at different productivity levels. I altered productivity levels in the model systems by altering the concentration of glucose in the incoming media. I established model

communities that were analogous to three different community modules: a simple food chain module (Chapter 2), a keystone predator module (Chapter 3) and a shared predator module (Chapter 4) (Figure 2). I tracked population densities of bacteria and bacteriophage over time and I estimated equilibrium population densities and stability in each treatment. The response of each model system was then compared to the predictions of mathematical models of the corresponding community module.

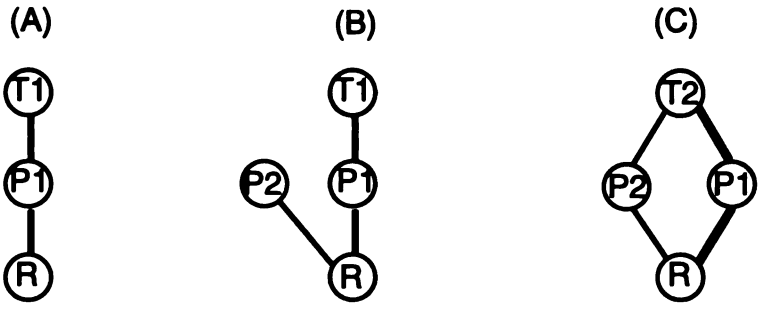


Figure 2. Experimental communities. (A) simple food chain, (B) community with keystone predator, (C) community with shared predator. Key: T1 = bacteriophage T4, T2 = bacteriophage T2, P1 = *E. coli* strain REL607, P2 = *E. coli* strain REL6584, R = glucose.

CHAPTER 2

EFFECT OF RESOURCE ENRICHMENT ON A CHEMOSTAT COMMUNITY OF BACTERIA AND BACTERIOPHAGE

INTRODUCTION

The dynamics of predator-prey and other exploitative interactions have long been recognized as fundamentally important to the structure of ecological communities (Hairston et al. 1960, Paine 1966, Lubchenco 1978). Nonetheless, there remains considerable debate over such basic issues as the effects of resource enrichment on these interactions and how best to model these effects (Arditi et al. 1991a, Ginzburg and Akçakaya 1992, Diehl et al. 1993, Abrams 1994a, Berryman et al. 1995). Classical predator-prey models (i.e., Lotka-Volterra models and modern variations thereof) make two controversial predictions concerning the effect of resource enrichment on prey and their predators. First, these models predict that enrichment will result in an increase in the equilibrium population density of the predator but have no effect on the equilibrium population density of the prey (Rosenzweig 1977) (Ehrlich and Birch 1967). Second, classical predator-prey models predict that enrichment can destabilize a predator-prey pair, increasing the amplitude and period of population oscillations (Rosenzweig 1971).

These classical models are considered "prey-dependent" because they assume that the attack rate of predators depends only on the instantaneous density of prey. Some theorists have argued that the attack rate is often better modeled as a function of the ratio of prey to predator density (Arditi and Ginzburg 1989). Such "ratio-dependent" models make very different predictions concerning the effect of enrichment on prey and their predators. Enrichment is not predicted to be destabilizing, and the equilibrium population sizes of both predators and prey are predicted to increase in response to enrichment.

Proponents of ratio-dependent models have suggested that this approach is superior because it captures the effects of heterogeneity on predator-prey dynamics. Such heterogeneity could include differences in the time scales of feeding by predators and reproduction by predators, discontinuous prey reproduction, spatial heterogeneity and heterogeneity in prey edibility (Arditi and Ginzburg 1989). The superiority of ratio-dependent models in these situations has been hotly debated (Oksanen et al. 1992, Diehl et al. 1993, Gleeson 1994, Abrams 1994a, Akçakaya et al. 1995, Berryman et al. 1995) . This debate has centered on whether ratio-dependent models do indeed capture the effects of heterogeneity, whether it is better to model heterogeneity by using a ratio-dependent model or by explicitly incorporating heterogeneity into a prey-dependent model, and what the tradeoffs are in using these two approaches.

There have been a number of attempts to answer these questions using field systems. Most of these attempts have involved comparing trophic structure and/or trophic level biomass across natural gradients of productivity (Arditi et al. 1991a, Ginzburg and Akçakaya 1992, Hansson 1992, Oksanen et al. 1992, Persson et al. 1992) or measuring the response of a natural community to enrichment (O'Brien et al. 1992, Wooton and Power 1993, Stow et al. 1995). The results of these attempts have been inconclusive. In some studies prey-

dependent models appeared to better predict the responses (Hansson 1992, Oksanen et al. 1992, Persson et al. 1992, Wooton and Power 1993) while in other studies the response appeared to be better predicted by ratio-dependent models (Arditi et al. 1991a, Ginzburg and Akçakaya 1992, O'Brien et al. 1992, Schmitz 1993). The limitations inherent in using field systems to test these models have been well discussed in the literature (Power 1992). These limitations include difficulty determining whether populations are at or near equilibrium, problems with quantifying trophic level biomass, and difficulty defining the physical boundaries of food chains.

Some of these limitations can be circumvented by using laboratory model systems. Ecological experiments with model laboratory systems can bridge the gap between mathematical models and natural communities, by allowing the predictions of mathematical models to be rigorously examined in a biological system that is easily manipulated, replicated and controlled before such models are applied directly to natural systems (Lawton 1995). Two attempts have been made to test prey-dependent and ratio-dependent models using laboratory model communities. In the first attempt, Harrison (Harrison 1995) reanalyzed the classic experiments of Luckinbill (Luckinbill 1973). Luckinbill observed that decreasing the concentration of nutrients in batch cultures of protozoan predators and prey increased the stability of the populations dramatically (although manipulating the interactions between predators and prey by thickening the media was also necessary to achieve persistence). Luckinbill was unable to compare equilibria between treatments because the predator populations went extinct in the higher nutrient treatment. However, in his reanalysis of these experiments, Harrison (1995) found no evidence for ratio-dependent predation.

In contrast, Balciunas and Lawler (Balciunas and Lawler 1995) found that in batch culture both bacteriovorus protozoans and prey bacteria increased in

abundance in response to increased nutrient input. However, they sampled the bacteria population only twice during their 52 day experiment (they were primarily interested in protozoan population dynamics); if the bacteria population cycled in response to predation, these estimates of population density could be inaccurate. In addition, Balciunas and Lawler used a heterogeneous population of bacteria in their experiments and the increase in bacteria abundance could be due to an increase in the abundance of less edible members of the mixed population. Balciunas and Lawler found some evidence for predator mutual interference and could not rule out ratio-dependent predation in their system.

Although most predator-prey theory assumes a "chemostat-like" environment (i.e., continuous input of resources, constant mortality, etc.), both studies above used batch culture systems rather than chemostats. In batch culture, an aliquot of the culture is transferred at regular intervals to fresh culture medium. The effect of such serial transfer is potentially confounding; it was considered by Harrison (1995) to be the major reason that he was unable to get a close fit between some of Luckinbill's data and the predictions of mathematical models.

I have built on these previous attempts by using chemostat communities of bacteria and bacteriophage (viruses that feed on bacteria) to test prey-dependent and ratio-dependent models. I observed the response of these communities to resource enrichment and compared this response to quantitative predictions of prey-dependent and ratio-dependent models. Both predator and prey persisted in all replicates and I was able to estimate equilibrium densities and quantify stability for all populations. In addition, bacteria and bacteriophage have sufficiently short generation times that I was able to observe the effect of enrichment on the evolution of predator-prey interactions during the course of my experiment.

METHODS

Experimental system

Bacteria and bacteriophages have been proposed as ideal experimental systems for studying predator-prey dynamics (Campbell 1961, Lenski and Levin 1985) and have been successfully used as such by a number of researchers (Paynter and Bungay 1969, Horne 1970, Paynter and Bungay 1971, Chao et al. 1977, Levin and Lenski 1983, Lenski and Levin 1985, Lenski 1988a). Although bacteriophage-bacteria interactions have been traditionally modeled using prey-dependent models (Levin et al. 1977), bacteriophage-bacteria interactions have a number of characteristics that could be modeled more simply using the ratio-dependent approach. Temporal heterogeneity is present, with bacteriophage feeding on bacteria on a time scale of seconds, but reproducing in bursts approximately every half an hour. Heterogeneity in the susceptibility of bacteria to bacteriophage attack is common, evolving rapidly even in populations of bacteria started from a single clone (Lenski 1988b). There is strong evidence that the chemostat environment is not spatially homogeneous, and that growth by bacteria on the wall of a chemostat can have a profound effect on population dynamics (Chao and Ramsdell 1985). Growth on the vessel wall has been observed to act as a refuge for bacteria from bacteriophage, leading to greater population stability than predicted by prey-dependent models (Schrag and Mittler 1996).

My experimental system consisted of *E. coli* B strain REL607 (Lenski et al. 1991) and the virulent bacteriophage T4 (kindly provided by L. Snyder) in

glucose-limited chemostats. My 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 $x10^{-6}$ g thiamine hydrochloride per liter and either 0.1 or 0.5 μg per ml glucose. These glucose concentrations were chosen because the predictions of the prey-dependent and ratio-dependent models differ dramatically within this range of concentrations (see below). The volume of the chemostats was maintained at approximately 30 ml, the flow rate at approximately 0.2 turnovers per hour and the temperature at 37°C. Three replicate chemostats at each glucose concentration were maintained simultaneously. Control chemostats, containing only *E. coli*, were established at each glucose concentration and maintained simultaneously with the treatment chemostats.

The population densities of *E. coli* and bacteriophage T4 were estimated twice daily by dilution and plating. *E. coli* cells were plated on Davis minimal agar supplemented with 2 $x10^{-6}$ g thiamine hydrochloride per liter and 4 mg per ml glucose. Heat-killed cells were mixed with each sample to inactivate free bacteriophage prior to plating, as described by Carlson and Miller (Carlson and Miller 1994). Bacteriophage T4 was plated on a lawn of *E. coli* using Davis minimal agar and the plate count technique described by Carlson and Miller (1994). I also estimated the population densities of *E. coli* mutants resistant to predation by bacteriophage T4. These T4-resistant cells were plated on Davis minimal agar supplemented as above. A concentrated bacteriophage T4 lysate was mixed with each sample to kill T4-sensitive *E. coli* prior to plating.

To estimate the population stability and equilibrium population densities of T4 and *E. coli*, I treated each chemostat as a single observational unit. I first calculated the mean and standard deviation of the T4 and *E. coli* population densities over time for each chemostat. I then estimated the stability of each

population as the mean coefficient of variation across replicate chemostats (the lower the coefficient of variation, the higher the stability). I estimated the equilibrium density of each population as the grand arithmetic mean of population density across replicate chemostats. I determined that the arithmetic mean was superior to the geometric mean as an estimator of equilibria by analyzing simulated population data. The arithmetic mean estimated the equilibria of simulated data more accurately than the geometric mean, and it was not systematically biased.

E. coli mutants resistant to predation by bacteriophage T4 eventually appeared in all chemostats. To remove the influence of these mutants on stability and equilibria, I excluded the last two time points before the appearance of resistant mutants from my calculations. I also excluded the first two time points after inoculation, to allow time for the populations to reach equilibria. In addition to these estimates, I also estimated population stability and equilibria for the time period after the T4-resistant mutants had reached equilibrium in the higher glucose treatment (the experiment was terminated before they reached equilibrium in the lower glucose treatment).

I compared population stability and equilibria with *t*-tests. One-tailed comparisons were used whenever the models made directional predictions. Prior to comparison I tested for homogeneity of variances. The data were log-transformed prior to comparison whenever the variances were found to be significantly different.

Mathematical models

I modeled my experimental system using modifications of the models developed by Levin et al. (1977). I solved these models analytically and

examined the behavior of the models numerically using STELLA II simulation software (High Performance Systems 1994). Because predator-resistant prey evolved in the chemostats during the experiment, prey-dependent and ratio-dependent models were also created for a system that included predator-resistant prey. The details of the models are described below.

Numerical simulations. I ran all numerical simulations using a time step of 0.05 hours. I tested the sensitivity of the simulations to time step size by running replicate simulations at step sizes of 0.1, 0.05 and 0.025 hours. Varying the size of the time steps had no detectable effect on the results of the simulations. I "sampled" the output of each simulation every 12 hours (the approximate sampling interval of my experiments) to produce the predictions depicted graphically.

Prey-dependent model. This model explicitly includes a time delay between consumption of prey and reproduction by the predator. This model differs from the model of Levin et al. in that it ignores the dynamics of infected cells (I consider infected cells to instantaneously become "dead" cells in my experimental system because infected cells will not produce colonies when plated). This model also ignores spatial heterogeneity. Although theorists have developed prey-dependent models that include spatial heterogeneity (Abrams and Walters 1996), these models cannot be easily adapted to my experimental system because they require parameters that I cannot yet estimate (e.g., cell transfer rates between wall and liquid populations). The prey-dependent food chain model is as follows,

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

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

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

where C_0 = concentration of glucose in the reservoir, C = concentration of glucose in the chemostat, ω = flowrate, ε = reciprocal of the yield of the bacteria, N = population size of uninfected bacteria, ψ = maximum specific growth rate, K = resource concentration at which the bacteria grow at one half ψ, $\alpha(N)$ = trophic function, P = population size of free bacteriophage, β = number of bacteriophage progeny per infected bacterial cell, τ = time lag between infection and release of bacteriophage progeny, $e^{-\tau\omega}$ = fraction of bacteria infected at time $t - \tau$ that has not washed out of the chemostat before releasing bacteriophage, N' = population size of uninfected bacteria at time $t - \tau$, and P' = population size of bacteriophage at time $t - \tau$.

The following parameter values were used for this model : C_0 = either 0.1 or 0.5 µg per ml, ω = 0.2 per hr, $\varepsilon = 2 \times 10^{-6}$ µg (Lenski 1988b), ψ = 0.7726 per hr (Vasi et al. 1994), K = 0.0727 µg per ml (Vasi et al. 1994), $\alpha = 3 \times 10^{-7}$ ml per hr (Lenski and Levin 1985), β = 80 viruses per bacterial cell and τ = 0.6 hr (Lenski and Levin 1985). The predictions for this model are presented in Figures 1A, 2A and 2B.

Ratio-dependent model. The ratio-dependent food chain model was similar to the prey-dependent model above with the exception that the trophic function $\alpha(N)$ was replaced with $\alpha(N/P)$. This model does not explicitly include a time delay, but it is presumed that the ratio-dependent functional response captures the effect of such temporal heterogeneity on predator-prey dynamics (Arditi and Ginzburg 1989).(Tilman and Downing 1994) The model was as follows,

$$dC/dt = (C_0 - C)\omega - \varepsilon N\psi C/(K+C)$$
$$dN/dt = N\psi C/(K+C) - \alpha(N/P)P - \omega N$$
$$dP/dt = \beta\{\alpha(N/P)P\} - \alpha(N/P)P - \omega P.$$

The parameter values used for the ratio-dependent model were the same as for the prey-dependent model with the exception of α. In the ratio-dependent model, α has different units than in the prey-dependent model, and thus must be estimated differently. Using the same value of α as that used in the prey-dependent model would result in predicted equilibria for my system that are orders of magnitude different from preliminary results for my system. Therefore I estimated α by fitting the ratio-dependent trophic function to preliminary estimates of equilibria for my system at a glucose input concentration of 0.1 µg per ml ($\alpha = 8.95 \times 10^{-3}$ per hr). I used this value to predict the equilibria of my system at 0.5 µg per ml and to produce the predictions depicted in Figures 1B, 2D and 2E.

Prey-dependent model (post-invasion). I used a modification of the prey-dependent model above to model my experimental system after T4-resistant mutants of *E. coli* had invaded the chemostats. This modified model consists of four differential equations,

$$dC/dt = (C_0 - C)\omega - \varepsilon N\psi C/(K+C) - \varepsilon_R R\psi_R C/(K_R+C)$$
$$dN/dt = N\psi C/(K+C) - \alpha(N)P - \omega N$$
$$dP/dt = \beta e^{-\tau\omega}\{\alpha(N')P'\} - \alpha(N)P - \omega P$$
$$dR/dt = R\psi_R C/(K_R+C) - \omega R$$

where, in addition to the variables described above, R = population size of T4-resistant bacteria, ε_R = reciprocal of the yield of the T4-resistant bacteria, ψ_R = maximum specific growth rate of T4-resistant bacteria, and K_R = resource concentration at which T4-resistant bacteria grow at one half ψ_R. In addition to the parameter values used in the prey-dependent model above, I used the following values: ψ_R = 0.7027 per hr and K_R = 0.123 µg per ml. These values were determined experimentally. ψ_R was estimated using the methods described by Vasi et al. (1994). I was unable to directly estimate K_R with consistent and meaningful results. Instead, I estimated K_R indirectly by first estimating the fitness of the T4-resistant mutants relative to their T4-sensitive ancestors in chemostats (as described by Lenski and Levin, 1985). The average relative fitness was 0.575, similar to values previously reported by Lenski and Levin (1985). From the relative fitness value I estimated the growth rates of the T4-sensitive (μ) and T4-resistant (μ_R) *E. coli* at steady state in the chemostats. The equilibrium glucose concentration in the chemostats (C^*) was assumed to be set by the superior competitor (the T4-sensitive *E. coli*). C^* was estimated from a rearrangement of the Monod (Monod 1949) model using parameters for the T4-sensitive *E. coli*,

$$C^* = K / (\psi / \mu - 1)$$

Finally, I estimated K_R from yet another rearrangement of the Monod model, in this case using parameters estimated for the T4-resistant *E. coli*,

$$K_R = C^*(\psi_R / \mu_R - 1).$$

The predictions of this model are summarized in Figure 2C.

Ratio-dependent model (post-invasion). Proponents of ratio-dependent models have argued that the ratio-dependent functional response incorporates the net effect of heterogeneity on population dynamics and that heterogeneous systems are more parsimoniously modeled by ratio-dependent models than by other types of models (Arditi and Ginzburg 1989). I tested this idea by developing a ratio-dependent model that combined the T4-sensitive and T4-resistant *E. coli* into one population that is heterogeneous in edibility. I used the same ratio-dependent model described above, with the exception that I fit the ratio-dependent trophic function to estimates of equilibria from previously published (Lenski and Levin 1985) observations of *E. coli* and T4 after invasion by T4-resistant mutants (α =2.28 x10^{-5} per hr). These observations were made in chemostats with glucose input concentrations of 300 μg per ml. The predictions of this model are summarized in Figure 2F.

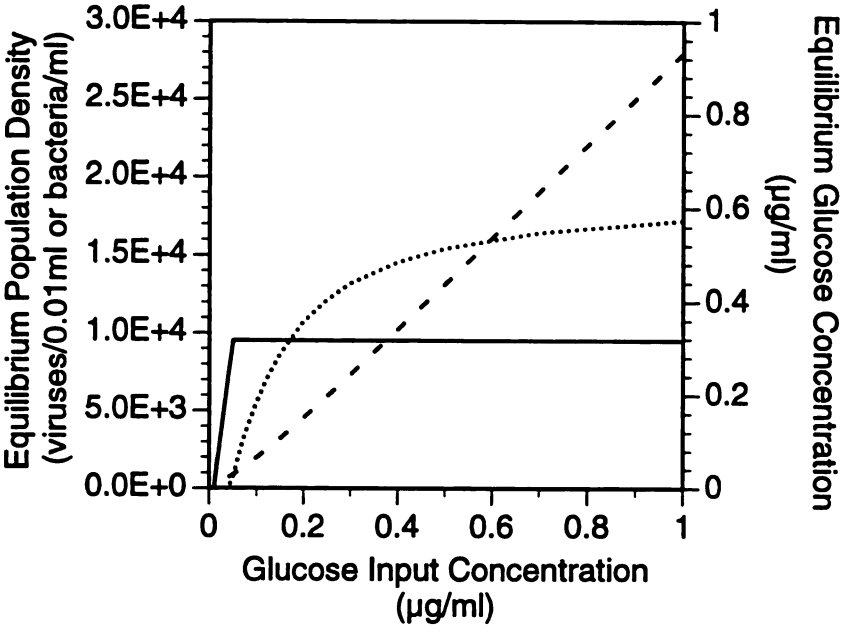
RESULTS

Ecological dynamics

Model predictions. The predictions made by the ratio-dependent and prey-dependent food chain models for the ecological dynamics in my system are presented in Figures 3 and 4. The predictions of the prey-dependent model vary depending on the concentration of glucose in the incoming media. At concentrations ranging from approximately 0.08 μg per ml to 1 μg per ml, the prey-dependent model predicts that: (1) the equilibrium population density of T4 will increase in response to enrichment and (2) the equilibrium population density of *E. coli* will not change in response to enrichment (Figure 3A). I conducted my

Figure 3. Relationship between glucose input concentration and equilibria. (A) prey-dependent model (note that bacteriophage equilibria are divided by 100), (B) ratio-dependent model. Key: solid line = equilibrium population density of T4-sensitive *E. coli*, dotted line = equilibrium population density of T4, dashed line = equilibrium concentration of glucose.

(A)


Equilibrium Population Density
(viruses/0.01ml or bacteria/ml)
3.0E+4
2.5E+4
2.0E+4
1.5E+4
1.0E+4
5.0E+3
0.0E+0
0
0.2
0.4
0.6
0.8
1
Glucose Input Concentration
(µg/ml)
Equilibrium Glucose Concentration
(µg/ml)
1
0.8
0.6
0.4
0.2
0

(B)

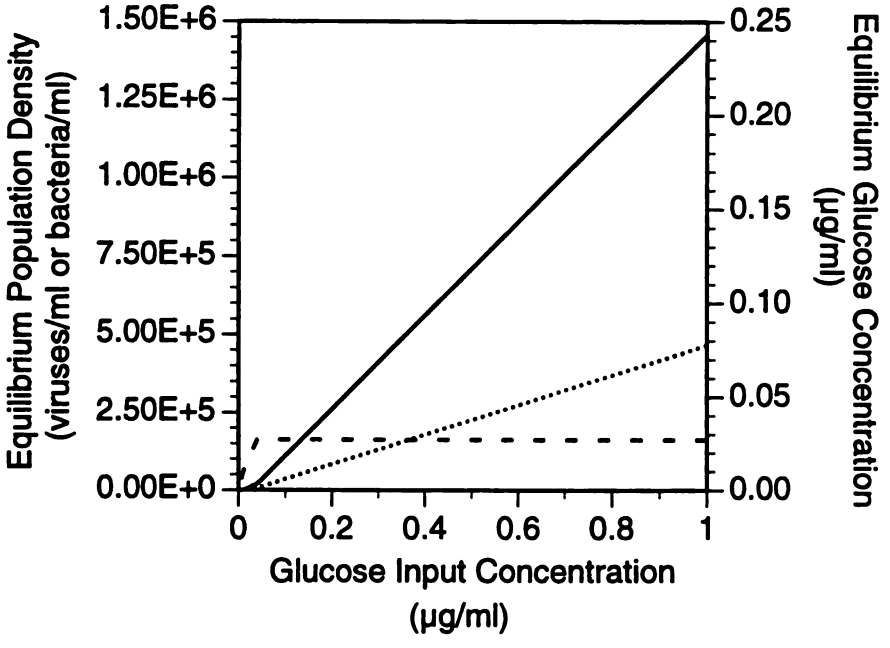
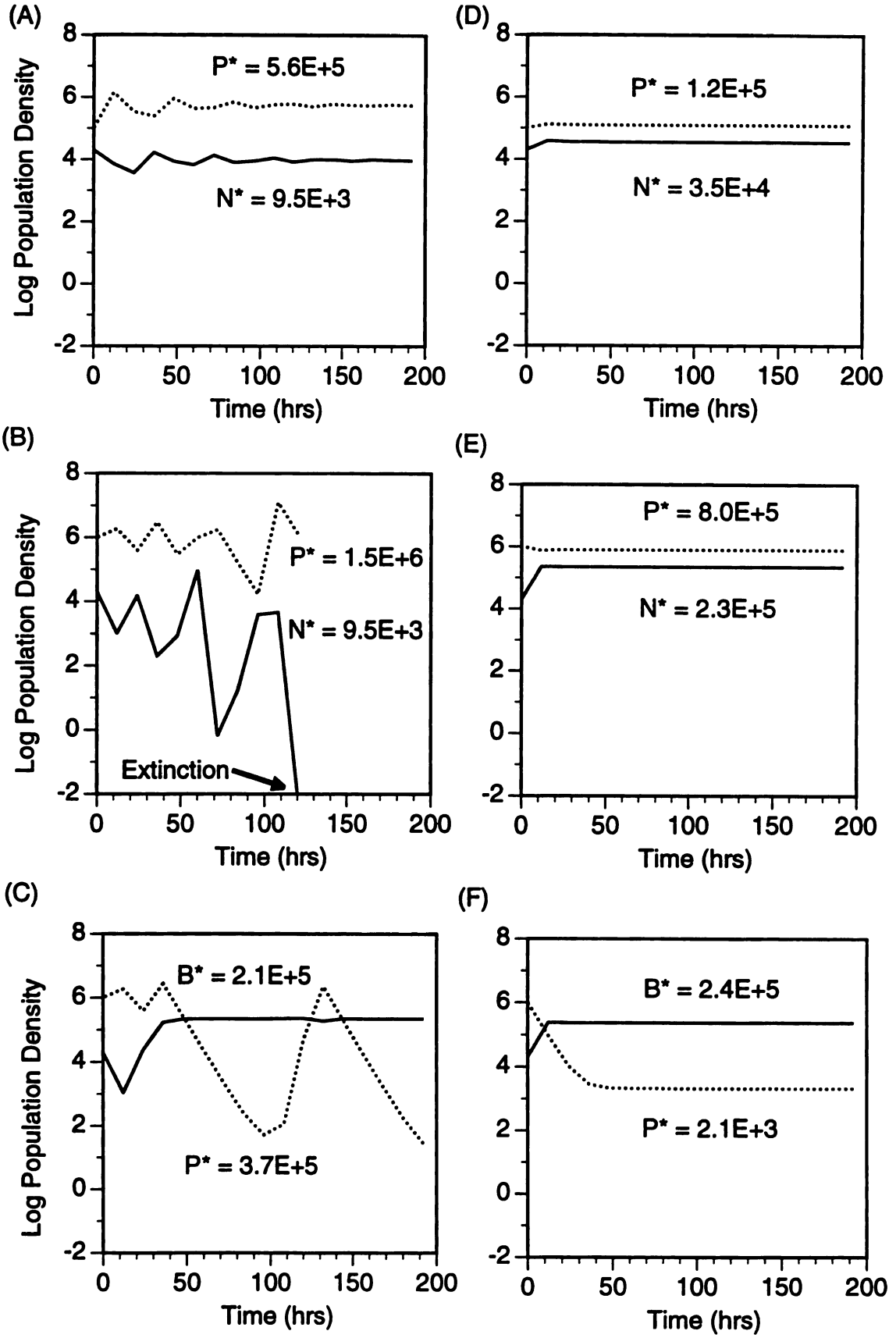

Equilibrium Population Density
(viruses/ml or bacteria/ml)
1.50E+6
1.25E+6
1.00E+6
7.50E+5
5.00E+5
2.50E+5
0.00E+0
0
0.2
0.4
0.6
0.8
1
Glucose Input Concentration
(µg/ml)
Equilibrium Glucose Concentration
(µg/ml)
0.25
0.20
0.15
0.10
0.05
0.00

Figure 4. Population equilibria and dynamics predicted by the models. Equilibria are from analytical solutions of the models; dynamics are from numerical simulations of the models, "sampled" at 12 hour intervals. The population densities (viruses per ml or bacteria per ml) have been log-transformed. (A) prey-dependent model with a glucose input concentration of 0.1 μg per ml, (B) prey-dependent model with a glucose input concentration of 0.5 μg per ml, (C) prey-dependent model (altered to include heterogeneity in prey edibility) with a glucose input concentration of 0.5 μg per ml, (D) ratio-dependent model with a glucose input concentration of 0.1 μg per ml, (E) ratio-dependent model with a glucose input concentration of 0.5 μg per ml, (F) ratio-dependent model (altered to include heterogeneity in prey edibility) with a glucose input concentration of 0.5 μg per ml. Key: N^* = equilibrium population density of T4-sensitive *E. coli*, P^* = equilibrium population density of T4, B^* = equilibrium population density of total *E. coli* (T4-sensitive and -resistant combined), solid line = *E. coli* population dynamics, dotted line = T4 population dynamics.


(A)
P* = 5.6E+5
N* = 9.5E+3
Log Population Density
Time (hrs)
(D)
P* = 1.2E+5
N* = 3.5E+4
Time (hrs)
(B)
P* = 1.5E+6
N* = 9.5E+3
Extinction
Log Population Density
Time (hrs)
(E)
P* = 8.0E+5
N* = 2.3E+5
Time (hrs)
(C)
B* = 2.1E+5
P* = 3.7E+5
Log Population Density
Time (hrs)
(F)
B* = 2.4E+5
P* = 2.1E+3
Time (hrs)

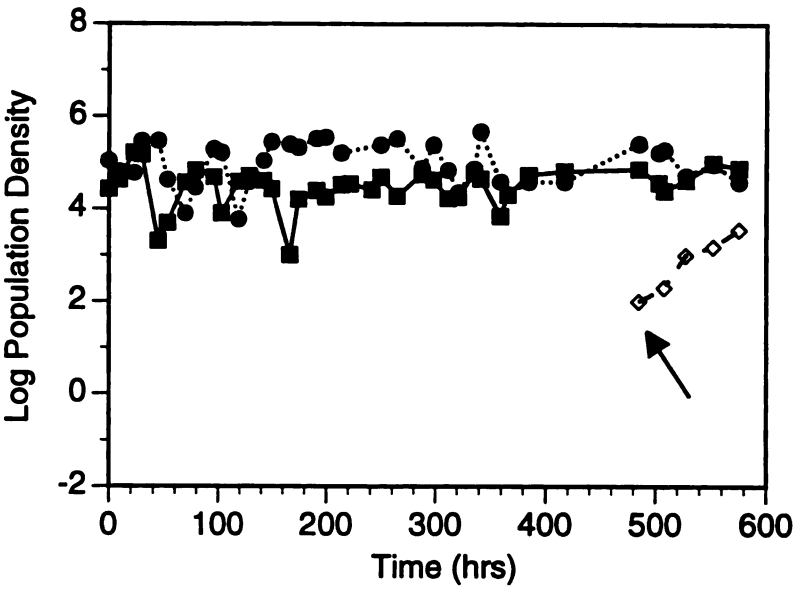
experiments within this input concentration range. As the glucose input concentration is lowered below this range the model predicts that the T4 population will become extinct first, and then the *E. coli* population. Above approximately 1 μg per ml, neither population equilibrium is predicted to increase appreciably in response to enrichment. In addition, this model predicts that within the range of concentrations used in my experiment both the T4 and *E. coli* populations will to be less stable as glucose input concentration is increased (Figures 4A and 4B).

In contrast, the ratio-dependent model predicts that the equilibrium population densities of both T4 and *E. coli* will increase in response to enrichment, regardless of the glucose input concentration range (Figure 3B). The ratio-dependent model also predicts that the stability of the populations will not be affected by enrichment (Figures 4D and 4E).

Empirical observations. The dynamics of the T4 and *E. coli* populations are shown in Figure 5 for representative chemostats with two different input concentrations of glucose. The populations persisted in all chemostats with apparent population cycles until the appearance of, and subsequent invasion by, T4-resistant bacteria. There was a large and highly significant increase in the equilibrium population density of bacteriophage T4 in response to enrichment (t = 18.225, df = 4, one-tailed $P < 0.0001$; Figure 6A). The equilibrium population density of *E. coli* responded to enrichment with a small but significant increase (t = 2.4699, df = 4, one-tailed $P = 0.0345$; Figure 6A). There was a large and highly significant decrease in stability (i.e., increase in coefficient of variation) for both T4 and *E. coli* populations in response to enrichment (t = 4.6092, df = 4, one-tailed $P = 0.0050$ for *E. coli*; t = 4.3042, df = 4, one-tailed P = 0.0063 for T4; Figure 6B). In control chemostats without bacteriophage, the *E. coli* population

Figure 5. Dynamics of *E. coli* and bacteriophage T4 populations in representative chemostats supplied with media containing different amounts of glucose. The population densities (viruses per ml or bacteria per ml) have been log-transformed. (A) 0.1 µg/ml glucose, (B) 0.5 µg/ml glucose. Key: solid line = total *E. coli* population, dotted line = T4 population, diamonds = T4-resistant *E. coli*, arrows indicate first detection of T4-resistant *E. coli*.

(A)


Log Population Density
8
6
4
2
0
-2
0
100
200
300
400
500
600
Time (hrs)

(B)

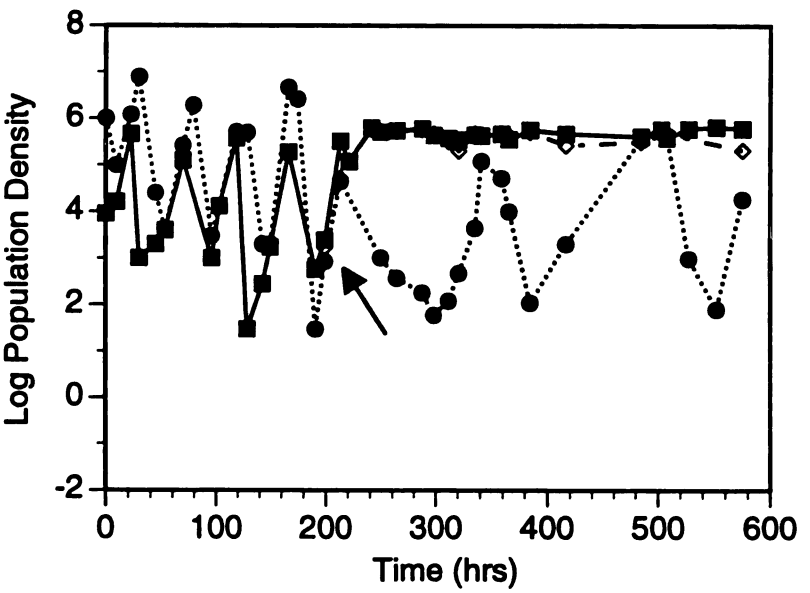
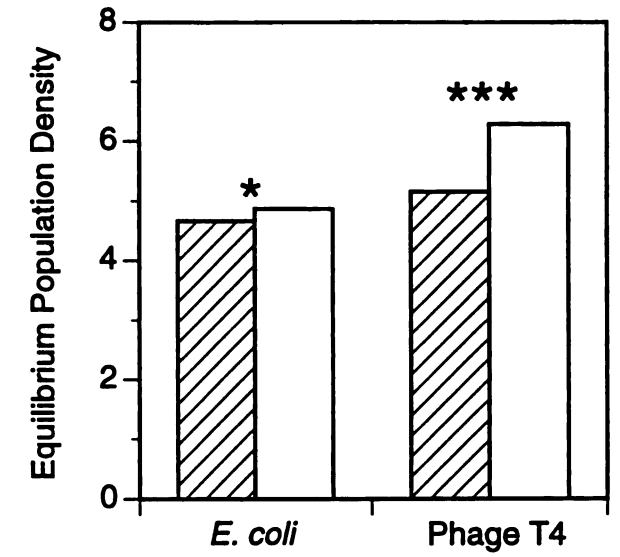
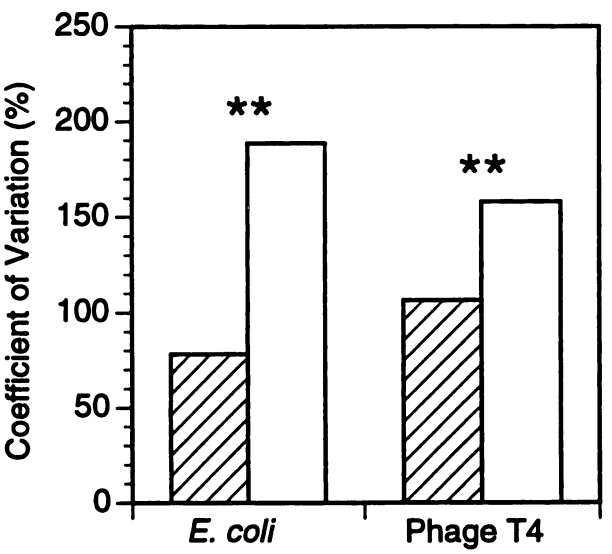

Log Population Density
8
6
4
2
0
-2
0
100
200
300
400
500
600
Time (hrs)

Figure 6. Effect of glucose input concentration on equilibrium densities and population instability of *E. coli* and bacteriophage T4 populations interacting in a chemostat. Equilibrium population density is estimated as the grand mean of the mean population densities in 3 replicate chemostats. The equilibrium population densities (viruses per ml or bacteria per ml) have been log-transformed in this figure. Instability is estimated as the coefficient of variation of population densities averaged across 3 replicate chemostats. Stars indicate statistical significance: * = $0.01 < P < 0.05$, ** = $0.001 < P < 0.01$, ***, $P < 0.001$. (A) equilibrium density, (B) instability. Key: striped bars = 0.1 μg per ml glucose treatment, solid bars = 0.5 μg per ml glucose treatment.

(A)


Equilibrium Population Density
8
6
4
2
0
*
***
E. coli
Phage T4

(B)


Coefficient of Variation (%)
250
200
150
100
50
0
**
**
E. coli
Phage T4

increased in response to enrichment. Population stability was unaffected by enrichment in the control chemostats.

Fit of observations to mathematical models. Qualitatively, the response of the predator and prey populations to enrichment was better predicted by the prey-dependent model than the ratio-dependent model. In response to enrichment: (1) the equilibrium population density of the predator increased, (2) the equilibrium population density of the prey changed slightly, and (3) the stability of both prey and predator populations decreased. However the quantitative agreement between the model and my data was far from perfect. The prey-dependent model predicted no change in the prey equilibrium; I observed a small but significant increase. The prey dependent model predicted an increase in the predator equilibrium of approximately three-fold; I observed approximately a 13-fold increase. The predator and prey populations were destabilized by increased resource input as predicted by the prey-dependent model, but the prey population did not go to extinction in the high glucose treatment as the model predicted (Figure 4B).

Evolutionary change

Model predictions. The prey-dependent and ratio-dependent food chain models also make predictions regarding evolutionary change in my experimental system. Evolutionary change in my system can be thought of as occurring in two phases: (1) appearance of, and invasion by, T4-resistant mutants of *E. coli*, and (2) persistence of T4-resistant mutants following invasion. I will discuss the predictions for phase 1 first. Both models predict that T4-resistant mutants of *E. coli* can invade the chemostat provided that they can grow fast enough at the equilibrium glucose concentration to offset washout. However, the prey-

dependent model predicts a substantially higher equilibrium glucose concentration than the ratio-dependent model predicts (Figure 3), resulting in much broader conditions for invasion by T4-resistant mutants. The prey-dependent model also predicts that the equilibrium glucose concentration will be proportional to the glucose input concentration and thus the rate of invasion by T4-resistant mutants will be faster in the higher glucose treatment. The ratio-dependent model does not predict this relationship. Both models predict that enrichment could decrease the amount of time required for T4-resistant mutants of *E. coli* to appear in the chemostats, either because the growth rate of *E. coli* is increased by enrichment (prey-dependent model) or because the equilibrium population size of *E. coli* is increased by enrichment (ratio-dependent model).

To produce predictions for phase 2, I modified the prey-dependent model by adding an additional equation to describe the dynamics of the T4-resistant mutants (see above). This model treats the resistant mutants as a separate population and assumes that there is a trade-off between T4-resistance and competitive ability. Such a trade-off has been previously reported (Lenski 1988a) and has been shown to result in coexistence of bacteriophage-resistant and bacteriophage-sensitive *E. coli* when bacteriophage is present (Chao et al. 1977). I measured the magnitude of this trade-off in my system and it is similar to previously reported trade-offs (see above). I also developed a ratio-dependent model of my system after invasion by fitting my ratio-dependent model to previously published observations of *E. coli* and T4 after invasion by T4-resistant mutants (see above). This model combines the T4-resistant and T4-sensitive *E. coli* into one heterogeneous population.

When the pre- and post-invasion versions of each model are compared, several predictions emerge (compare Figures 4B and 4C for the prey-dependent model, Figures 4E and 4F for the ratio-dependent model). The ratio-dependent

model predicts a 4% increase in the equilibrium population density of total *E. coli* (T4-sensitive and -resistant combined) following invasion, while the prey-dependent model predicts a 22-fold increase. The ratio-dependent model predicts no change in the stability of the total *E. coli* population following invasion; in contrast, the prey-dependent model predicts that the population will increase in stability. Both models predict a decrease in the population density of T4 following invasion; however, the ratio-dependent model predicts a decrease of approximately 300-fold, while the prey-dependent model predicts a decrease of approximately 5-fold. Neither model predicts that the stability of the T4 population will change following invasion. However, the ratio-dependent model predicts a stable equilibrium both before and after invasion by T4-resistant mutants, while the prey-dependent model predicts that the population will exhibit undamped oscillations both before and after invasion. In addition, the prey-dependent model predicts that the T4 population oscillations will increase in period following invasion.

Empirical observations. T4-resistant bacteria were eventually detected in all treatment chemostats (they were not detected in control chemostats without bacteriophage). T4 was unable to make visible plaques on a lawn of these bacteria, indicating that the bacteria were completely resistant to predation by T4 (see also Lenski and Levin 1985). I tested these bacteria for genetic markers present in REL607. The T4-resistant bacteria were identical to REL607 and were therefore presumed to be T4-resistant mutants of REL607 (as opposed to contaminating bacteria). These mutants appeared significantly sooner in the high glucose treatment than in the low glucose treatment ($t = 8.999$, df = 4, one-tailed $P = 0.0004$). I calculated the rate of invasion of these mutants by first fitting a line to the log-transformed time series data for the mutants. Only those data points occurring before the mutants reached equilibrium were used. The slopes

of the best fit lines were then compared between treatments (only one replicate of the lower glucose treatment had a sufficient number of data points to determine the invasion rate; this was compared to the three replicates of the high glucose treatment). The rate of invasion by these mutants was significantly faster in the high glucose treatments than in the low glucose treatments (t = 7.1117, df = 2, one-tailed P = 0.0096).

The invasion of the chemostats by T4-resistant mutants had a significant effect on the equilibrium density and stability of the *E. coli* population. The *E. coli* population increased dramatically in population density (t = 10.944, df = 4, one-tailed P = 0.0002, Figure 7) and stability (t = 7.8563, df = 4, one-tailed P = 0.0014) following invasion. The equilibrium density of the total *E. coli* population after invasion was not significantly different from the equilibrium population density of *E. coli* in the control chemostat without bacteriophage (indicating that the population was now resource-limited, rather than predator-limited).

There was a moderate but significant decrease in equilibrium population density of T4 (t = 11.199, df = 2, one-tailed P = 0.0039, Figure 7) following invasion. The T4 population continued to cycle even after equilibrium was reached by the T4-resistant *E. coli* in the higher glucose treatment (the experiment was terminated before T4-resistant *E. coli* had reached equilibrium in the lower glucose treatment). This persistence of T4 has also been observed by other researchers; it is hypothesized to be due to the bacteriophage feeding on a minority population of T4-sensitive *E. coli*, which coexists with resistant cells because of a trade-off between resistance and competitive ability (Chao et al. 1977, Lenski and Levin 1985). Although I did observe a trade-off between resistance and competitive ability in my system, I was unable to detect the minority population of T4-sensitive cells (presumably because it was too small relative to the resistant population to detect directly). In two of the three replicate

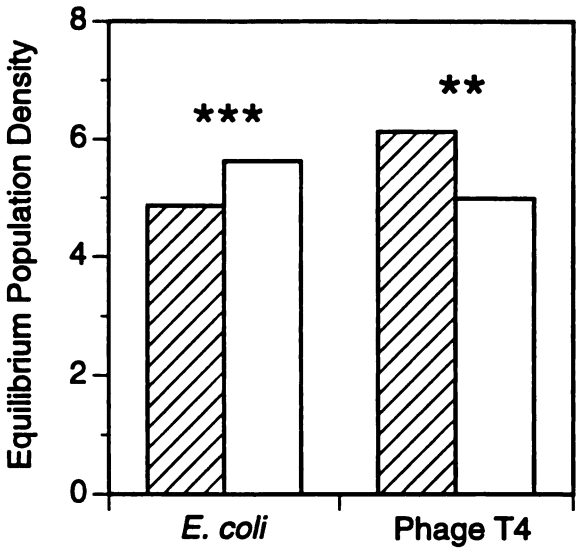


Figure 7. Effect of invasion by T4-resistant mutants of E. coli on the equilibrium densities of total E. coli (T4-sensitive and -resistant cells) and bacteriophage T4 populations interacting in a chemostat. Equilibrium population density is estimated as the grand mean of the mean population densities in 3 replicate chemostats. The equilibrium population densities (viruses per ml or bacteria per ml) have been log-transformed in this figure. Stars indicate statistical significance: ** = $0.001 < P < 0.01$, ***, $P < 0.001$. Key: striped bars = before invasion by T4-resistant mutants, shaded bars = after invasion by T4-resistant mutants.

chemostats the period of T4 population oscillations increased following invasion (there was not enough data points in the third replicate to determine the period length after invasion).

Fit of observations to mathematical models. Qualitatively, evolutionary change occurred in my system in a manner most consistent with the predictions of the prey-dependent model. The invasion by the mutants was faster in the higher glucose than lower glucose treatments, and, following invasion by the T4-resistant mutants: (1) the total equilibrium density of *E. coli* increased substantially, (2) the stability of the total *E. coli* population increased dramatically, (3) there was a moderate decrease in the equilibrium density of the T4 population, and (4) the T4 population continued to cycle, with oscillations longer in period than before invasion. Quantitatively, both models predicted the total equilibrium population density of *E. coli* within a factor of 2; however, the prey-dependent model better predicted the equilibrium population size of T4 (within a factor of 5) than did the ratio-dependent model (which under-estimated the equilibrium by two orders of magnitude).

DISCUSSION

Currently there is great debate among ecologists over the strengths and weaknesses of ratio-dependent predator-prey models. This debate has centered on whether it is better to model the effects of heterogeneity on predator-prey dynamics using ratio-dependent models or by altering prey-dependent models. I believe that there are at least two important reasons why these issues remain contentious. One reason is the profound difficulty of obtaining high quality empirical data on predator-prey interactions. This is due at least in part to

insufficient data to distinguish equilibrium from non-equilibrium dynamics and an inability to perform critical manipulative experiments to test key predictions of the alternative models. I have been able to circumvent each of these typical limitations by studying the dynamics of bacteria and their predatory bacteriophages in chemostats. I have population dynamics that extend for several hundred hours, equivalent to some 100 generations under the maximum generation time that is set by flow through the chemostat. I have manipulated the key variable (rate of resource input) while being confident that all other extrinsic factors (temperature, etc.) have remained unchanged.

A second reason for the lack of consensus on these issues may reflect the diverse goals of model builders and users in ecology. Levins (Levins 1966) distinguished three sometimes conflicting goals: realism, generality, and precision. My own tastes favor a primary emphasis on mechanistic realism, but I recognize that others may prefer, for example, generality to enable robust inferences under conditions where it is not feasible to pursue a mechanistic understanding. Nonetheless, it seems worthwhile to us to ask whether (and in what respects) a more complex model that maintains mechanistic realism might perform as well as, or better than, a simpler model that ignores mechanism.

Temporal and Spatial Heterogeneity

The chemostat is often assumed to be an environment homogeneous in time and space, but this is an oversimplification. The presence of the vessel wall introduces spatial heterogeneity into the chemostat, and growth on the vessel wall can profoundly influence the dynamics of chemostat populations (Chao and Ramsdell 1985, Schrag and Mittler 1996). Moreover, temporal heterogeneity is present in chemostat communities of bacteria and bacteriophage because there

is a latent period between prey consumption (i.e., infection) and predator reproduction (i.e., cell lysis) (Lenski 1988b). The effects of these heterogeneities can be modeled using a ratio-dependent model or by altering a prey-dependent model. The ratio-dependent model is presumed to capture the effects of temporal and spatial heterogeneity simply by virtue of the ratio-dependent functional response. The prey-dependent model is more complex, requiring additional terms to capture the effect of temporal heterogeneity; capturing the effects of spatial heterogeneity using this model would require even more complexity, and the inclusion of parameters not yet possible to estimate (see above). Thus, with a chemostat community of bacteria and bacteriophage I can ask: Does a prey-dependent model that explicitly incorporates temporal heterogeneity (via a time delay) perform better than a ratio-dependent model that incorporates temporal heterogeneity phenomenologically? Does a prey-dependent model that ignores spatial heterogeneity perform better than a ratio-dependent model that incorporate spatial heterogeneity phenomenologically? And what are the trade-offs between these approaches?

The prey-dependent and ratio-dependent models make numerous distinct predictions with respect to the effects of resource enrichment on the dynamics of predators and prey. I estimated the response of chemostat populations of bacteriophage and bacteria to enrichment and compared the response to these predictions. In almost all respects, the predictions of the prey-dependent models were fulfilled, whereas those of the ratio-dependent model were not. In summarizing these points, I will gloss over one notable exception, which I will then discuss in greater detail.

Both models predicted that resource enrichment would cause the equilibrium population of the predator (bacteriophage T4) to increase substantially, as indeed I observed. The equilibrium ratio of predators to prey

also increased substantially in response to resource enrichment, as predicted by the prey-dependent model but not the ratio-dependent model. Moreover, resource enrichment destabilized the interaction, producing greater temporal fluctuations of both prey and predator densities, an outcome predicted by the prey-dependent model but not the ratio-dependent model.

In addition to these effects on the equilibrium and stability properties of the system, as originally constituted, resource enrichment influenced the evolutionary dynamics of my system. Both models predicted (but for different underlying reasons) that T4-resistant bacteria might appear sooner at high than at low resource inputs, and indeed I observed that effect. However, resource enrichment also increased the rate at which resistant mutants subsequently invaded the sensitive bacterial population, as predicted by the prey-dependent model but not its ratio-dependent counterpart.

In short, the results of my experiments on resource enrichment in a simple microbial community provide very strong support for several distinct predictions of the prey-dependent model. At the same time, many predictions of the ratio-dependent model are flatly contradicted by my results. However, as I noted earlier there is one exception. That is, I observed an increase in the density of sensitive bacteria in response to resource enrichment, a prediction of the ratio-dependent model but not the prey-dependent model. This result was just barely significant, with $P = 0.0345$ using a one-tailed test. I used a one-tailed test because, in all fairness, the ratio-dependent model makes a directional prediction (while the prey-dependent model predicts no effect). I can think of at least three possible reasons for this result. First, this significant difference could be due to chance (Rice 1989). I performed many statistical tests in this paper, and all of the other tests that were judged significant had associated *P*-values of less than 0.01. Thus, it seems possible that a spuriously significant result would be

obtained, and this one is the most likely candidate given its large *P*-value and the fact that it alone contradicts a qualitative prediction of the prey-dependent model. However, when I correct for the number of comparisons using the Sequential Bonferroni method, this result remains significant (Sokal and Rohlf 1995).

The second potential explanation is that the difference between equilibria is actually larger and highly significant, but that this effect is masked by differences in mass per bacterial cell between treatments. I tracked population density over time rather than biomass because density is much easier to estimate accurately in my system. However, the prey-dependent model predicts that the growth rate of the bacteria population will increase with increasing glucose input, and faster growing bacterial cells tend to be larger (Bremer and Dennis 1987, Mongold and Lenski 1996). Thus, it is possible that the mass per bacterial cell could vary between my treatments, potentially affecting the accuracy of my estimates of equilibria. The relationship between cell mass and growth rate has been determined for my *E. coli* strain (Mongold and Lenski 1996), and the maximum growth rate difference possible between my treatments can be estimated from the prey-dependent model. Using these estimates, the cells in my 0.5 μg per ml treatment could be no more than 15% larger than the cells in the 0.1 μg per ml treatment. Even if I account for this potential cell mass difference in my estimates of equilibrium population densities of *E. coli*, the difference in equilibria between treatments and the significance of this difference increases only slightly. In addition, this slight increase in mass per cell would likely be offset by a concomitant increase in the adsorption rate of bacteriophage to the cell (because the cell would also be larger in volume and would thus be a bigger "target").

The third possible explanation is that this difference is due to the spatial heterogeneity present in my system. Recent research (Schrag and Mittler 1996)

suggests that even in well-mixed chemostats, growth of *E. coli* on the vessel wall can shelter *E. coli* cells from predation by bacteriophage. Wall growth was not visible in my chemostats, but the growth need not be dense to make an impact. Other researchers have demonstrated theoretically (Abrams and Walters 1996) that the presence of prey refuges can lead to increases in equilibrium prey density in response to enrichment. Thus, the presence of such a physical refuge from predation could explain the slight increase in the equilibrium density of the sensitive prey population at higher resource inputs. The presence of such a refuge is ignored by my prey-dependent model; however, the ratio-dependent model is presumed to capture the effects of such heterogeneity in the ratio-dependent functional response (Arditi et al. 1991b, Arditi and Saiah 1992).

While the prey-dependent model accurately predicts many qualitative effects of resource enrichment not predicted by the ratio-dependent model, the quantitative agreement between the prey-dependent model and my empirical observations is far from perfect. For example, the equilibrium density of the predator population increases to a greater extent than is predicted by the prey-dependent model. According to the prey-dependent model, the five-fold experimental increase in the resource supply rate should have produced a three-fold increase in the equilibrium density of the predator population, whereas I observed an increase of about thirteen-fold. And while the prey-dependent model predicts oscillations in prey and predator densities, as I observed, the model also predicts that these oscillations should be of increasing amplitude, leading to eventual extinction of one or both populations, whereas I did not witness any such extinctions. These quantitative discrepancies could be explained by the spatial heterogeneity in my system. Schrag and Mittler (1996) have shown, both theoretically and empirically, that wall growth can stabilize the oscillations of sensitive bacteria and their viral predators. By reducing the

average vulnerability of the bacteria, and hence the average adsorption rate, wall growth might simultaneously increase the expected equilibrium density of both the prey and predator populations, as well as stabilize their interaction.

The ratio-dependent model did not provide a better quantitative fit to my experimental observations than the prey-dependent model. The ratio-dependent model predicted an approximately seven-fold increase in the prey and predator populations in response to enrichment; I observed an approximately 13-fold increase in predators and approximately a 1.5-fold increase in prey.

In summary, although the ratio-dependent model may have predicted the qualitative effects of enrichment on the equilibrium densities of predator and prey, it failed to predict the effects of enrichment on the stability of predator and prey populations, as well as the effects of enrichment on the evolution of prey defenses. A prey-dependent model altered to include temporal heterogeneity (in the form of a time delay) did a much better job, even though it ignored spatial heterogeneity in my system. But what of other forms of heterogeneity? For example, can the effects of heterogeneity in prey edibility be captured by the ratio-dependent model, as some have argued (Arditi et al. 1991a, Samelle 1994). Or is it better to alter a prey-dependent model to include this heterogeneity? I can address this question with my system because heterogeneity developed in prey edibility due to the evolution of T4-resistant *E. coli*.

Heterogeneity in Prey Edibility

The presence of heterogeneity in prey edibility has been recognized by a number of ecologists as a factor that could alter the response of a food chain to enrichment (McCauley et al. 1988, Leibold 1989, Abrams 1993, Kretzschmar et al. 1993, Samelle 1994, Abrams and Walters 1996, Leibold 1996, Polis and

Strong 1996). The addition of inedible (or less edible) individuals to a prey population has been shown theoretically to result in a shift in population regulation, from limitation primarily by predators to limitation primarily by resources (Leibold 1989, Abrams 1993, Leibold 1996). I observed this shift in my experimental system. The total *E. coli* population went from being primarily predator-limited to primarily resource-limited due to the evolution of T4-resistance.

The ratio-dependent model can easily capture the effect of heterogeneity in prey edibility (albeit phenomenologically) by changing the value of the coefficient in the functional response. In contrast, the prey-dependent model describes this heterogeneity mechanistically, but it requires the inclusion of a separate equation with multiple parameters to accomplish this. The prey-dependent and ratio-dependent models make numerous distinct predictions with respect to the effects of heterogeneity in prey edibility on the dynamics of predators and prey. In almost all respects, the predictions of the prey-dependent models were fulfilled by my experimental system, whereas those of the ratio-dependent model were not.

Qualitatively, both models predicted that the equilibrium density of the *E. coli* population would increase following invasion by T4-resistant *E. coli*; however, only the prey-dependent model predicted that the total *E. coli* population would increase in stability following invasion. Both models predicted that the equilibrium density of T4 would decrease following invasion by T4-resistant *E. coli*; however, only the prey-dependent model predicted that the T4 population would continue to cycle. Moreover, the prey-dependent model accurately predicted that the oscillations of the T4 population would increase in period in response to invasion. Quantitatively, both models adequately predicted the total population density of *E. coli* as well as the relatively high stability of the

heterogeneous population. However, the prey-dependent model adequately predicted the equilibrium density of T4 , while the ratio-dependent model underestimated the equilibrium density by orders of magnitude.

As was the case for temporal and spatial heterogeneity, the ratio-dependent model predicted the qualitative effects of heterogeneity in prey edibility on equilibrium population density, but it failed to predict the effects of heterogeneity in edibility on population stability. The prey-dependent model (altered to include heterogeneity in edibility) was superior, predicting not only the effect of heterogeneity on equilibria and stability, but even predicting the effect of heterogeneity in edibility on the period of population cycles. However, the prey-dependent model was much more complex, requiring not only additional parameters but an additional equation.

Mechanistic Realism versus General Applicability

Since it was first proposed by Arditi and Ginzburg, the idea that ratio-dependent models could parsimoniously model the effects of heterogeneity on predator-prey dynamics has been hotly debated. This debate has centered on whether the effects of heterogeneity could indeed be captured by such a simple model, and what the limitations of using this approach might be. Using a laboratory model system, I have demonstrated that although some qualitative effects of heterogeneity may be captured by a ratio-dependent model, this approach overlooks a number of important aspects of predator-prey dynamics. In particular, the effects of resource enrichment on population stability and evolution were not predicted by the ratio-dependent models. In contrast, the prey-dependent models did a superior job of predicting the response of my model system to enrichment, but at the cost of simplicity. The prey-dependent models

are more complex than the ratio-dependent models and require detailed information about the communities of interest. It is certainly not possible always (perhaps even usually) to develop a fully mechanistic model of complex communities. Ratio-dependent models may be very useful, for example, to managers concerned with the effects of resource enrichment on the structure of complex communities. While ratio-dependent models may be sold as able to predict equilibrium responses in complex communities, they should also come with the explicit warning that certain complications may be missed (e.g., unstable equilibria) by virtue of the lack of mechanistic realism.

CHAPTER 3

EFFECT OF HETEROGENEITY IN PREY EDIBILITY ON THE RESPONSE OF A MODEL FOOD CHAIN TO RESOURCE ENRICHMENT

INTRODUCTION

Ecologists have long debated the relative importance of population regulation by resources (bottom-up control) and population regulation by predators (top-down control) (Hairston et al. 1960, Murdoch 1966, Ehrlich and Birch 1967, Slobodkin et al. 1967). This debate has recently focused 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.

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: (1) a homogeneous and highly edible population and (2) 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 can also have a profound effect on how a predator population is regulated. Less edible prey can essentially "siphon off" resources that would otherwise be eventually converted 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 making 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). 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, Leibold 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, due to the tradeoff between edibility and competitiveness(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 extinct ("apparent competition" sensu Holt 1977). However, this cannot occur if the less edible prey is completely inedible (Levin et al. 1977). A "chemostat-like" environment (e.g., constant volume, continuous input of resources, etc.) 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 consistently observed only when higher trophic levels (i.e., predators of *Daphnia* and their respective predators) are present, suggesting that these responses may be due not 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, I demonstrate that heterogeneity in prey edibility can alter the balance between bottom-up and top-down forces. I 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. The results are compared with the predictions of two alternative mathematical models that are constructed using different approaches to modeling heterogeneity. My experimental system consisted of populations of the bacterium *Escherichia coli* and the bacteriophage T4 (a virus that feeds on *E. coli*) interacting in chemostats. I 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 to predation by T4. Bacteria and bacteriophages have been proposed as ideal experimental systems for studying predator-prey dynamics (Campbell 1961, Lenski and Levin 1985) and have been successfully used as such by a number of researchers (Paynter and Bungay 1969, Horne 1970, Paynter and Bungay 1971, Chao et al. 1977, Levin and Lenski 1983, Lenski and Levin 1985, Lenski 1988a).

The use of laboratory model communities such as mine has several advantages. I am able to use organisms with short generation times, so that

steady state responses to enrichment are achieved relatively quickly. I am able to unambiguously quantify 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 rigorously examined 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

My experimental system consisted of *E. 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. REL6584 is identical to REL607 with the exceptions that (1) it is resistant to predation by bacteriophage T4, and (2) it cannot utilize the sugar arabinose. The ability to utilize arabinose has been previously shown to confer neither a competitive advantage nor disadvantage in a glucose-limited environment (Lenski 1988a). I used this trait as a neutral marker to distinguish the two *E. coli* strains. T4-resistant mutants of E. coli have been shown to be completely invulnerable to predation by T4 (Lenski

and Levin 1985). 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 1988a). 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 1988a).

I 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. I calculated the competitive disadvantage as described by Lenski and Levin (1985). The disadvantage was approximately 35% for REL6584. I also checked the neutrality of the arabinose-utilization marker by coinoculating REL607 and REL606 into glucose-limited chemostats. REL606 is the T4-sensitive progenitor of REL6584; it is identical to REL607 with the exception of its inability to utilize arabinose. I detected no effect of the arabinose-utilization marker on competitive ability.

My 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^{-6} g thiamine hydrochloride per liter and either 0.1 or 0.5 μg per ml glucose. These glucose concentrations were chosen so that my results could be compared to previous experiments at these concentrations (see Chapter 2 above). The volume of the chemostats was maintained at approximately 30 ml, the flow rate at approximately 0.2 turnovers per hour 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 hours 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. REL607 cells were plated on Davis minimal agar supplemented with 2×10^{-6} g thiamine hydrochloride per liter and 4 mg per ml arabinose (this media 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 using Davis minimal agar and the plate count technique described by Carlson and Miller (1994). REL6584 cells were plated on Davis minimal agar supplemented with 2×10^{-6} g thiamine hydrochloride per liter and 4 mg per 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, I treated each chemostat as a single observational unit. I first calculated the mean and standard deviation of the T4 and *E. coli* population densities over time for each chemostat. I then estimated the equilibrium density of each population as the grand arithmetic mean of population density across replicate chemostats. I had previously determined that the arithmetic mean is superior to the geometric mean as an estimator of equilibria in my system (see Chapter 2 above). I estimated stability as the mean coefficient of variation of population densities across replicate chemostats (the higher the coefficient of variation, the lower the stability). I excluded the first six time points after inoculation of REL6584 from my calculations of equilibria and stability, to allow time for the populations to reach equilibria.

I compared population equilibria and stability between the resource treatments with *t*-tests. One-tailed comparisons were used whenever the models made directional predictions. Prior to comparison I tested for homogeneity of variances. The data was 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.

I tracked population density over time rather than biomass because density is much easier to accurately estimate in my system. I 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 either mathematical model described below.

Mathematical models

There is a great deal of controversy over how best to mathematically model food chains with heterogeneous prey. Two approaches have received a great deal of attention recently. The first approach is to modify traditional prey-dependent models (i.e., Lotka-Volterra models and variations thereof) to incorporate such heterogeneity. These models are considered "prey-dependent" because they assume that the attack rate of predators depends only on the instantaneous density of prey. Heterogeneity is incorporated into these models by additional terms that explicitly describe the heterogeneous population. An alternative approach is to use ratio-dependent predator-prey models. 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 asserted that this ratio

incorporates the "net effect" of heterogeneity on population dynamics (Arditi and Ginzburg 1989). The ratio-dependent approach is simpler mathematically than the prey-dependent approach; however, this simplicity may come at the cost of mechanistic realism. In an attempt to explore these possible tradeoffs, I modeled my experimental system using both the prey-dependent and ratio-dependent approaches.

I modeled my experimental system using the models first developed by Levin et al. (1977). The prey-dependent 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 = flowrate, 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, 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 $t - \tau$, P' = population size of T4 at time $t - \tau$, R = population size of inedible *E. coli*, ε_R = reciprocal of the yield of the inedible *E.*

coli, ψ_R = maximum specific growth rate of inedible *E. coli*, and K_R = resource concentration at which inedible *E. coli* grow at one half ψ_R.

I used the following parameter values for this model : C_O = either 0.1 or 0.5 μg per ml, $\omega = 0.2$ per hr, $\varepsilon = 2 \times 10^{-6}$ μg (Lenski 1988b), $\psi = 0.7726$ per hr (Vasi et al. 1994), $K = 0.0727$ μg per ml (Vasi et al. 1994), $\alpha = 3 \times 10^{-7}$ ml per hr (Lenski and Levin 1985), (Power 1992) , $\beta = 80$ viruses per bacterial cell infected (Lenski and Levin 1985), $\tau = 0.6$ hr (Lenski and Levin 1985), and $\varepsilon_R = 2 \times 10^{-6}$ μ. I estimated ψ_R (= 0.7027 per hr) and K_R (= 0.123 μg per ml) as described in Chapter 2 above.

The ratio-dependent model consisted of three differential equations,

$$dC/dt = (C_0 - C)\omega - \varepsilon B\psi C/(K+C)$$
$$dB/dt = B\psi C/(K+C) - \alpha(B/P)P - \omega B$$
$$dP/dt = \beta\{\alpha(B/P)P\} - \alpha(B/P)P - \omega P$$

where in addition to the above parameters, B = the total population size of uninfected edible and inedible *E. coli*. This model therefore combines the T4-sensitive and T4-resistant *E. coli* into one population that is heterogeneous in edibility. I fit the ratio-dependent trophic function (i.e., $\alpha B/P$) to estimates of equilibria from previously published observations (Lenski and Levin 1985) of coexisting populations of T4-sensitive *E. coli*, T4-resistant *E. coli*, and T4. These observations were made in chemostats with glucose input concentrations of 300 μg per ml, and they gave an estimated value for α of 2.28×10^{-5} per hour. I used the average of ψ and ψ_R above (0.7701 per hour) as the value for ψ in the ratio-dependent model and the average of K and K_R above (0.0986 μg per ml) as the value of K. Proponents of ratio-dependent models have argued 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. All other parameters were the same as in the prey-dependent model.

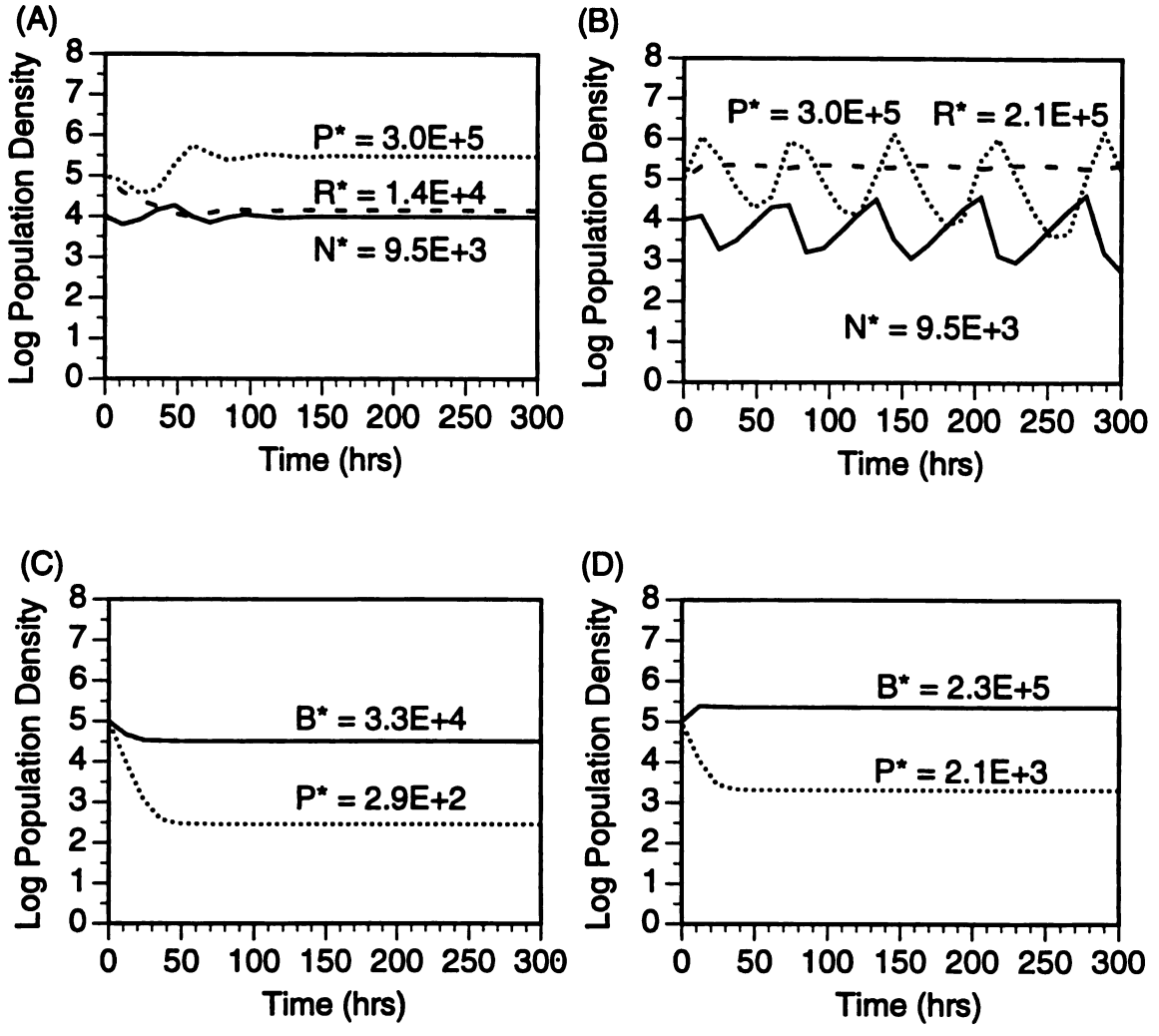
I solved these models analytically and examined the behavior of the models numerically using STELLA II simulation software (High Performance Systems 1994). A time-step of 0.05 hours was used in the simulations. However, I "sampled" the output of each simulation every 12 hours (the approximate sampling interval of my experiments) to produce the dynamical predictions shown in Figure 8.

RESULTS

Model Predictions

The prey-dependent model predicts that the equilibrium density of the inedible *E. coli* will increase in response to enrichment (Figure 8A and B). It also predicts that the equilibrium densities of the edible *E. coli* and the predator will be unaffected by resource enrichment. In contrast, 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 (Figure 8C and D). The prey-dependent model also predicts that the edible prey and predator populations will be destabilized (i.e., the amplitude of oscillations will increase) by enrichment (Figure 8A and B). The ratio-dependent model does not make this prediction (Figure 8C and D).

Figure 8. Population equilibria and dynamics predicted by prey-dependent and ratio-dependent food chain models. Equilibria are from analytical solutions of the models; dynamics are from numerical simulations of the models, "sampled" at 12 hour intervals. The population densities (viruses per ml or bacteria per ml) have been log-transformed. (A) prey-dependent model with a glucose input concentration of 0.1 μg per ml , (B) prey-dependent model with a glucose input concentration of 0.5 μg per ml , (C) ratio-dependent model with a glucose input concentration of 0.1 μg per ml , (D) ratio-dependent model with a glucose input concentration of 0.5 μg per ml . Key: R^* = inedible *E. coli* equilibrium density, N^* = edible *E. coli* equilibrium density, P^* = T4 equilibrium density, B^* = total *E. coli* equilibrium density; solid line = edible *E. coli* or total *E. coli* dynamics, dashed line = inedible *E. coli* dynamics, dotted line = T4 dynamics.


(A)
Log Population Density
P* = 3.0E+5
R* = 1.4E+4
N* = 9.5E+3
Time (hrs)
(B)
Log Population Density
P* = 3.0E+5
R* = 2.1E+5
N* = 9.5E+3
Time (hrs)
(C)
Log Population Density
B* = 3.3E+4
P* = 2.9E+2
Time (hrs)
(D)
Log Population Density
B* = 2.3E+5
P* = 2.1E+3
Time (hrs)

Empirical Observations

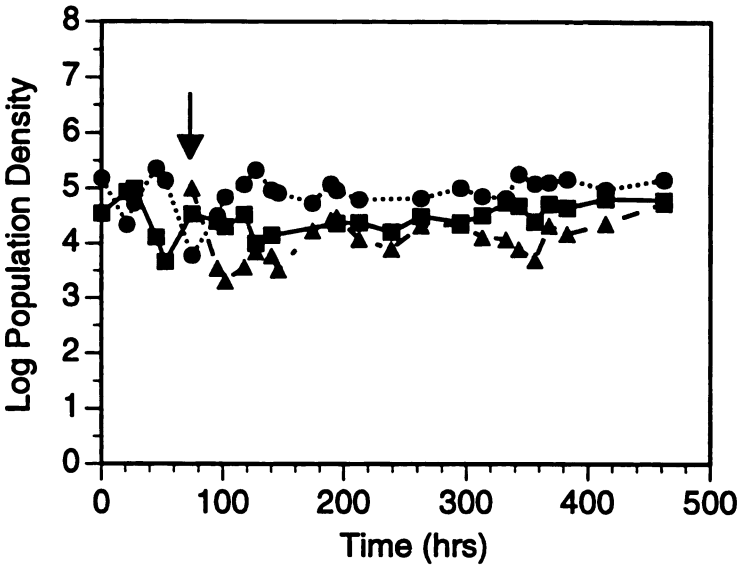
The dynamics of the T4 and *E. coli* populations are shown in Figure 9 for representative chemostats with two different input concentrations of glucose. The populations persisted in all chemostats with apparent population cycles. There was a large and highly significant increase in the equilibrium density of the inedible *E. coli* in response to enrichment ($t = 15.238$, $df = 4$, one-tailed $P < 0.0001$; Figure 10A). The equilibrium density of the edible E. coli did not change significantly in response to enrichment (Welch's approximate $t = 0.4976$, $df = 2$, one-tailed $P = 0.3340$; Figure 10A) nor did the predator population ($t = 1.2421$, $df = 4$, one-tailed $P = 0.1410$; Figure 10A). I 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 (see below).

Enrichment significantly destabilized the edible *E. coli* ($t = 2.4503$, $df = 4$, one-tailed $P = 0.0352$; Figure 10B), and the predator population ($t = 2.7991$, $df = 4$, one-tailed $P = 0.0244$; Figure 10B). The inedible *E. coli* became more stable with enrichment ($t = 7.0875$, $df = 4$, one-tailed $P = 0.0010$; Figure 10B). The population oscillations of the edible *E. coli* in the higher glucose treatment appeared to be damped in Figure 9B and could possibly 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) were essentially identical to those reported earlier for edible *E. coli* and T4 (see Chapter 2 above). Enrichment appeared to destabilize both the *E. coli* and T4 populations in these chemostats. Enrichment also resulted in a large increase in the equilibrium population density of T4 (approximately fourteen-fold) and a very small increase in *E. coli* (approximately 1.5-fold). *E. coli* and T4

Figure 9. Dynamics of edible *E. 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 ml or bacteria per ml) have been log-transformed. (A) 0.1 μg/ml glucose, (B) 0.5 μg/ml glucose.

(A)


Log Population Density
Time (hrs)
0
1
2
3
4
5
6
7
8
0
100
200
300
400
500

(B)

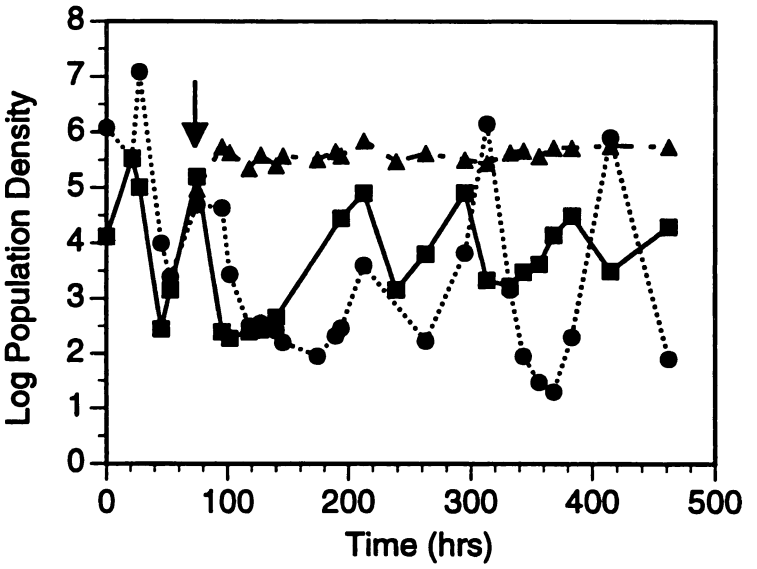
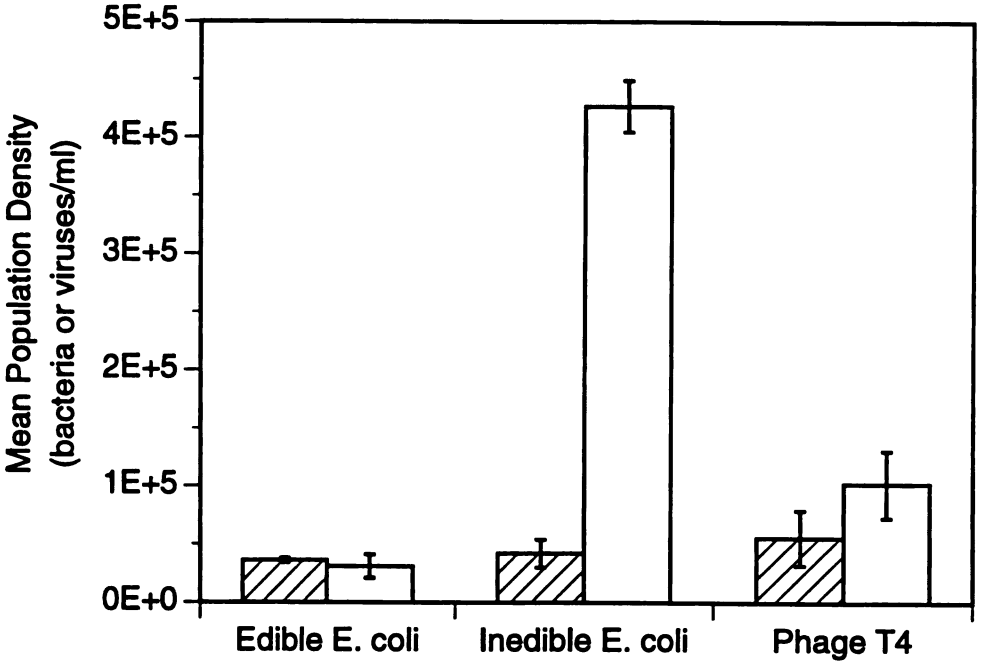
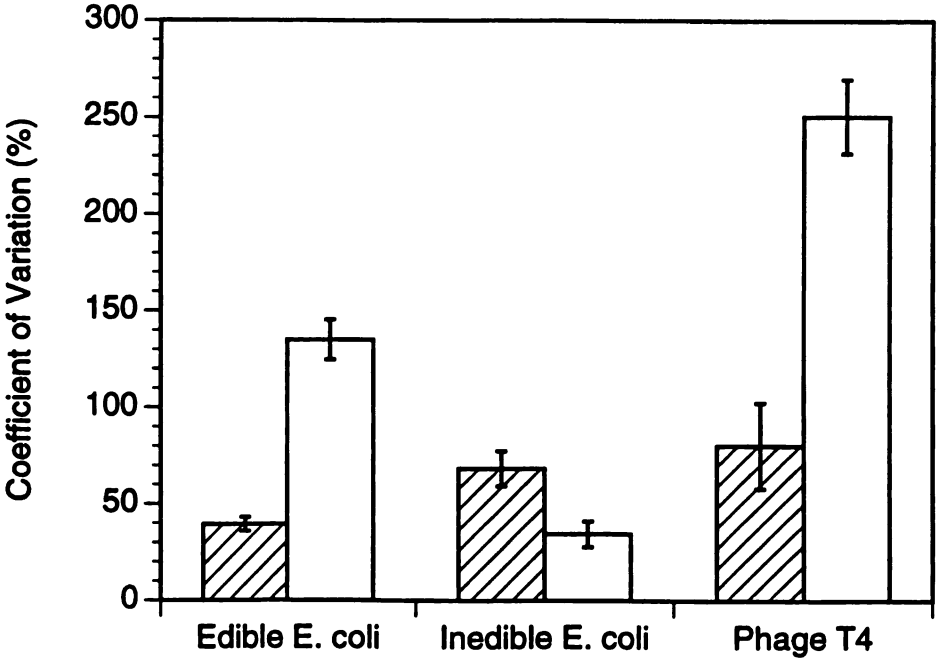

Log Population Density
Time (hrs)
0
1
2
3
4
5
6
7
8
0
100
200
300
400
500

Figure 10. Effect of glucose input concentration on equilibrium population densities and instability of edible *E. 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 3 replicate chemostats. Population instability is estimated as the mean of the coefficient of variation in 3 replicate chemostats. Error bars are standard error of the mean. (A) equilibrium density, (B) instability.

(A)


Mean Population Density
(bacteria or viruses/ml)
5E+5
4E+5
3E+5
2E+5
1E+5
0E+0
Edible E. coli
Inedible E. coli
Phage T4

(B)


Coefficient of Variation (%)
300
250
200
150
100
50
0
Edible E. coli
Inedible E. coli
Phage T4

persisted in the control chemostats in apparent population cycles until the appearance of, and subsequent invasion by, T4-resistant mutants of *E. coli*. T4-resistant *E. coli* mutants appeared much sooner in the higher glucose treatment than in the lower glucose treatment. Following invasion by the T4-resistant *E. coli* mutants, the T4 population continued to persist in cycles.

Fit of observations to mathematical models

Qualitatively, the responses of the predator and prey populations to enrichment were better predicted by the prey-dependent model than the ratio-dependent model. In response to enrichment: (1) the equilibrium density of the inedible *E. coli* increased, (2) the equilibrium density of the edible *E. coli* was unchanged, (3) the equilibrium density of the bacteriophage T4 population was also unchanged, (4) the stability of the edible *E. coli* and bacteriophage T4 populations decreased and (5) the stability of the inedible *E. coli* population increased.

DISCUSSION

The relative importance of population regulation by resources (bottom-up control) and population regulation 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, Abrams 1993, Leibold 1996). 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 clearly linked to processes. In this study, I have clearly demonstrated 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 equilibrium density of my model heterogeneous prey population (edible and inedible *E. coli*) increased strongly in response to an enrichment of its resources (Figure 10A). In contrast, the model prey population without heterogeneity (edible *E. coli* only) increased only marginally, if at all (Figure 6A). The model predator population (bacteriophage T4) did not significantly increase when its prey were heterogeneous (Figure 10A), even though it increased strongly in response to enrichment when its prey were homogeneous (Figure 6A). The mechanism underlying this change in regulation can be seen vividly in Figure 10A. 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.

Enrichment significantly destabilized the edible *E. coli* and bacteriophage T4 populations in my model food chain (Figure 10B). The presence of inedible prey reduced the degree to which the food chain was destabilized by enrichment. The edible *E. coli* exhibited large and undamped oscillations when the inedible *E. coli* were absent (Figure 9B before arrow; also Figure 5B) but relatively small

population oscillations in the higher glucose treatment when the inedible *E. coli* were present (Figure 9B after arrow). The T4 population oscillations were also larger in the higher glucose treatment when the inedible *E. coli* were absent (Figure 9B before arrow; also Figure 5B) than when the inedible *E. coli* were present (Figure 9B before arrow). The presence of inedible *E. coli* also effected the evolutionary stability of my food chain. I did not detect inedible (i.e., T4-resistant) mutants of the edible *E. coli* when inedible *E. coli* were already present, but such mutants appeared consistently when inedible *E. coli* were absent.

Food chains with heterogeneous prey populations can be modeled in different ways. The prey-dependent approach involves building on traditional predator-prey models (i.e., Lotka-Volterra models and their derivatives) by adding an equation for each distinctly different subpopulation (e.g., each level of edibility), as well as terms that describe the interactions between these subpopulations. The ratio-dependent approach is much simpler; it involves changing the form of the predator's functional response from a function of the prey's density only, to a function of the ratio of prey to predator densities. This ratio is assumed to incorporate the "net effect" of heterogeneity on population dynamics. I used both of these approaches and found that the prey-dependent model better predicted the response of my model system to enrichment. The prey-dependent model was a better predictor of both changes in equilibrium density and changes in population stability. I believe that this occurred for several reasons. The first reason is that the prey population in my system had truly inedible individuals. Some ecologists have argued that the damping of top-down forces that results from the presence of "inedible" or "invulnerable" prey can be expressed as ratio-dependence (Sarnelle 1994). However, ratio-dependent models predict that both predator and prey will increase in response to enrichment, a pattern that I did not observe in my study. An increase in both

predator and prey could occur if less edible (but not inedible) prey are present (Abrams 1993, Leibold 1996). It is possible that ratio-dependent models could 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 my system (edible and inedible prey). Secondly, I have previously noted that ratio-dependent models tend to overestimate the stability of simple systems (see Chapter 2 above). This may explain the inability of these models to predict the changes in population stability that I observed in my 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, etc.) may lead to strong bottom-up control and possibly greater population stability (Polis and Strong 1996), and these could possibly be approximated by ratio-dependent models. However, trade-offs between simplicity and mechanistic realism need to be carefully considered before using the ratio-dependent approach (Diehl et al. 1993).

A third approach to modeling food chains with heterogeneous prey has also 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 using prey-dependent predator-prey equations and modeling the interactions between inedible prey and edible prey using 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 my observations. Enrichment is predicted to be destabilizing to edible prey and predators, the presence of inedible prey is predicted to reduce these destabilizing effects, 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 my observations.

CHAPTER 4

EFFECT OF PRODUCTIVITY ON THE IMPORTANCE OF APPARENT COMPETITION IN A MODEL COMMUNITY

INTRODUCTION

Most species are embedded in a web of interactions with other species. Changes in the abundance of one species can ripple through this web and indirectly effect other species. Although ecologists have recognized for nearly half a century that such indirect effects can occur (e.g., Andrewartha and Birch 1954), the comprehensive study of this phenomenon is relatively recent (Kerfoot and Sih 1987). Recent research has focused on two goals: 1) to clarify the types of indirect effects that occur in nature and 2) to determine the importance of indirect effects in community dynamics. Ecologists have been relatively successful in meeting the first goal, and numerous examples of indirect effects have been reported (see review in Wooton 1993). However, the second goal has been more difficult to accomplish, and identifying when and where indirect effects are important remains a challenge.

One of the best studied examples of indirect effects are those that occur between species that share a predator (Kerfoot and Sih 1987, Holt and Lawton 1994). Theoretically various interactions are possible between species that share a predator, even when the prey species do not interact directly. Changes

in the population density of one prey species may indirectly affect the instantaneous growth rate, equilibrium population size, existence and magnitude of population cycles, and evolution of prey defenses in the other prey species (Abrams 1987). Of these possible effects, a negative relationship between the equilibrium population sizes of prey has received the most attention in the literature (see reviews in Jeffries and Lawton 1984, Holt and Lawton 1994). Holt has labeled this type of indirect effect "apparent competition" (Holt 1977). If the prey species differ in vulnerability to predation, apparent competition can result in exclusion of the more vulnerable prey species. This effect occurs because the density of predators at equilibrium must be sufficiently high to prevent the less vulnerable prey type from increasing, and that same density of predators may drive the more vulnerable prey type to extinction.

Several theorists have hypothesized that the importance of apparent competition should vary with productivity (Holt et al. 1994, Leibold 1996). For example, imagine a simple community consisting of two prey species competing for a common resource and sharing a common predator. Theory predicts the following response of this simple community to productivity. At high productivity apparent competition will be the primary determinant of community structure, with the less vulnerable prey excluding the more vulnerable prey. At low productivity apparent competition will be relatively unimportant and exploitative competition for resources will be the primary determinant of community structure, with the superior competitor excluding the inferior competitor. At moderate levels of productivity neither apparent nor exploitative competition are predicted to be the primary influence on community structure, and both prey types can coexist if a trade-off exists between competitive ability and vulnerability to predation. Thus, the relative importance of apparent competition in this simple community is controlled by productivity.

Although the influence of productivity on apparent competition has been theorized, this idea has not been tested experimentally. In this chapter, I report a test of this theory using a laboratory model community. Apparent competition has been shown to occur in laboratory communities of protozoan predators and protozoan prey (Lawler 1993), protozoan predators and prey bacteria (Nakajima and Kurihara 1994a, Nakajima and Kurihara 1994b), and bacteriophage and bacteria (Levin et al. 1977, Lenski 1984). However, the effect of changes in productivity on the importance of apparent competition has not previously been demonstrated in laboratory model communities. In previous work, I used laboratory model communities of *E. coli* and bacteriophage to determine the effect of changes in productivity on the population dynamics in simple food chains (see Chapter 2) and in communities with keystone predators (see Chapter 3). In this chapter, I expand on my earlier work and use a chemostat community of *E. coli* and bacteriophage T2 to test a mathematical model of competition between prey types with a shared predator. I assembled communities that consisted of bacteriophage T2 and two *E. coli* strains that differed in their susceptibility to this bacteriophage. I manipulated the productivity of this model community and compared the observed responses to the predictions of a mathematical model that includes both actual competition for resources and apparent competition through a shared predator.

Chemostat communities of *E. coli* and bacteriophage T2 are excellent model systems with which to test shared predator models for a number of reasons. First, T2 is one of the few bacteriophages against which *E. coli* has been observed to evolve *partial* resistance (i.e., reduced vulnerability). *E. coli* can evolve complete resistance (i.e., invulnerability) to many bacteriophages; however, partial resistance is rare. Because partial resistance is possible, model communities that consist of prey populations that differ in susceptibility to a

shared predator can be constructed using *E. coli* and bacteriophage T2. Second, *E. coli* mutants that are partially resistant to bacteriophage T2 have the correlated trait of complete resistance to bacteriophage T4. This relationship allows the partially resistant mutants to be easily obtained (by selecting for T4-resistant mutants) and easily tracked in a model community (by screening for T4-resistant bacteria). Third, chemostat communities of *E. coli* and bacteriophage T2 share the advantages of other microbial model systems. These advantages include the ease with which experimental variables such as productivity can be manipulated and the relatively short time period necessary to observe steady state responses to changes such as increased productivity.

METHODS

Experimental system

My experimental system consisted of *E. coli* B strain REL607 (Lenski et al. 1991), *E. coli* B strain REL6584, and the virulent bacteriophage T2 (kindly provided by L. Snyder) in glucose-limited chemostats. REL6584 is identical to REL607 with the exceptions that (1) it cannot utilize the sugar arabinose, and (2) it is invulnerable to predation by bacteriophage T4. The ability to utilize arabinose has been previously shown to confer neither a competitive advantage nor disadvantage in a glucose-limited environment (Lenski 1988a). I used this trait as a neutral marker to distinguish the two *E. coli* strains. Invulnerability to predation by T4 has been shown to result in a competitive disadvantage in a glucose-limited environment when phage is not present (Lenski and Levin 1985, Lenski 1988a). Mutants of *E. coli* that are invulnerable to predation by T4 have

also been shown to be less vulnerable to predation by bacteriophage T2 (Lenski 1984). This effect occurs because T4-invulnerable mutants of *E. coli* achieve invulnerability through the loss of the cell surface receptor to which T4 initially attaches (Lenski 1988a). This cell surface receptor is also one of two receptors used for attachment by T2 (Lenski 1984). The loss of this receptor reduces the rate at which T2 infects *E. coli* by approximately 50% (Lenski 1984). Thus, mutants of *E. coli* that have reduced vulnerability to T2 can be detected by screening for the correlated trait of invulnerability to T4 .

I measured the competitive disadvantage associated with reduced vulnerability to T2 by co-inoculating REL6584 and REL607 into phage-free, glucose-limited chemostats and tracking their respective population densities. I calculated the competitive disadvantage as described by Lenski and Levin (1985). The disadvantage was approximately 35% for REL6584. I also checked the neutrality of the arabinose-utilization marker by co-inoculating REL607 and REL606 into glucose-limited chemostats. REL606 is the T4-sensitive progenitor of REL6584; it is identical to REL607 with the exception of its inability to utilize arabinose. I detected no effect of the arabinose-utilization marker on competitive ability.

My 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^{-6} g thiamine hydrochloride per liter and various concentrations of glucose (see below). The volume of the chemostats was maintained at approximately 30 ml, the flow rate at approximately 0.2 turnovers per hour and the temperature at 37°C. Replicate chemostats at each glucose concentration were maintained simultaneously. The chemostats were inoculated with the vulnerable prey (*E. coli* strain REL607), the less vulnerable prey (*E. coli* strain REL6584), and the predator (bacteriophage T2) simultaneously. Control

chemostats, containing each of the *E. coli* strains alone with the predator, were established at each glucose concentration and maintained simultaneously with the treatment chemostats.

I manipulated productivity in this experiment by running replicate chemostats at different input concentrations of glucose. Each glucose input concentration represented a different level of productivity. Three blocks of chemostats were run. The first block consisted of two replicates with a glucose input concentration of 0.1 μg per ml, two replicates with a glucose input concentration of 0.5 μg per ml, and four control chemostats. These resource levels were chosen so that my results could be compared to previous experiments at these concentrations (see Chapters 2 and 3). The second block consisted of two replicates with a glucose input concentration of 0.09 μg per ml, two replicates with a glucose input concentration of 0.5 μg per ml, and four control chemostats. The third block consisted of two replicates with each of the following glucose input concentrations: 0.07, 0.08, 0.09, 0.10, 0.11, and 0.12 μg per ml, and four control chemostats. The productivity levels in blocks two and three were chosen to explore productivity levels slightly lower and slightly higher than 0.1 μg per ml, in an effort to detect the transition between coexistence and exclusion. I ran blocks 1 and 2 for 200 hours and block 3 for 100 hours.

The population densities of the *E. coli* strains and phage T2 were estimated twice daily in blocks 1 and 2, and daily in block 3. Population densities were estimated by dilution and plating. REL607 cells were plated on Davis minimal agar supplemented with 2 $x10^{-6}$ g thiamine hydrochloride per liter and 4 mg per ml arabinose (this media 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 T2 was plated on a lawn of REL607

using Davis minimal agar and the plate count technique described by Carlson and Miller (1994). REL6584 cells were plated on Davis minimal agar supplemented with 2 $x10^{-6}$ g thiamine hydrochloride per liter and 4 mg per ml glucose; a concentrated phage T4 lysate was mixed with each sample to kill REL607 cells prior to plating.

In past laboratory studies, the vulnerability of *E. coli* to predation by T2 has been observed to change due to evolution (Lenski 1984). Therefore, in each chemostat I tracked the evolution of mutants with reduced vulnerability and complete invulnerability to T2. I estimated the total population density of mutants invulnerable to T2 in each chemostat by mixing concentrated phage T2 lysate with an aliquot of each chemostat sample and plating on minimal glucose. The fraction of these mutants derived from REL607 was estimated by mixing concentrated phage T2 lysate with an aliquot of each sample and plating on minimal arabinose (REL6584 cannot grow on minimal arabinose media). The fraction of the invulnerable mutants derived from REL6584 was then determined by subtraction of the REL607-derived mutants from the total. As described above, invulnerability to predation by phage T4 can be used as a marker for detecting partial invulnerability to phage T2. I estimated the density of mutants of REL607 that were partially invulnerable to T2 by mixing concentrated phage T4 lysate with an aliquot of each sample and plating on minimal arabinose. This media allows the growth of both partially invulnerable and completely invulnerable mutants; I estimated the density of partially invulnerable mutants by subtracting the density of invulnerable mutants (estimated as described above) from the total.

Mathematical models

I modeled my experimental system using modifications of the models developed by Levin et al. (1977). I analyzed these models graphically and examined the behavior of the models numerically using STELLA II simulation software (High Performance Systems 1994).

Mathematical model. The model consisted of four coupled differential equations,

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

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

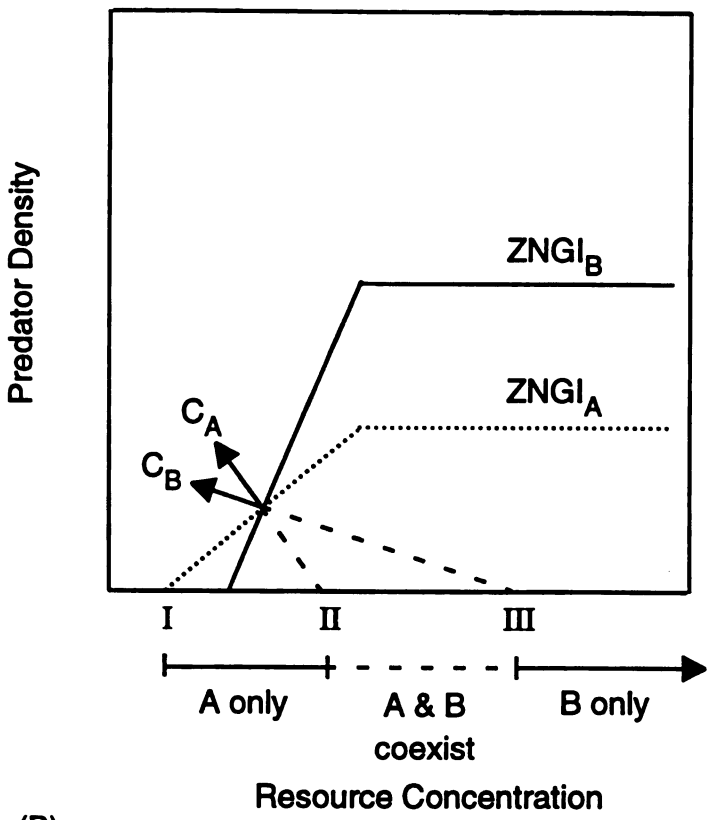
$$dR/dt = R\psi_R C/(K_R + C) - \alpha_R RP - \omega R$$

$$dP/dt = \beta e^{-\tau\omega}(\alpha N'P') + \beta_R e^{-\tau R\omega}(\alpha_R R'P') - \alpha NP - \alpha_R RP - \omega P$$

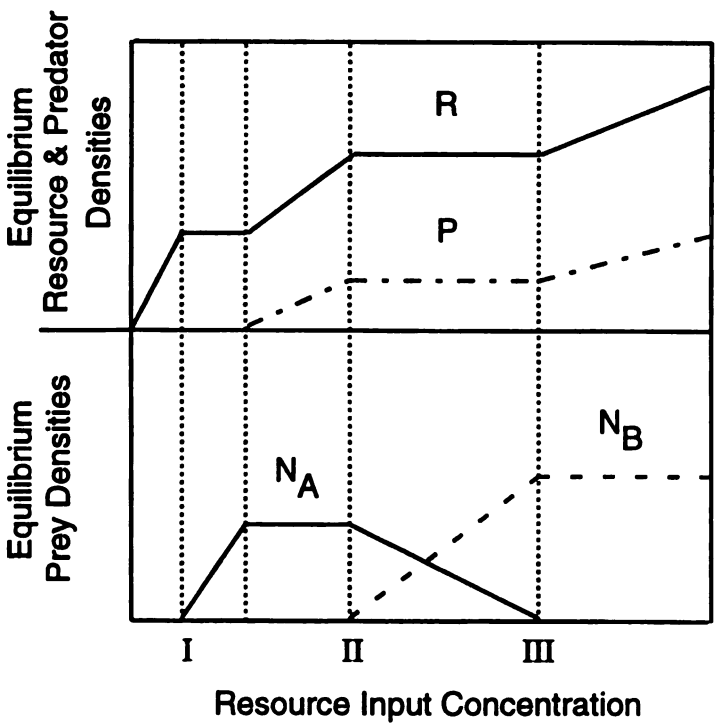
where C_O = concentration of glucose in the reservoir, C = concentration of glucose in the chemostat, ω = flowrate, ε = reciprocal of the yield of the bacteria, N = population density of the more vulnerable bacteria, R = population density of the less vulnerable bacteria, P = population density of the bacteriophage, ψ = maximum specific growth rate, K = resource concentration at which the bacteria grow at one half ψ, $\alpha(N)$ = trophic function, β = burst size of the bacteriophage, τ = latent period of the phage, $e^{-\tau\omega}$ = fraction of bacteria infected at time $t - \tau$ that has not washed out before lysing, N' = population density of the more vulnerable bacteria at time $t - \tau$, P' = population density of bacteriophage at time $t - \tau$, and R' = population density of less vulnerable bacteria at time $t - \tau$. Parameters that are specific to the less vulnerable population are followed by the subscript R. Note that equations describing the dynamics of the infected bacteria could also be written. Such equations were not included in this model because infected

Figure 11. Effect of productivity on the density of two prey types, a shared predator, and a shared resource. (A) Graphical analysis of an idealized community model (modified from Leibold, 1996) showing zero net growth isoclines (ZNGI's) and net consumer-prey impact vectors (C) for two prey types (A and B). Coexistance is possible because the ZNGI's intersect. The zone of coexistance is determined by the slope of the impact vectors and their intersection with the resource axis. (B) The predicted pattern of equilibrium population densities across a gradient of productivity (modified from Leibold, 1996). Productivity is assumed to be proportional to resource input concentration. The resource levels labeled I, II and III are identical to those in panel A. R = equilibrium resource concentration, P = equilibrium predator density, N_A = equilibrium density of more vulnerable prey, N_B = equilibrium density of less vulnerable prey.

(A)


Predator Density
ZNGI$_B$
ZNGI$_A$
C_A
C_B
I
II
III
A only
A & B coexist
B only
Resource Concentration

(B)


Equilibrium Resource & Predator Densities
R
P
Equilibrium Prey Densities
N_A
N_B
I
II
III
Resource Input Concentration

bacteria cannot be easily tracked in the chemostats. The inclusion of infected cell populations has very little effect on the dynamics of the other populations (none if infected cells neither consume resources nor adsorb additional phage).

I used the following parameter values for this model : C_O = either 0.1 or 0.5 μg per ml, $\omega = 0.2$ per hr, $\varepsilon = 2 \times 10^{-6}$ μg (Lenski 1988b), $\psi = 0.7726$ per hr (Vasi et al. 1994), $K = 0.0727$ μg per ml (Vasi et al. 1994), $\alpha = 2 \times 10^{-7}$ ml per hr (Lenski 1984), $\beta = 98$ viruses per bacterial cell infected (Levin et al. 1977), $\tau = 0.5$ hr (Levin et al. 1977), $\alpha_R = 1 \times 10^{-7}$ ml per hr (Lenski 1984), $\varepsilon_R = 2 \times 10^{-6}$μg (Chapter 3), $\psi_R = 0.7027$ per hr (Chapter 3) and $K_R = 0.123$ μg per ml (Chapter 3).

Graphical analysis. I analyzed the model graphically as described by Leibold (1996). This approach consists of plotting zero net growth isoclines and net consumer-prey impact vectors for both prey types (Figure 11A). The relationship between the impact vectors and the isoclines determines the overall relationship between community structure and productivity (Figure 11B). I calculated the net consumer-prey impact vectors for each prey type in my experimental system by taking the vector sum of the per capita feeding rate of each prey type (calculated as $\varepsilon\psi C/\{K + C\}$ or $\varepsilon_R\psi_R C/\{K_R + C\}$) and the per capita contribution of each prey type to predator growth (calculated as $\beta\{\alpha P\} + \beta_R\{\alpha_R P\}$) as described by Leibold (1996).

Numerical simulations. I ran all numerical simulations using a time step of 0.05 hours. I tested the sensitivity of the simulations to time step size by running replicate simulations at step sizes of 0.1, 0.05 and 0.025 hours. Varying the size of the time steps had no detectable effect on the results of the simulations. I "sampled" the output of each simulation every 12 hours (the approximate sampling interval of my experiments) to produce the predictions depicted graphically.

RESULTS

Model predictions

The graphical analysis of the models revealed that coexistence of both prey types would occur only within a very small range of productivity levels (Figure 12). The net consumer-prey impact vectors for the two types were very similar in slope (2.85×10^7 viruses/μg glucose for the more vulnerable prey; 1.99×10^7 viruses/μg glucose for the less vulnerable prey) resulting in predicted coexistence only within the range of 0.157 μg per ml glucose to 0.178 μg per ml glucose. Below this range, the more vulnerable prey were predicted to displace the less vulnerable prey. Above this range, the less vulnerable prey were predicted to displace the more vulnerable prey.

Numerical simulations of the model are presented in Figure 13. The two input concentrations I chose for this experiment are predicted to lie on either side of the narrow range of coexistence as described above. In chemostats with an input glucose concentration of 0.1 μg per ml, the more vulnerable prey is predicted to displace the less vulnerable prey (Figure 13A). In chemostats with an input glucose concentration of 0.5 μg per ml, the less vulnerable prey is predicted to displace the more vulnerable prey (Figure 13B). These results are due solely to the indirect interactions between the *E. coli* strains, because both *E. coli* strains are predicted to persist for the duration of the experiment at both glucose input concentrations when the other competitor is removed (Figure 14).

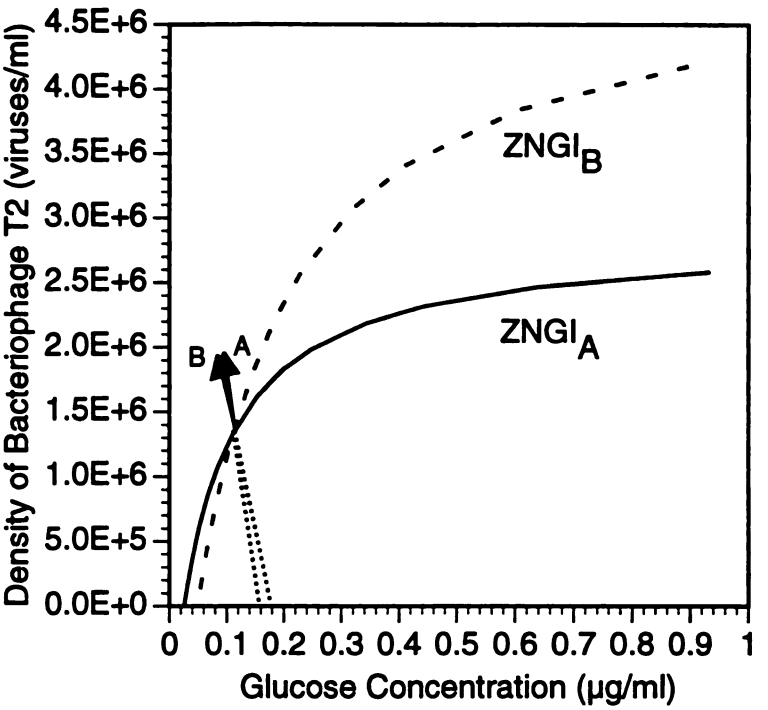
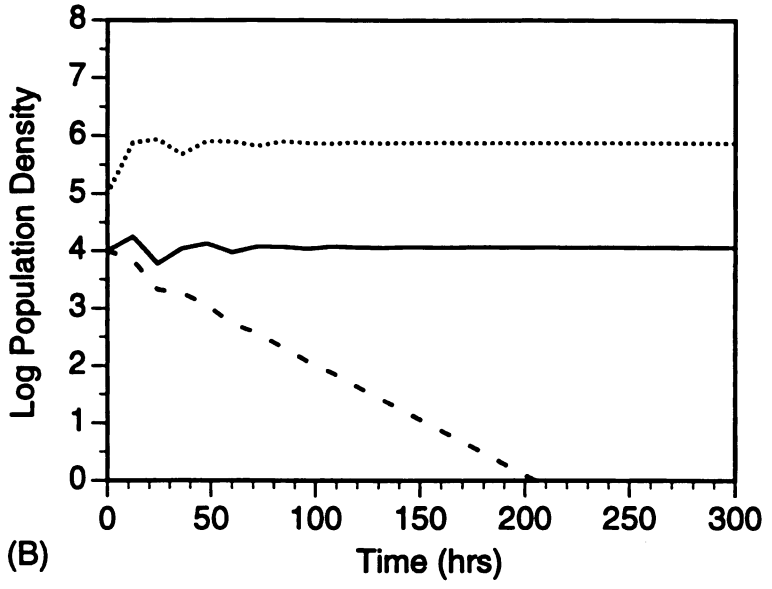


Figure 12. Graphical analysis of the community model for T2, prey bacteria and glucose. The zero net growth isoclines and the consumer impact vectors for the more vulnerable prey (strain REL607) and the less vulnerable prey (strain REL6584) are shown. Key: zero net growth isocline for strain REL607 = ZNGI$_A$, zero net growth isocline for strain REL6584 = ZNGI$_B$, consumer impact vector for strain REL607 = A, consumer impact vector for strain REL6584 = B.

Figure 13. Population equilibria and dynamics predicted by the model for the treatment chemostats. Dynamics are from numerical simulations of the model, "sampled" at 12 hour intervals. The population densities (viruses per ml or bacteria per ml) have been log-transformed. (A) model with a glucose input concentration of 0.1 μg per ml , (B) model with a glucose input concentration of 0.5 μg per ml. Solid line = more vulnerable *E. coli* dynamics, dashed line = less vulnerable *E. coli* dynamics, dotted line = T2 dynamics.

(A)


Log Population Density
0
1
2
3
4
5
6
7
8
0
50
100
150
200
250
300
Time (hrs)

(B)

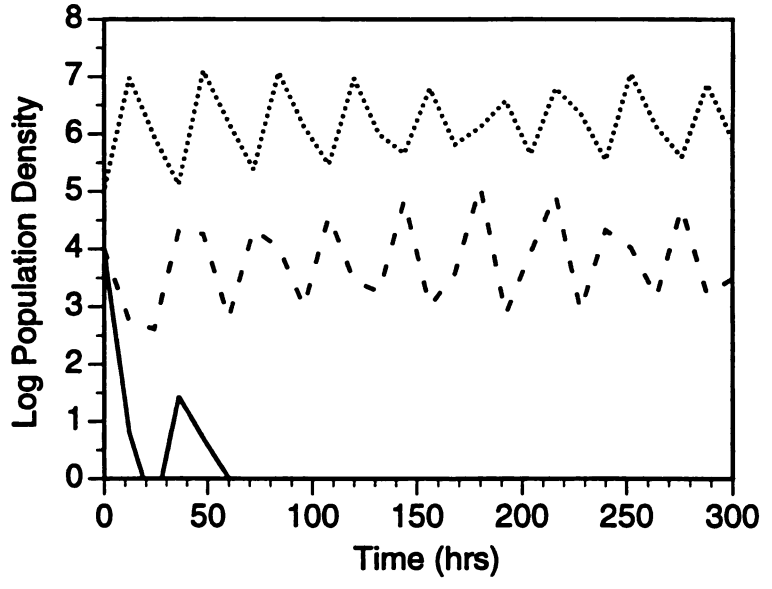
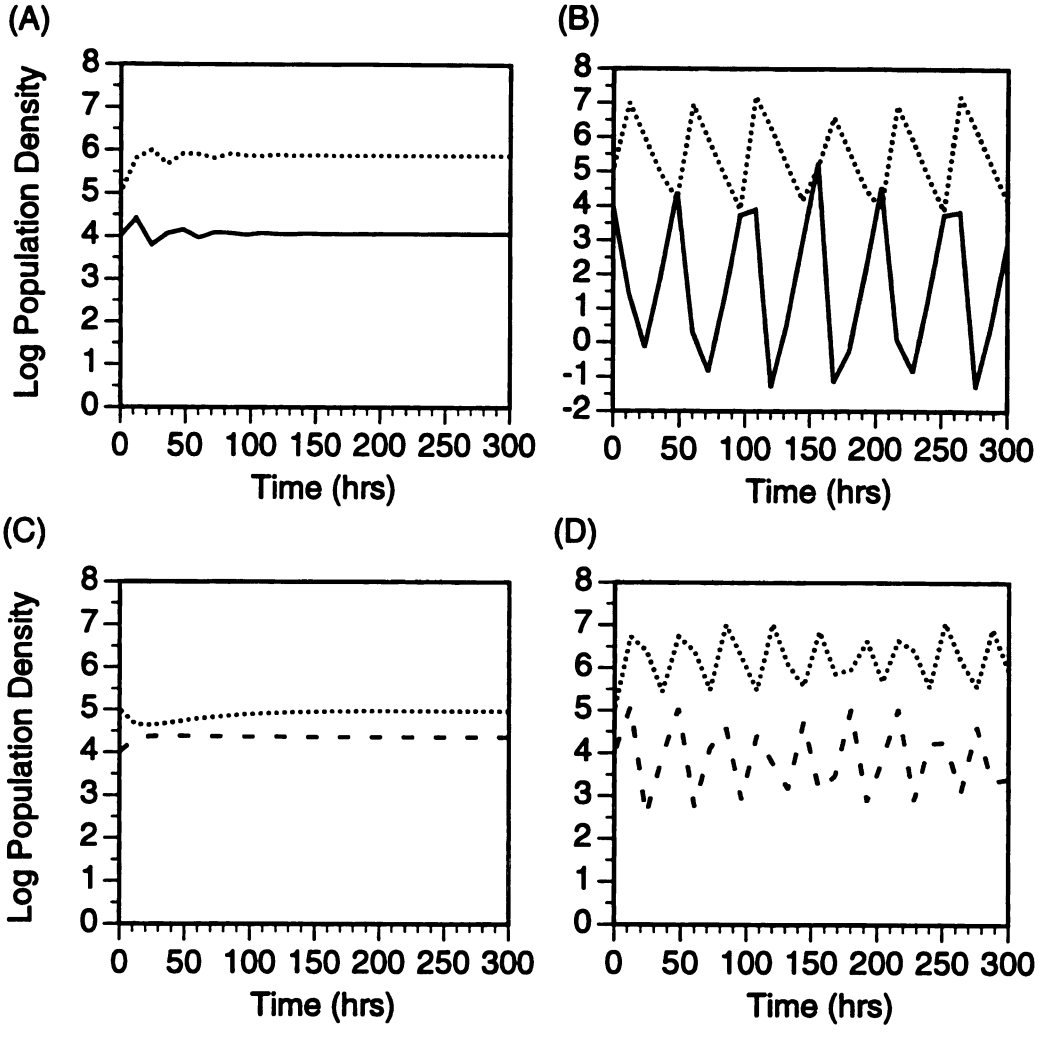

Log Population Density
0
1
2
3
4
5
6
7
8
0
50
100
150
200
250
300
Time (hrs)

Figure 14. Population equilibria and dynamics predicted by the model for the control chemostats. Dynamics are from numerical simulations of the model, "sampled" at 12 hour intervals. The population densities (viruses per ml or bacteria per ml) have been log-transformed. (A) T2 and more vulnerable prey with a glucose input concentration of 0.1 μg per ml , (B) T2 and more vulnerable prey with a glucose input concentration of 0.5 μg per ml, (C) T2 and less vulnerable prey with a glucose input concentration of 0.1 μg per ml , (D) T2 and less vulnerable prey with a glucose input concentration of 0.5 μg per ml. Solid line = more vulnerable *E. coli* dynamics, dashed line = less vulnerable *E. coli* dynamics, dotted line = T2 dynamics.


(A)
(B)
(C)
(D)
Log Population Density
Time (hrs)
0 50 100 150 200 250 300
0 1 2 3 4 5 6 7 8
-2 -1 0 1 2 3 4 5 6 7 8

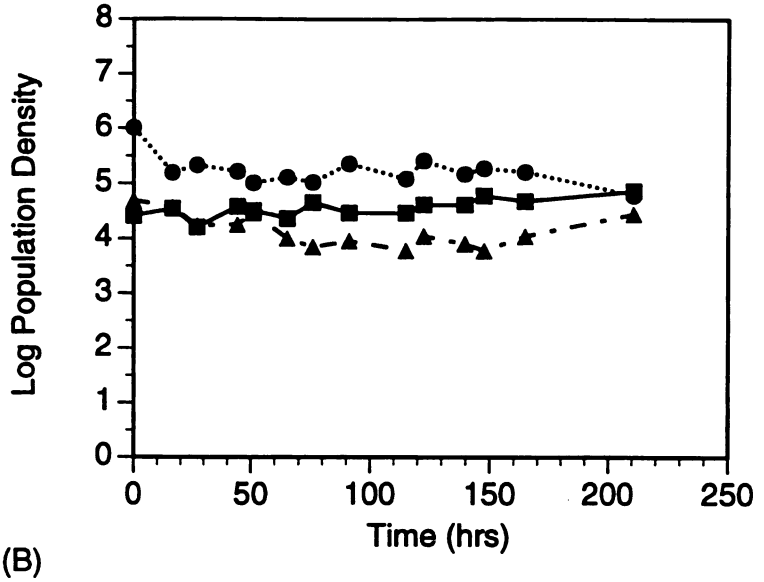
Empirical observations

The dynamics of the viral predator T2 and *E. coli* prey populations are shown in Figure 15 for representative chemostats with two different input concentrations of glucose. In chemostats with an input glucose concentration of 0.1 μg per ml all three populations persisted in all four replicates (Figure 15A), contrary to the predictions of the model (Figure 13A). Invulnerable *E. coli* mutants were not detected in any of the replicates of this treatment. In chemostats with an input glucose concentration of 0.5 μg per ml, the more vulnerable prey initially declined in all four replicates (Figure 15B), as predicted by the model (Figure 13B). The evolution of invulnerable *E. coli* (from a "less vulnerable" ancestor) occurred in all four replicates of this higher glucose treatment. The invasion of the chemostats by these invulnerable mutants initially halted the decline in density of the more vulnerable *E. coli*. However, once the invulnerable *E. coli* population reached its equilibrium, the more vulnerable *E. coli* resumed their population decline in three of the four replicates. The phage persisted in all four replicates of the higher glucose treatment, including the three replicates where the invulnerable *E. coli* population reached its equilibrium and the more vulnerable *E. coli* declined. The persistence of phage in these three chemostats indicates that a minority population of the less vulnerable *E. coli* persisted in these chemostats as well.

Control chemostats with T2 and each of the *E. coli* strains were run simultaneously with the treatment chemostats. The dynamics of the T2 and *E. coli* populations in representative control chemostats are shown in Figures 16 and 17. All populations persisted in all chemostats. Neither less vulnerable nor invulnerable *E. coli* mutants were detected in the lower glucose controls (0.1 μg

Figure 15. Dynamics in treatment chemostats containing more vulnerable *E. coli* (squares), less vulnerable *E. coli* (triangles), and bacteriophage T2 (circles) in chemostats supplied with media containing different amounts of glucose. The dynamics of invulnerable mutants of strain 6584 (the less vulnerable strain) are indicated with open triangles. The population densities (viruses per ml or bacteria per ml) have been log-transformed. (A) 0.1 μg/ml glucose, (B) 0.5 μg/ml glucose.

(A)


Log Population Density
0
1
2
3
4
5
6
7
8
0
50
100
150
200
250
Time (hrs)

(B)

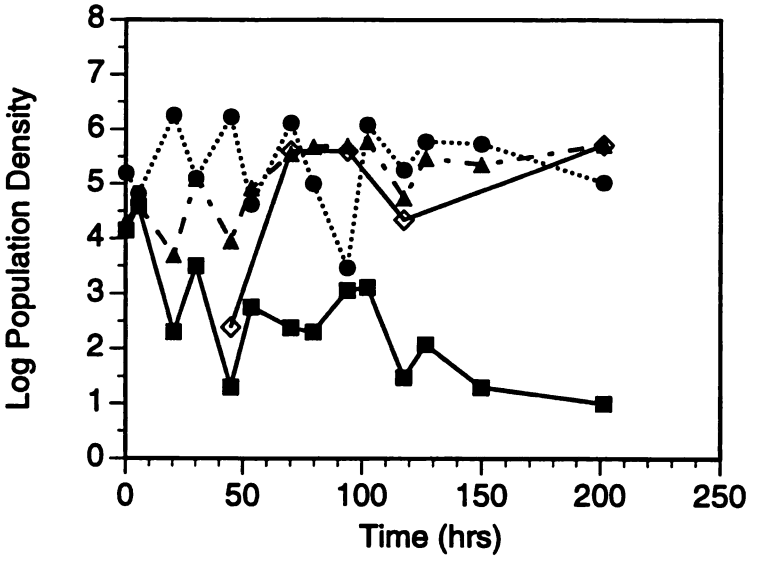
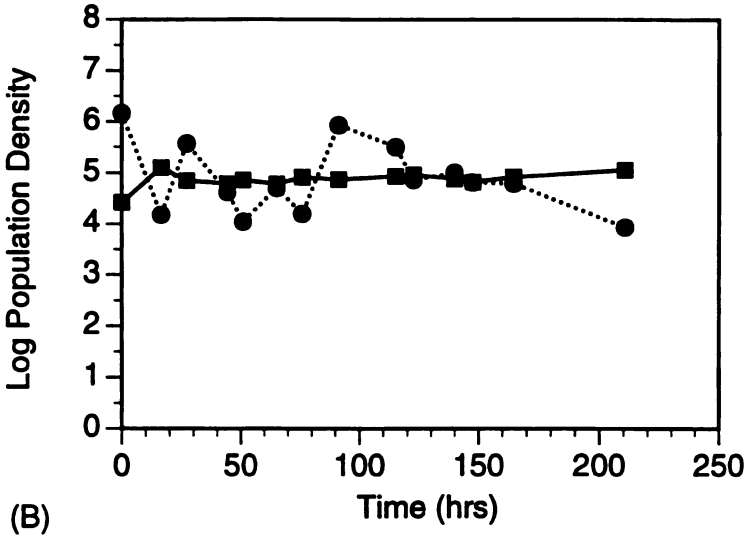

Log Population Density
0
1
2
3
4
5
6
7
8
0
50
100
150
200
250
Time (hrs)

Figure 16. Dynamics in control chemostats supplied with media containing 0.1 μg per ml of glucose. The population densities (viruses per ml or bacteria per ml) have been log-transformed. (A) bacteriophage T2 (circles) and more vulnerable *E. coli* (squares), (B) bacteriophage T2 (circles) and less vulnerable *E. coli* (triangles).

(A)


Log Population Density
0
1
2
3
4
5
6
7
8
0
50
100
150
200
250
Time (hrs)

(B)

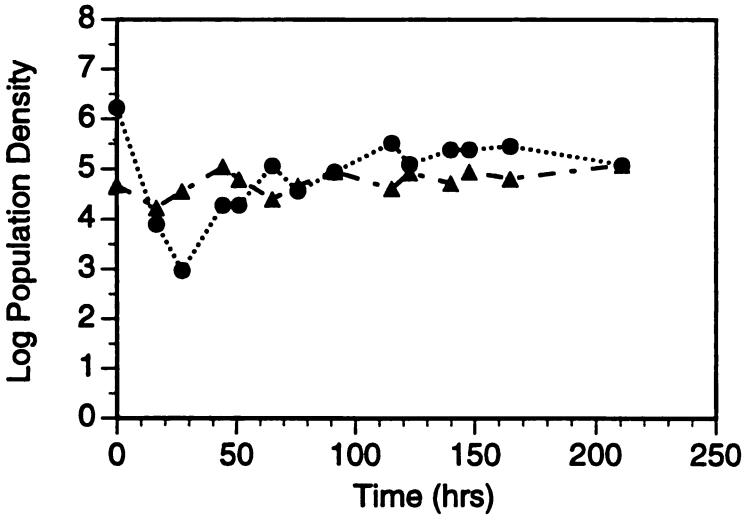
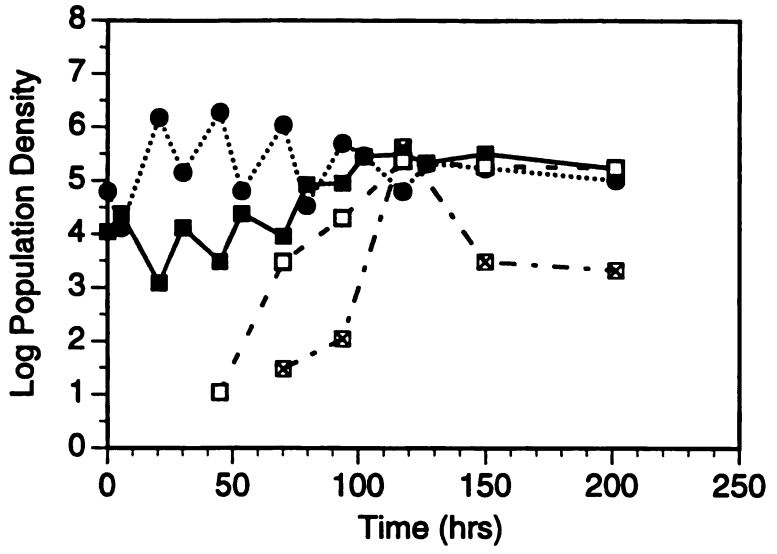
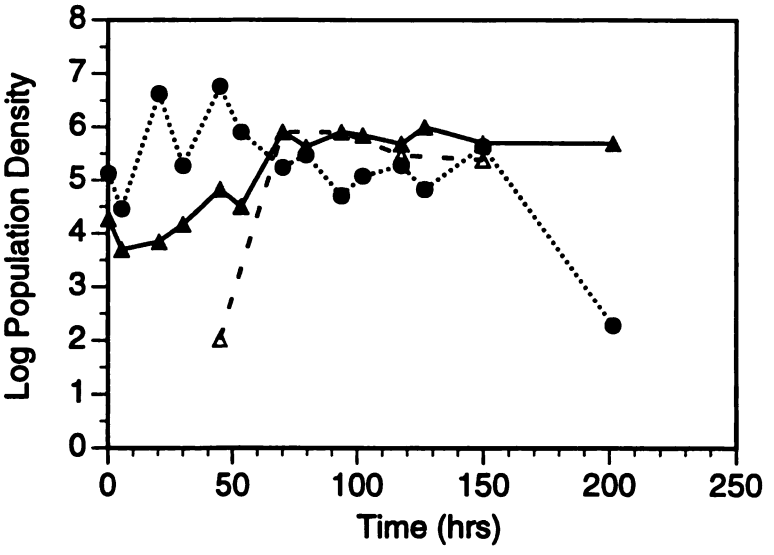

Log Population Density
0
1
2
3
4
5
6
7
8
0
50
100
150
200
250
Time (hrs)

Figure 17. Dynamics in control chemostats supplied with media containing 0.5 μg per ml of glucose. The population densities (viruses per ml or bacteria per ml) have been log-transformed. (A) bacteriophage T2 (circles), more vulnerable *E. coli* (squares), less vulnerable *E. coli* mutants (open squares) and invulnerable *E. coli* mutants (hatched squares). (B) bacteriophage T2 (circles), less vulnerable *E. coli* (triangles) and invulnerable *E. coli* mutants (open triangles).

(A)


Log Population Density
0
1
2
3
4
5
6
7
8
0
50
100
150
200
250
Time (hrs)

(B)


Log Population Density
0
1
2
3
4
5
6
7
8
0
50
100
150
200
250
Time (hrs)

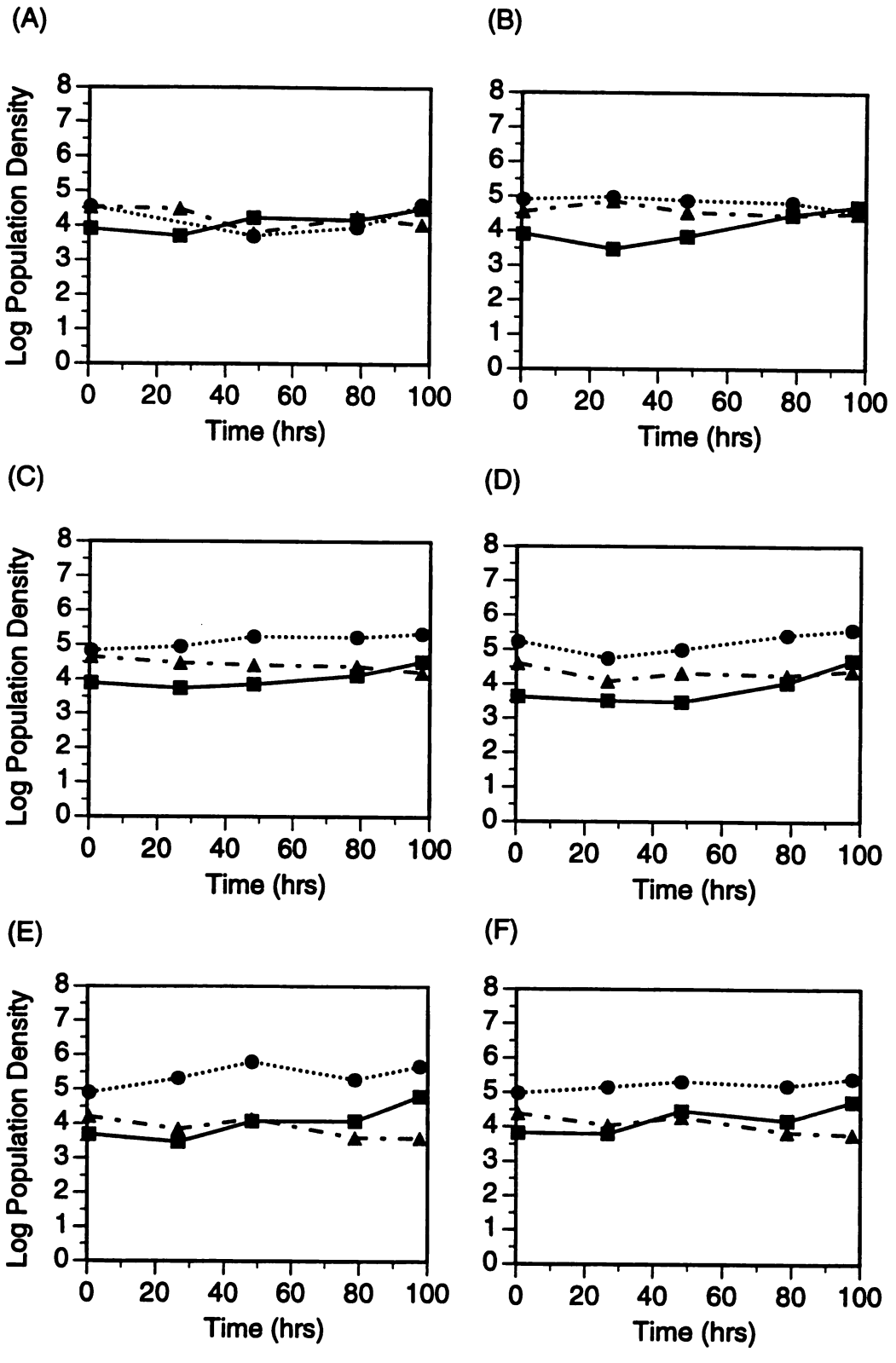
per ml and below); however, both kinds of mutants were eventually detected in the higher glucose controls.

Lowering the glucose input concentration from 0.1 to 0.07 μg per ml did not result in exclusion of the less vulnerable *E. coli* (Figure 18). All three populations persisted at all of the lower glucose input concentrations used, with the exception of one replicate of the 0.09 μg per ml treatment run for 200 hours. In this replicate, the T2 population underwent an initial rapid decline (from which it later recovered). During this decline the less vulnerable prey also declined sharply and it continued to decline even after the T2 population had recovered. In the other replicate of the 0.09 μg per ml treatment run for 200 hours, the less vulnerable prey population appeared to be declining slightly, but this decline was not evident until after 100 hours (Figure 19).

DISCUSSION

Ecologists have documented the existence of indirect effects in a number of biological communities. However, identifying the factors that determine the importance of indirect effects remains elusive, at least in part because of the complexity of natural communities. One approach to identifying such factors is to study simple community "modules" (i.e., abstract communities with 2 to 4 interacting populations) that have potential indirect interactions. Several theorists have predicted that productivity can control the relative importance of indirect effects in such community modules (Holt et al. 1994, Leibold 1996). For example, apparent competition mediated by shared predators is predicted to be an important determinant of community structure primarily at higher productivity levels, while exploitative competition for resources is predicted to be important

Figure 18. Dynamics in treatment chemostats containing more vulnerable *E. coli* (squares), less vulnerable *E. coli* (triangles), and bacteriophage T2 (circles) in chemostats supplied with media containing different amounts of glucose. The population densities (viruses per ml or bacteria per ml) have been log-transformed. (A) 0.07 μg/ml glucose, (B) 0.08 μg/ml glucose, (C) 0.09 μg/ml glucose, (D) 0.1 μg/ml glucose, (E) 0.11 μg/ml, and (F) 0.12 μg/ml glucose.


(A)
(B)
(C)
(D)
(E)
(F)
Log Population Density
Time (hrs)
0
1
2
3
4
5
6
7
8
0
20
40
60
80
100

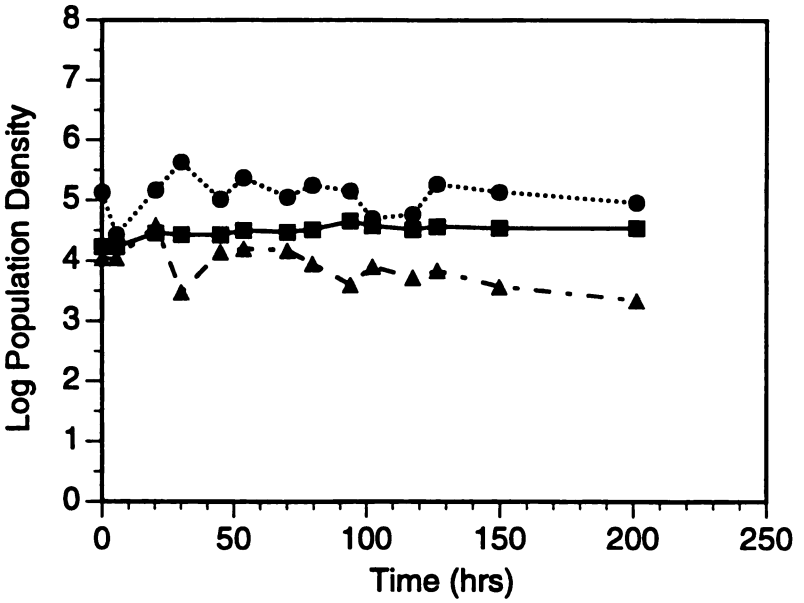


Figure 19. Dynamics of more vulnerable *E. coli* (squares), less vulnerable *E. coli* (triangles), and bacteriophage T2 (circles) in a chemostat supplied with media containing 0.09 µg/ml glucose. The population densities (viruses per ml or bacteria per ml) have been log-transformed.

primarily at lower productivity levels. I tested this theory using chemostat communities of bacteria and bacteriophage. Using these chemostat communities, I demonstrated that the importance of apparent competition increases with rising productivity. Apparent competition resulted in exclusion of the more vulnerable prey at a higher productivity level (0.5 μg per ml glucose input concentration) but not at a lower productivity level (0.1 μg per ml glucose input concentration). However, contrary to the predictions of the model, exploitative competition did not result in the exclusion of the inferior competitor (the less vulnerable prey) at the lower productivity level.

The graphical analysis of my mathematical model predicted that the productivity range within which both prey types were predicted to coexist was very narrow. One explanation for the lack of competitive exclusion at lower productivity levels is that my estimates of model parameters were inaccurate and that the lower productivity treatment actually fell within the coexistence range. For example, the range of coexistence would be predicted to be broader and to begin at a lower glucose concentration if the cost of reduced vulnerability is actually lower than estimated (i.e., if the ratio ψ_R: ψ is higher than estimated and/or the ratio K_R:K is lower than estimated). To determine if the range of coexistence was indeed broader (as well as lower) than predicted, I repeated the experiment using input concentrations of glucose that were slightly lower and slightly higher than 0.1 μg per ml. Concentrations of 0.07, 0.08, 0.09, 0.11 and 0.12 μg per ml glucose were used. All populations persisted at all glucose input treatments, with the exception of one replicate of the 0.09 μg per ml treatment. Thus, it appears that the range of coexistence of the two prey types is lower and broader than predicted theoretically.

Another explanation for the unexpectedly broad range of coexistence is that growth of the prey on the chemostat wall may have prevented competitive

exclusion at lower productivity levels. Other researchers have observed that wall growth can prevent competitive exclusion (Chao and Ramsdell 1985). I did not observe growth on the chemostat walls in these experiments, but growth need not be visible to have an effect. However, if wall growth was preventing exclusion by exploitative competition at lower productivity levels, then coexistence of the two prey types should be possible even when the predator is absent. Yet, I observed competitive exclusion exactly as expected when the two prey types were inoculated in chemostats in which T2 was absent (Figure 20). Thus, coexistence of the two prey types does not occur in the case of pure exploitative competition. Evidently, coexistence of the two prey types depends on a combination of exploitative and apparent competition, even though it remains unclear why coexistence occurs over a lower and broader range of resource concentrations than predicted by the model.

Although exclusion due to exploitative competition did not occur at low productivity in the presence of T2 (contrary to the predictions of the model), it should be emphasized that the general trends in the data are consistent with the model. In the long-term lower productivity treatments (those run for 200 hours; e.g., Figure 15A) the less vulnerable prey persisted at a *lower* average population density than the more vulnerable prey, while in the higher productivity treatment the less vulnerable prey persisted at a *higher* average population density than the more vulnerable prey (and eventually excluded them). In the short-term lower productivity treatments (those run for 100 hours; e.g., Figure 18) the ratio of less vulnerable prey to more vulnerable prey *declined* over time, while in the higher productivity treatment this ratio *increased* (until the more vulnerable prey was excluded). Thus, the competitive relationship between the prey was reversed as productivity was changed, in general agreement with the model.

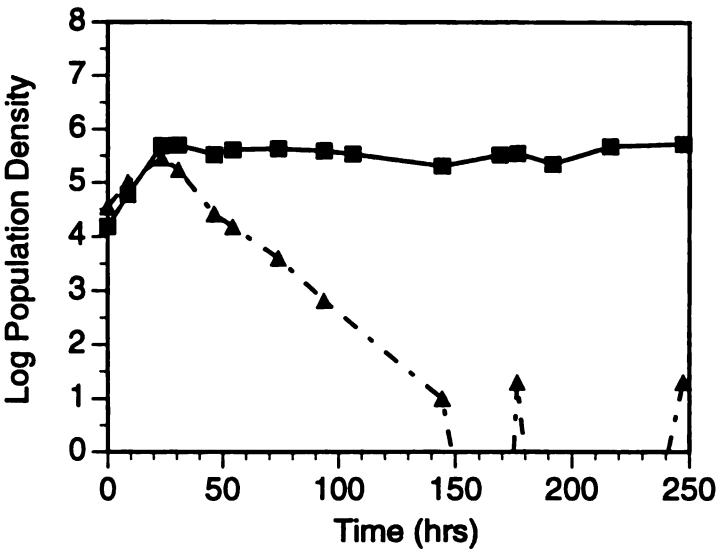


Figure 20. Dynamics of more vulnerable *E. coli* (squares) and less vulnerable *E. coli* (triangles) in a chemostat without predators. The population densities (viruses per ml or bacteria per ml) have been log-transformed.

It should be noted that starting population densities were not always identical among replicates in this experiment. However, my mathematical model does not predict any major effect of starting densities on population dynamics. Numerical simulations of the model predict the exclusion of the less vulnerable *E. coli* in the 0.1 μg per ml treatment and the exclusion of the more vulnerable *E. coli* in the 0.5 μg per ml treatment, regardless of starting population densities. None of my experiments showed any discernible effect of starting population densities on subsequent dynamics.

The Evolutionary Ecology of Shared Predation

I was able to observe the evolutionary ecology of shared predation in my experimental communities because invulnerable *E. coli* evolved in the highest glucose treatment (e.g., Figure 15B). The invasion of the chemostats by these invulnerable mutants had a profound effect on population dynamics, initially halting the decline of the more vulnerable *E. coli* population. One likely explanation for this result is that the invasion by the invulnerable mutants depressed the equilibrium glucose concentration in the chemostats, and that this decrease in equilibrium glucose concentration reversed the competitive advantage between the more vulnerable *E. coli* and the less vulnerable *E. coli*. This reversal would occur because at low glucose concentrations the more vulnerable *E. coli* would have an advantage, since it is a superior competitor for glucose. The reversal of the competitive advantage would halt the decline of the more vulnerable *E. coli* population. This hypothesis could be tested in future research by tracking the concentration of glucose in the chemostats.

It is interesting that the invasion had only a temporary effect on the outcome of apparent competition in the majority of the replicate chemostats; once

the invulnerable *E. coli* population reached its equilibrium density, the more vulnerable *E. coli* resumed their population decline in three of the four replicates. A likely explanation for this observation is that the initial invasion of the chemostats by the invulnerable mutants resulted in an overshoot of the invulnerable population's equilibrium density and a severe (but temporary) decrease in the glucose concentration in the chemostats. This severe decrease could confer a transient advantage to the more vulnerable *E. coli.* However, as the invulnerable population approached a stable equilibrium density the glucose concentration in the chemostat would rise, restoring the competitive advantage of the less vulnerable *E. coli* and resulting in the continued decline of the more vulnerable *E. coli.* This hypothesis could be tested in future studies by tracking the glucose concentrations in the chemostats during the invasion period.

The observation that the more vulnerable *E. coli* population resumed its decline in only three of the four replicates suggests that there are significant differences among the invulnerable mutants in the different chemostats. Differences in the magnitude of the trade-off between invulnerability and competitive ability for glucose could explain the results observed. The magnitude of this trade-off would determine the equilibrium glucose concentration in the chemostats and thus whether the less vulnerable or more vulnerable *E. coli* would be competitively superior after the invulnerable mutants reached their equilibrium. This idea could be tested in future experiments by measuring the magnitude of this trade-off in mutants from the different replicates.

Implications for Other Areas of Ecological Theory

My experiments also shed light on two contentious areas of ecological theory: the relationship between productivity and species diversity, and the

debate over prey-dependent *versus* ratio-dependent predator-prey models. The nature of the relationship between productivity and species diversity has been vigorously debated by ecologists (Abrams 1995, Rosenzweig 1995). In many studies, species diversity has been observed to decline with increasing productivity (see review in Goldberg and Miller 1990), at least within certain productivity ranges. It is unclear what mechanisms may be responsible for this decline. One explanation is that as productivity is increased apparent competition becomes more important, resulting in the exclusion of more vulnerable species and a decline in diversity (Leibold 1996). My experiments have shown that this explanation is biologically plausible. Increasing productivity resulted in a decline in diversity in my experimental system (from two prey types to one prey type) through apparent competition.

My approach to modeling the interactions between a predator and two or more prey that differ in their vulnerability is one of several different approaches that have been described in the literature (see Chapter 3). For example, some theorists have argued that shared predation by prey that differ in vulnerability can be modeled using ratio-dependent functional responses (Arditi et al. 1991a, Samelle 1994). Ratio-dependent models combine multiple prey populations with different vulnerabilities into one population that is heterogeneous in vulnerability. However, ratio-dependent models do not predict the exclusion of the more vulnerable prey as productivity is increased, as I observed in this experiment and as predicted by a model with a prey-dependent functional response. Thus, the dynamics of community modules with simple food chains (Chapter 2), with keystone predators (Chapter 3), and with apparent competition (this Chapter) are all better predicted by more traditional prey-dependent models than by newer ratio-dependent models.

CHAPTER 5

SUMMARY AND CONCLUSIONS

SUMMARY

I studied the effect of increased productivity on laboratory model communities that differed in community structure. Three different model communities were used. The first community I used was a simple food chain. This community consisted of bacteriophage T4, a strain of *E. coli* vulnerable to predation by bacteriophage T4, and the limiting nutrient glucose. The second community I used was a food web with a keystone predator. This community consisted of bacteriophage T4, a strain of *E. coli* vulnerable to predation by bacteriophage T4, a strain of *E. coli* invulnerable to predation by bacteriophage T4, and the limiting nutrient glucose. The third community I used was a food web with a shared predator. This community consisted of bacteriophage T2, a strain of *E. coli* highly vulnerable to predation by bacteriophage T2, a strain of *E. coli* less vulnerable to predation by bacteriophage T2, and the limiting nutrient glucose. All of these model communities were maintained in chemostats. Productivity was manipulated in these model communities by altering the concentration of glucose in the incoming media.

In the simple food chain, the bacteriophage population responded to increased productivity with a large and highly significant increase in equilibrium

density, while the *E. coli* population responded with a small but significant increase in equilibrium density. Both populations had a significant decrease in stability in response to increased productivity. Mutants of *E. coli* invulnerable to bacteriophage were detected in both higher and lower productivity treatments. These mutants appeared significantly sooner, and invaded at a faster rate, in the higher productivity treatment than in the lower productivity treatment. The bacteriophage population continued to oscillate in the higher productivity treatment after the invasion by invulnerable *E. coli* mutants; however, the bacteriophage population oscillated with a longer period and at a lower equilibrium density following this invasion.

In the keystone predator food web, neither the bacteriophage nor the vulnerable *E. coli* population changed in equilibrium density in response to increased productivity. Only the invulnerable *E. coli* responded to increased productivity with an increase in equilibrium density. However, both the bacteriophage and the vulnerable *E. coli* populations decreased in stability in response to increased productivity, although to a lesser degree than the populations in the simple food chain. The invulnerable *E. coli* population increased in stability in response to increased productivity. I did not observe bacteriophage-invulnerable mutants of the vulnerable *E. coli* in any productivity treatment, presumably because this niche was already filled.

In the shared predator food web, increased productivity resulted in the exclusion of the more vulnerable *E. coli* due to apparent competition. Mutants of *E. coli* invulnerable to bacteriophage were not detected in the lower productivity treatments; however, they were detected in the higher glucose treatment. Invasion of the higher glucose treatment by invulnerable mutants temporarily halted the exclusion of the more vulnerable *E. coli.*

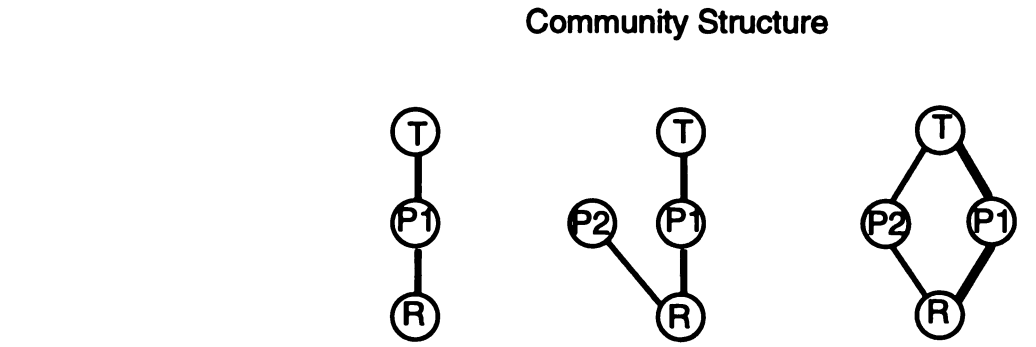
CONCLUSIONS

1. *The response of microbial populations to changes in productivity can be strongly influenced by community structure.*

In my microbial model communities, community structure determined how a given population responded to productivity (Figure 21). For example, *E. coli* strain REL607 (the strain most vulnerable to phage predation) had strikingly different responses to increased productivity when it was imbedded in a simple food chain, a keystone predator web or a shared predator web. REL607 responded to increased productivity with an increase in equilibrium population density when imbedded in a simple food chain, but no change whatsoever in equilibrium population density when imbedded in a food web with a keystone predator. Furthermore, when REL607 was imbedded in a shared predator web it responded to increased productivity by being driven toward extinction by apparent competition.

2. *The response of microbial populations to changes in productivity is better predicted by prey-dependent models than by ratio-dependent models.*

In almost all regards, the prey-dependent mathematical models were superior to the ratio-dependent mathematical models at predicting the responses of my model communities to increased productivity. In a simple food chain, the prey-dependent model successfully predicted the responses of all but one of the population variables I measured (Table 1). The prey-dependent model even predicted the response of the simple food chain to invasion by invulnerable *E. coli* mutants (Table 2). The response of the keystone predator community to



Predator (T):	Persistence (++)	Persistence (0)	Persistence
Prey (P1):	Persistence (+)	Persistence (0)	Exclusion
Prey (P2):		Persistence (++)	Persistence

Figure 21. Effect of community structure on the response of predator (T) and prey (P1 and P2) populations to increased productivity. Key: ++ = large increase in equilibrium density, + = small increase in equilibrium density, 0 = no significant change in equilibrium density, Persistence = persistence of population for duration of experiment, Exclusion = competitive exclusion of population.

TABLE 1. Predicted and observed responses of *E. coli* and phage T4 populations in a simple food chain to increased productivity.

	Model Predictions*		Experimental
Population Variable	Prey-dependent	Ratio-dependent	Results
Equilibrium density of *E. coli*	–	↑	↑
Equilibrium density of phage T4	↑	↑	↑
Ratio of phage T4 to *E. coli*	↑	–	↑
Instability of *E. coli* population	↑	–	↑
Instability of phage T4 population	↑	–	↑
Time to appearance of T4-resistant *E. coli*	↑	↑	↑
Rate of invasion by T4-resistant *E. coli*	↑	–	↑

* ↑ = increase, – = no change.

TABLE 2. Predicted and observed responses of *E. coli* and phage T4 populations in a simple food chain to invasion by T4-resistant mutants of *E. coli*.

	Model Predictions*		Experimental
Population Variable	Prey-dependent	Ratio-dependent	Results
Equilibrium density of total *E. coli*	↑ ↑	↑	↑ ↑
Equilibrium density of phage T4	↓	↓ ↓	↓
Instability of total *E. coli* population	↓ ↓	—	↓ ↓
Instability of phage T4 population	↓ ↓	—	↓ ↓

* ↑ ↑ = increase (large), ↑ = increase (small), ↓ ↓ = decrease (large), ↓ = decrease (moderate), — = no change.

increased productivity was accurately predicted by the prey-dependent model but not the ratio-dependent model (Table 3). The prey-dependent model accurately predicted the response of the shared predator community to a higher level of productivity, although it did not predict the persistence properties of the community at lower productivity (Table 4).

The degree to which the predictions of the prey-dependent mathematical models fit the dynamics of my experimental communities was satisfying but also somewhat surprising. There are a number of complexities in these experimental systems that the prey-dependent models ignore, and that could have led to a departure from the model predictions. These complexities include spatial heterogeneity (Schrag and Mittler 1996), the possibility that population parameters such as burst size and adsorption rate could vary with bacterial growth rate (Hadas et al. 1997) and the potential for population parameters to change over time due to evolution (Lenski 1988c). The prey-dependent models did a reasonably good job of predicting the responses of my experimental communities despite these complexities.

The ratio-dependent models did a particularly poor job of predicting the response of population stability to increased productivity in my experimental communities. Polis and Strong (1996) have suggested that the response of population stability to increased productivity may decrease as community complexity increases, although this idea has been challenged (Hairston and Hairston 1997). If Polis and Strong are correct, then ratio-dependent models might accurately predict the responses of populations in more complex communities than those I constructed.

TABLE 3. Predicted and observed responses of *E. coli* and phage T4 populations in a keystone predator system to increased productivity.

	Model Predictions*		Experimental
Population Variable	Prey-dependent	Ratio-dependent	Results
Equilibrium density of vulnerable *E. coli*	–		–
Equilibrium density of invulnerable *E. coli*	↑		↑
Equilibrium density of total *E. coli*	↑	↑	↑
Equilibrium density of phage T4	–	↑	–
Instability of vulnerable *E. coli* population	↑		↑
Instability of invulnerable*E. coli* population	↑		↑
Instability of total *E. coli* population	↑	–	↑
Instability of phage T4 population	↑	–	↑

* ↑ = increase, – = no change.

TABLE 4. Predicted and observed responses of *E. coli* and phage T4 populations in a shared predator system to increased productivity.

	Model Predictions*				Experimental	
	Prey-dependent		Ratio-dependent		Results	
Productivity Level	low	high	low	high	low	high
Population Variable						
Persistance of more vulnerable *E. coli*	Y	N	Y	Y	Y	N
Persistance of less vulnerable *E. coli*	N	Y	Y	Y	Y	Y
Persistance of phage T2	Y	Y	Y	Y	Y	Y

* Y = persistance of population, N = exclusion of population.

3. *Chemostat communities of E. coli and bacteriophage are excellent laboratory model systems for studying community ecology.*

Using chemostat communities of bacteria and phage, I was able to obtain abundant and high-quality empirical data on population responses to increased productivity. I was able to track populations for several hundred hours, equivalent to some 100 generations under the maximum generation time that is set by flow through the chemostat. Population oscillations were evident, and the resolution of the time-series data was high enough that I was even able to detect shifts in the period of oscillations following an invasion. Because of the short generation time of my study organisms, I was also able to witness the evolution of the interactions within my experimental communities and the effect increased productivity had on evolutionary change. With this system I was able to increase community complexity in a controlled manner, and measure the effect of added complexity on the community's response to increased productivity. This system also allowed me to manipulate the key variable (productivity) while being confident that all other extrinsic factors (temperature, etc.) remained unchanged.

Thus, chemostat communities of bacteria and phage are excellent systems for doing rigorous, manipulative experiments in community ecology and for testing community module theory. Community module theory now forms the theoretical backbone of community ecology. Great strides in our theoretical understanding of community ecology have been made through the careful and detailed analysis of relatively simple community models. I believe that applying this approach to experimental community ecology will prove equally fruitful.

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