



# The dynamics of top-down and bottom-up effects in food webs of varying prey diversity, composition, and productivity

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Prey diversity is thought to mediate the strength of top-down and bottom-up effects, but few experiments directly test this hypothesis. I assembled food webs of bacteria and bacterivorous protist prey in laboratory microcosms with all combinations of five productivity levels, two top predator treatments (present or absent), and three prey compositions. Depauperate food chains contained one of two edible prey species, while more diverse food webs contained both edible prey species plus two additional less-edible/inedible prey. Equilibrium theory predicts that prey diversity should weaken the top-down and bottom-up effects on trophic level biomasses, due to density compensation among prey species. Top-down effects should increase with productivity in food chains, but decrease with productivity in food webs. Results revealed highly dynamic top-down effects, the strength of which varied more over time than among treatments. Further, top-down effects did not merely vary in absolute strength over time, but also in relative strength across different prey compositions and productivity levels. It might be expected that equilibrium models would qualitatively reproduce time-averaged results. However, time-averaged data largely failed to support equilibrium predictions. This failure may reflect strong temporal variability in treatment effects combined with nonlinear density dependence of species' per-capita growth rates. Strong temporal variability in the strength of top-down effects has not previously been demonstrated, but likely is common in nature as well.

Both top-down (predation) and bottom-up forces (resource availability) are important determinants of the distribution of biomass among trophic levels (Shurin et al. 2002, Borer et al. 2005). Less clear is how diversity within trophic levels influences the propagation of top-down and bottom-up effects. Several lines of evidence suggest that prey diversity may weaken top-down and bottom-up effects (King and Pimm 1983, Leibold 1989, Abrams 1993, Leibold et al. 1997, Chase et al. 2000, Schmitz et al. 2000). High prey species richness creates the potential for density compensation within the prey trophic level, since prey species often interact through exploitative and apparent competition (Leibold 1996). Density compensation among prey can weaken the propagation of top-down and bottom-up effects by buffering total prey biomass (Leibold 1989, 1996, Strong 1992, Abrams 1993). For this reason, results of predator

removal and resource addition experiments often are better-predicted by models that explicitly incorporate details of food web structure than by models that assume homogeneous trophic levels (Briand and McCauley 1978, Leibold 1989, Hulot et al. 2000, Persson et al. 2001, Steiner 2001, Jiang and Morin 2005, but see Wootton and Power 1993).

However, empirical evidence on the role of within-trophic level diversity is largely comparative or indirect (Schmitz 2000, Shurin et al. 2002, Borer et al. 2005). Disagreement over whether Spiller and Schoener (1994) describe a trophic cascade in a “highly speciated” system (as suggested by Pace et al. 1999) or a “relatively simple” system (as suggested by Chase 2000) illustrates the difficulty of interpreting indirect evidence. Diverse (“highly speciated”) systems are presumably those that are too diverse to behave approximately like a linear food chain, but how diverse

a system must be to meet this standard is unknown. The most recent quantitative meta-analyses do not find significant effects of within-trophic level diversity on cascade strength (Shurin et al. 2002, Borer et al. 2005), although these negative results must be interpreted cautiously because cross-study variation in diversity inevitably is confounded with variation in other factors. The few experimental studies that directly manipulate within-trophic level diversity find mixed effects of diversity on the strength of trophic cascades (Balčiūnas and Lawler 1995, Cochran-Stafira and von Ende 1998, Mikola 1998, Bohannan and Lenski 1999, 2000, Steiner 2001). Also unclear is the relative importance of prey species richness per se vs prey species composition in determining the strength of top-down and bottom-up effects. Teasing apart the effects of prey species richness and composition requires manipulative experiments, since otherwise prey species richness and composition likely will be confounded.

Theoretical predictions about the strength of trophic cascades typically assume equilibrium (Abrams 1993, Chase et al. 2000). However, density compensation is a dynamic process, and food webs can exhibit complex transient dynamics even in the absence of destabilizing factors like nonlinear functional responses (Noonburg and Abrams 2005). Intuitively, we might expect that experimental manipulations of top-down or bottom-up factors (e.g. resource addition, predator removal) in diverse webs would have initially-strong effects that gradually decline over time as prey species composition shifts. Contrasting results of short-term experiments and long-term “natural” experiments provide some support for this intuition (Leibold et al. 1997), but variation in cascade strength among manipulative experiments of differing duration does not (Shurin et al. 2002, Borer et al. 2005). However, little theory or empirical data directly addresses the question of how the strength of top-down and bottom-up effects will vary over time. Recent studies show that changes in prey diversity can have a variety of effects on predator–prey dynamics (e.g. altered predator–prey cycle period; Yoshida et al. 2003). It is unclear whether equilibrium predictions about the effect of prey diversity on trophic cascades will hold, even approximately, in temporally-varying systems.

I tested the effect of within-trophic-level diversity and species composition on the strength of top-down and bottom-up effects by assembling communities of protist predators, protist prey, and a bacterial resource base in laboratory microcosms. The short generation times of these organisms (< 48 h for all species in this study) facilitate collection of long-term population dynamic data, necessary for detecting density compensation. Many studies of trophic cascades last only one or a few prey generations (Borer et al. 2005). I assembled communities with and without predators at each of five

productivity levels. Communities varied in prey diversity and composition, containing either of two edible prey species (depauperate food chains), or both of these species plus two additional less-edible/inedible species (more diverse food webs). I tested three predictions drawn from equilibrium theory (Oksanen et al. 1981, Leibold 1989, 1996, Abrams 1993): (1) increased prey diversity (species richness) will weaken both top-down and bottom up effects, (2) top-down effects will increase with productivity in food chains, but decrease with productivity in diverse food webs, and (3) variation in prey composition within the lowest diversity level will affect the strength of top-down and bottom-up effects.

## Material and methods

### Choice of species and culture methods

All protists used in this study are common species that coexist in nature, typically on the bottoms of ponds (personal observation). I selected four bacterivorous ciliate prey: *Tetrahymena thermophila*, mating type VII (American Type Culture Collection, Rockville, MD, stock no. 30307), *Colpidium striatu* (Carolina Biological Supply, Burlington, NC), *Paramecium tetraurelia*, mating type VII (ATCC no. 30568), and *Paramecium caudatum* (Carolina Biological Supply). I chose prey of varying body size to facilitate identification, and because prey of different sizes vary in their vulnerability to predators (Fox 2000, and see below). Prey competed for bacteria, which comprised the basal trophic level. Elsewhere I describe competitive interactions among prey along the productivity gradient in the absence of predators (Fox 2002).

Although the range of prey diversity used here (one or four species) is low in absolute terms, it is large in relative terms as it represents a 4-fold range of prey species richness. Food web theory predicts that altering within-trophic level diversity by even a single species can have major effects on trophic cascade strength (Abrams 1993, Leibold 1996).

The predator was the heliozoan protist *Actinosphaerium* (= *Echinospaerium*) *nucleofilum* (Carolina Biological Supply). *Actinosphaerium* is a generalist “sit-and-wait” predator that adheres to a substrate and uses axopods (stiff pseudopods) to capture prey that bump into it. Likelihood of prey capture depends on the relative size of predator and prey (Fox 2000, pers. obs.). *Actinosphaerium* of all sizes readily consume the two smallest ciliates, *Tetrahymena* and *Colpidium* (Fox 2000). Only larger *Actinosphaerium* can capture *P. tetraurelia*, and not at a sufficiently high rate to reliably support a predator population when *P. tetraurelia* is the only food source (Fox 2000, pers. obs.). *Actinosphaerium*

cannot consume the largest ciliate, *P. caudatum* (Fox 2000). *Actinosphaerium* does not consume bacteria in significant quantities, cannot survive on bacteria alone, and is not cannibalistic (Suzaki et al. 1980, Fox 2000, pers. obs.).

All protists used here reproduce continuously and asexually via binary fission, and have generation times of 5–48 h (Fenchel 1987, Fox 2000). None of the prey encyst under the experimental conditions, while the predator *Actinosphaerium* does so only rarely (pers. obs.). None of the species exhibit phenotypically-plastic switching between alternative morphotypes, as do some other protists.

Microcosm assembly, maintenance, and sampling followed well-established methods (Fox 2002). Microcosms were 260 ml screw-capped glass bottles containing 100 ml of growth medium (crushed Protozoan Pellets [PP, Carolina Biological Supply] autoclaved in well water). Protozoan Pellets are standardized pellets of dried plant matter that provide carbon and nutrients for bacteria. Sterile wheat seeds provided an additional, slow-release carbon source. I created a productivity gradient by varying PP concentration and the number of wheat seeds (see below for productivity levels used). Higher PP concentrations and more wheat seeds increase bacterial carrying capacity (Kaunzinger and Morin 1998, Fox 2002). I added bacteria (*Serratia marcescens* [Carolina Biological Supply]) at low initial density 48 h before protist prey. Because protist stock cultures contained other bacterial taxa, I also added 0.3 ml of 1.2  $\mu\text{m}$ -filtered mixed medium, comprised of equal parts from each stock culture, to homogenize the bacterial inoculum among treatments.

I added prey as 0.3 ml inocula from agitated, high-productivity single-species stock cultures (0.84 g PP  $\text{l}^{-1}$ ). Prey therefore began the experiment at low density ( $<1\%$  of carrying capacity). Bottles with predators received 35 *Actinosphaerium* (added via micropipet) 5 d after prey addition, when prey had reached high density. Weekly replacement of 10% of the medium (and any organisms in it) with fresh, sterile medium of the same PP concentration prevented

accumulation of waste products or exhaustion of carbon supplies. Cultures were maintained in an incubator at 20°C.

Sampling procedures for protists and bacteria are described elsewhere (Fox 2002). I sampled protists every 2–4 d. Logistical constraints required sampling of bacteria on different schedules in different treatments (described below). I converted prey density to biovolume (an index of biomass) by multiplying density of each species by the mean biovolume of 10 cells. I estimated cell biovolume by measuring cells and treating them as standard geometric shapes (Wetzel and Likens 1999).

## Experimental design, sampling regime, and statistical analyses

The experiment comprised three factors (prey species composition, predator presence/absence, and productivity level) in a factorial design (Table 1). There were three prey species compositions: depauperate linear food chains of each of the two edible prey species (*Tetrahymena* and *Colpidium*), and diverse food webs with these two species plus the *Paramecium* spp. Each of these three prey species compositions (*Tetrahymena*, *Colpidium*, or four prey species) was grown with and without the predator at five productivity levels: 0.14, 0.28, 0.42, 0.56, or 0.84 g PP  $\text{l}^{-1}$ . There were one, two, three, four, or five wheat seeds/bottle, with more seeds corresponding to higher PP concentrations. The entire experiment initially comprised 105 bottles. However, the predator failed to establish on *Tetrahymena* at the highest productivity level, and on *Colpidium* at the two highest productivity levels. *Actinosphaerium* also occasionally failed to establish in the diverse food webs: the predator failed to establish in one replicate each at 0.14 and 0.28 g PP  $\text{l}^{-1}$  and three replicates at 0.84 g PP  $\text{l}^{-1}$ . The reason for these failures to establish is unknown, but could not be related to the results in any obvious way. Replicates in which predators failed to establish were excluded from all analyses, leaving a total of 91 bottles (Table 1).

Table 1. Experimental design. The experiment comprised both depauperate food chains (one prey species), and diverse food webs (four prey species), with and without the predator. For food chains there were two prey species compositions, *Colpidium* or *Tetrahymena*. Each food chain and web was grown at each of 3–5 productivity levels, with 2–5 replicates/level. Productivity level is the concentration of Protozoan Pellets (PP) in the medium. Predators failed to establish in some treatment combinations (which are not listed), and some replicates of other treatment combinations; see text for details.

Trophic structure	Prey species compositions	Predator pres./abs.	Prod. levels (g PP $\text{l}^{-1}$ )	Replicates/prod. level
Food chains, -pred.	<i>Colp.</i> , <i>Tet.</i>	absent	0.14, 0.28, 0.42, 0.56, 0.84	3
Food web, -pred.	four prey species	absent	0.14, 0.28, 0.42, 0.56, 0.84	4
Food chains, +pred.	<i>Colp.</i> , <i>Tet.</i>	present	<i>Colp.</i> : 0.14, 0.28, 0.42; <i>Tet.</i> : 0.14, 0.28, 0.42, 0.56	3
Food web, +pred.	four prey species	present	0.14, 0.28, 0.42, 0.56, 0.84	2–5

Data on predator-free food chains and webs were previously published as part of a study of resource competition among these prey species (Fox 2002). Originally, the plan for the present study was to compare these data to predator treatments conducted at the same time. However, the predator-free food chains in Fox (2002) only ran for  $\sim 14$  d, and so cannot be used to analyze the long-term dynamics of top-down and bottom-up effects without making unwarranted extrapolations. Therefore, after completion of the first run of the experiment I re-ran the predator-free food chains, collecting data for a longer period of time (see below for length of experiment). Protist densities in the predator-free food chains on day 14 were not significantly different between the two runs of these treatments (results not shown). There is no reason to think that the dynamics of the first run would not have continued to match those of the second run had the first run continued for  $>14$  d. Comparing predator food chains to predator-free food chains conducted later therefore should not be misleading. More generally, the high repeatability of protist microcosm experiments is widely recognized as one of their strengths as a model system (Morin 1998). Repeated runs of the same experiment almost invariably produce very similar results (pers. obs.).

I sampled each bottle with predators until predators were absent from at least five consecutive samples, with a few predator populations persisting until the experiment was terminated after 120 d. I sampled predator-free bottles for 56 d. Because many predator populations went extinct by day 44, I only used data from day 5 (predator addition) to day 44 in the analyses of protist data ( $\sim 20$  predator generations). At least one predator population persisted until day 44 in every treatment. Because of logistical constraints different treatments could not be sampled on precisely the same schedules. I therefore restricted analysis of protist data to those sampling days on which all treatments were sampled, giving a total of 13 sampling days from day 5 to day 44 (12 days for predators, since the first of the 13 sampling days was the day of predator addition).

Bacteria were sampled via epifluorescence microscopy (Fox 2002). Prey densities with predators were quite variable over time. Since bacteria have much shorter generation times than protists, bacterial densities should track prey densities. However, sampling bacteria at the same frequency as protists was infeasible due to time constraints. In treatments with predators, I sampled bacteria shortly after *Actinospaerium* was added and prey were at high density, and again when *Actinospaerium* first attained a peak density and reduced prey to low density. These samples should bracket the range of bacterial variability. I took 1–2 additional bacterial samples after day 44 from bottles that settled down to an approximate equilibrium with

*Actinospaerium*. I sampled bacteria in all predator-free treatments on day 42, and again in predator-free food webs on day 56.

The general analytical approach for protist data was to conduct two analyses on each response variable. ANOVAs on the time-averaged data (means over time within bottles) addressed whether equilibrium theory correctly predicts the average states of temporally-variable systems. MANOVAs treated data from each sampling day as a separate response variable, with follow-up univariate ANOVAs on the data from each sampling day used to aid interpretation of the MANOVAs. The MANOVAs (and follow-up ANOVAs) addressed whether treatment effects varied over time. Testing for temporal variation using MANOVAs rather than repeated-measures ANOVAs requires less-restrictive assumptions on the form of temporal correlation among samples (von Ende 1993). The slight imbalance in the design (Table 1) means that some of the variation in the response variables cannot be uniquely attributed to any single factor in a statistical model, so that results depend on order of model terms when using sequential sums of squares. However, alternative orderings of model terms produced no qualitative change in the results, and only minor quantitative changes. Data were square root transformed as necessary to reduce heteroscedasticity.

Using this general analytical approach, I tested the effects of productivity level (considered a continuous variable, measured as  $\text{g PP l}^{-1}$ ), predator presence/absence, and prey composition (*Tetrahymena*, *Colpidium*, or four species) on total prey biomass. I also tested effects of productivity level (considered as a categorical variable), predator presence/absence, and their interaction, on the proportion of prey biomass comprised of *Colpidium* in the food webs. Preliminary inspection of the data indicated that the proportional biomass of *Colpidium* was not linearly related to productivity, so treating productivity as a continuous variable would have been inappropriate. These analyses test how prey diversity and composition mediate the strength of top-down and bottom-up effects on prey. I tested effects of productivity level (considered as a continuous variable), prey composition, and their interaction on *Actinospaerium* density. This analysis tests how prey diversity and composition mediate bottom-up effects on predators.

To allow comparison with recent reviews of top-down effects (Schmitz et al. 2000, Shurin et al. 2002, Borer et al. 2005), I also calculated the effect size of predators on total prey biomass. I estimated effect size as  $\ln(X_{+p}/X_{-p})$ , where  $X_{+p}$  and  $X_{-p}$  are the mean values of total prey biomass with and without predators, respectively (Hedges et al. 1999, Schmitz et al. 2000, Borer et al. 2005). I calculated effect size and its

standard error for each combination of prey composition and productivity level on each sampling day.

Since bacteria were sampled different numbers of times on different days in different treatments, a MANOVA on bacterial density is impossible due to missing data. While a repeated-measures ANOVA can be conducted with missing data, the missing data points must be a random sample of all the data and cannot be too numerous relative to the observed data points. Neither assumption was met in the present experiment, since on some days only a few bottles were sampled for bacteria and on no day was every bottle sampled. A repeated-measures ANOVA therefore was impossible. Instead, I conducted a multiple regression of bacterial density on productivity, total prey biomass, and proportion of prey biomass comprised of the competitive dominant *Colpidium* (=1 in *Colpidium* food chains, and 0 in *Tetrahymena* food chains). This analysis directly addresses the proximate causes of variation in bacterial density. The multiple regression treats multiple samples from the same bottle as independent. While perhaps not strictly valid, this assumption probably was not seriously violated. The substantial amount of time between bacterial samples within any bottle (>7 d, equivalent to dozens or hundreds of bacterial generations) suggests that autocorrelation is unlikely. Further, averaging over time within bottles to eliminate any non-independence would average away much of the variation in the data. Regression results are very highly significant, and so are unlikely to reflect any modest inflation of error degrees of freedom that might result from treating non-independent samples as independent.

## Results

Prey dynamics in the absence of predators resembled convergence to equilibrium (Fig. 1a, 1c, Fox 2002). Predators greatly increased population dynamic variability in both food chains and webs (Fig. 1), as in previous studies of food chains in this system (Lawler and Morin 1993). Predator persistence times varied widely and did not vary systematically among treatments (Fox 2000). Predators always persisted for at least 10 d in all replicates (generally much longer; Fig. 1b, 1d, 1f, 1h, Fox 2000).

Productivity, predators, prey composition, and all interactions (save that between productivity and predators) had highly significant effects on time-averaged square root transformed total prey biomass (all  $p < 0.01$ ). Time-averaged total prey biomass increased with productivity, decreased with predators present, and was lower in *Tetrahymena* chains than in other prey compositions (Fig. 2). Significant interaction terms arose because time-averaged total prey biomass in-

creased with productivity less strongly in *Tetrahymena* chains (with or without predators), and in food chains with *Colpidium* and predators, than in other treatments (Fig. 2). All main effects and their interactions were highly significant in the MANOVA (all  $p < 0.001$ ), in a manner broadly consistent with the ANOVA on time-averaged data. However, ANOVAs for each sampling day revealed that statistical interactions between predators and other treatments varied in form from day to day (Fig. 3). Predator effect size on total prey biomass also varied with productivity and prey composition in different ways on different days (Fig. 4).

Time-averaged *Actinosphaerium* density increased significantly with productivity ( $p < 0.001$ ), and was significantly higher in *Colpidium* food chains than in other prey compositions ( $p < 0.05$ ), with the productivity  $\times$  prey composition interaction marginally non-significant ( $p = 0.10$ ) (Fig. 5). However, inspection of the data suggests time-averaged *Actinosphaerium* density actually increases more strongly with productivity in food webs than in food chains, until it saturates or declines at the highest productivity level (Fig. 5). The linear effect of productivity assumed in the ANOVA may not be an adequate description of the data over the entire productivity gradient, preventing detection of a significant productivity  $\times$  prey composition interaction. The MANOVA also reveals significant effects of productivity and prey composition ( $p < 0.001$ ) with no interaction ( $p = 0.12$ ). However, individual ANOVAs for each sampling day indicate that treatment effects on mean predator density varied over time. For instance, *Colpidium* food chains did not support the highest predator density at all times (Fig. 1b, 1d, 1f, 1h).

In a multiple regression, square root-transformed bacterial density increased significantly with productivity, and decreased significantly with total prey biomass and proportional biomass of *Colpidium* ( $p < 0.001$  for all partial regression coefficients; Fig. 6). The overall multiple regression was highly significant ( $R^2 = 0.58$ ,  $p < 0.001$ ). Productivity, total prey biomass, and proportional biomass of *Colpidium* respectively explained 37%, 18% and 3% of the variation in bacterial density (Fig. 6). Restricting the multiple regression analysis to bacterial samples taken before day 44 did not qualitatively alter the results, and produced only minor quantitative changes.

In the absence of predators, *Colpidium* dominated all productivity levels in food webs (Fig. 1e, 1g, Fox 2002). Predators significantly reduced time-averaged proportional biomass of *Colpidium* ( $p < 0.001$ ), with significant but less important effects (as judged by magnitudes of F-values) of productivity and the productivity  $\times$  predator interaction ( $p < 0.05$ ) MANOVA also found a strong main effect of predators and significant but less important effects of productivity and the productivity  $\times$  predator interaction ( $p < 0.001$ ).

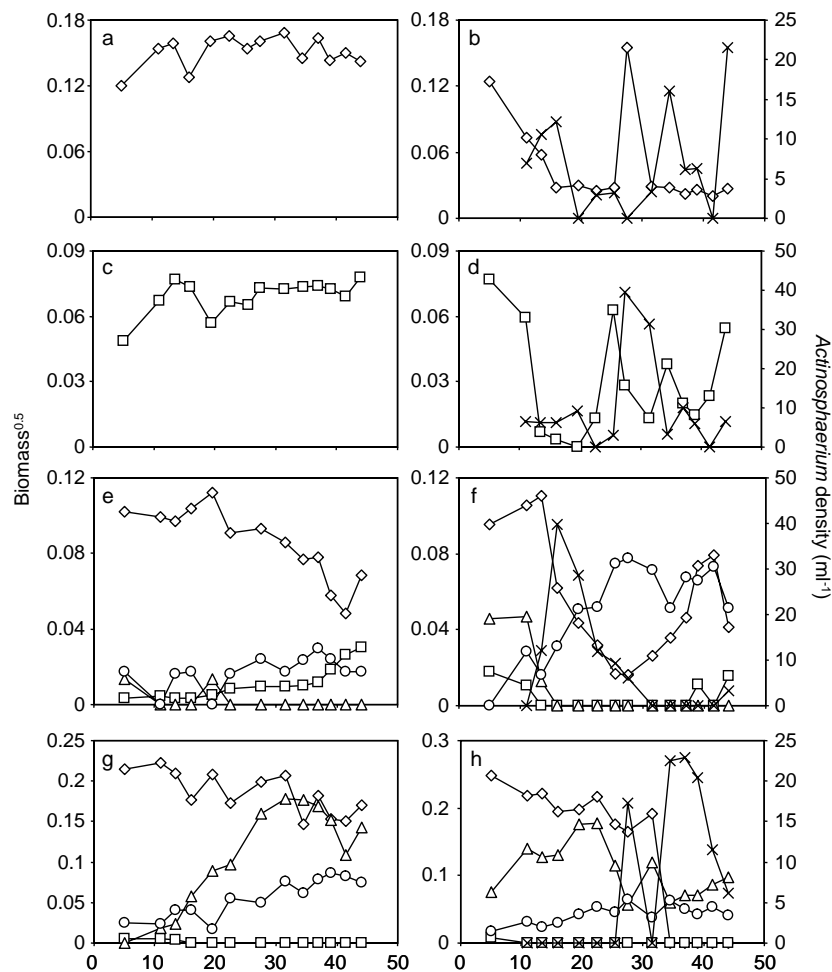


Fig. 1. Population dynamics from representative replicates of different treatments: (a–b) *Colpidium* food chains,  $0.28 \text{ g PP l}^{-1}$ ; (c–d) *Tetrahymena* food chains,  $0.28 \text{ g PP l}^{-1}$ ; (e–f) low productivity food webs,  $0.14 \text{ g PP l}^{-1}$  (e) or  $0.28 \text{ g PP l}^{-1}$  (f); (g–h) high productivity food webs,  $0.84 \text{ g PP l}^{-1}$ ; (a, c, e, g) without predators; (b, d, f, h) with predators. Left vertical axis scale gives prey square-root transformed prey biomasses, right vertical axis scale gives predator densities. Symbols indicate different species: diamonds, *Colpidium*; squares, *Tetrahymena*; triangles, *P. tetraurelia*; circles, *P. caudatum*, crosses, *Actinosphaerium* (predator). Note that vertical scales vary among panels. Only those sampling days included in the statistical analyses of prey biomass and predator density are shown.

However, ANOVAs for the individual sampling days indicated that treatment effects on proportional biomass of *Colpidium* varied in magnitude and direction over time (Fig. 1e–h).

## Discussion

The experiment revealed both top-down and bottom-up effects. Predators reduced time-averaged total prey biomass and shifted prey composition toward more resistant, less-effective grazers, leading to increased bacterial density (a top-down trophic cascade). Increasing productivity increased time-averaged total prey

biomass and predator density (a bottom-up effect). These broad trends accord with findings in many other studies (Shurin et al. 2002), but testing theoretical predictions requires more detailed exploration of the results.

### Temporal variability in protist dynamics

Equilibrium theory predicts that increased prey diversity (species richness) will weaken both top-down and bottom up effects, and that top-down effects will increase with productivity in food chains, but decrease with productivity in diverse food webs. None of these

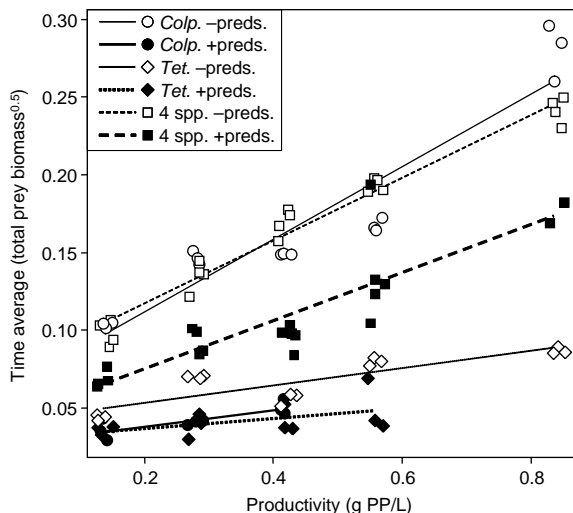


Fig. 2. Time-averaged square root-transformed total prey biomass ( $\text{mm}^3 \text{ ml}^{-1}$ ) as a function of productivity. Each point gives data from a different replicate. Symbols indicate different prey and predator treatments, as shown in the legend; lines are linear regressions.

predictions was consistently supported at all times. Results for effect size of predators on total prey biomass provide a particularly striking example. Depending on the sampling day, predator effect size on total prey biomass could be higher either in food chains or food webs, and could either increase or decrease with productivity. Time-averaging eliminates much of the variation in the data. In particular, within-treatment temporal variation in predator effects on prey biomass and composition often exceeds among-treatment variation in time-averaged means.

Temporal variation in treatment effects reflects asynchrony in predator dynamics among treatments. Similar dynamics probably occur in nature. Theory and experiments show that changes in prey diversity and productivity alter other features of predator dynamics besides time-averaged mean (or equilibrium) predator densities (Luckinbill 1979, Yoshida et al. 2003). Further, natural systems are subject to greater abiotic variability than the microcosms used here, with potentially complex effects on predator–prey dynamics (Brassil 2006). For these reasons, top-down and bottom-up effects generally will be temporally dynamic. The experiment reported here provides the first demonstration of this temporal dynamism in a controlled experiment lacking the complicating factor of abiotic environmental variation.

Temporally-dynamic top-down and bottom-up effects are a ubiquitous feature of even the simplest theoretical models. For instance, the very simple model of Noonburg and Abrams (2005; their Eq. 1) lacks many factors expected to generate persistent oscillations

or complex transient dynamics (e.g. nonlinear functional responses, time lags, abiotic variability) but nevertheless generates long-lasting fluctuations in the strength of trophic cascades for most parameter values (results not shown). The temporally-dynamic top-down and bottom-up effects observed in the present study therefore do not require a special explanation. Rather, it would have been surprising had such dynamism not been observed.

### Time-averaged protist dynamics and equilibrium theory

While temporal variability is ubiquitous in ecological systems, the hope is that equilibrium models will correctly describe time-averaged behavior. Equilibrium theory predicts that (1) increased prey diversity (species richness) will weaken both top-down and bottom up effects, (2) top-down effects will increase with productivity in food chains, but decrease with productivity in diverse food webs, and (3) variation in prey composition within the lowest diversity level will affect the strength of top-down and bottom-up effects. Predictions (1–2) arise because predators and changes in productivity should produce prey density compensation in diverse food webs. Only prediction (3) was supported by the time-averaged data.

There was no evidence that prey diversity and the associated potential for density compensation consistently reduces predator effect size on total prey biomass (prediction 1). Predators reduced dominance of *Colpidium*, the most effective bacterivore, but reduction in *Colpidium* was not associated with strong density compensation by the less-edible species. This result may reflect the fact that competing prey can coexist even in the absence of the predator (Fox 2002), and so do not compete for resources as intensely as assumed by some theoretical models predicting strong density compensation (Leibold 1996). However, only linear food chains exhibited predator effect sizes  $< -3$ , so it may be that prey diversity in food webs buffered against very strong transient top-down effects on total prey biomass.

Increasing prey diversity did not alter the bottom-up effect of productivity on total prey biomass in the presence of predators (prediction 1). The slope of the relationship between productivity and time-averaged total prey biomass in the presence of predators is broadly similar in both food chains and food webs. Nor did increasing prey diversity reduce bottom-up cascading effects on predators (prediction 1). Time-averaged predator density actually increases more strongly with productivity in diverse food webs than in depauperate food chains over most of the productivity gradient. In some replicates, stronger bottom-up effects on

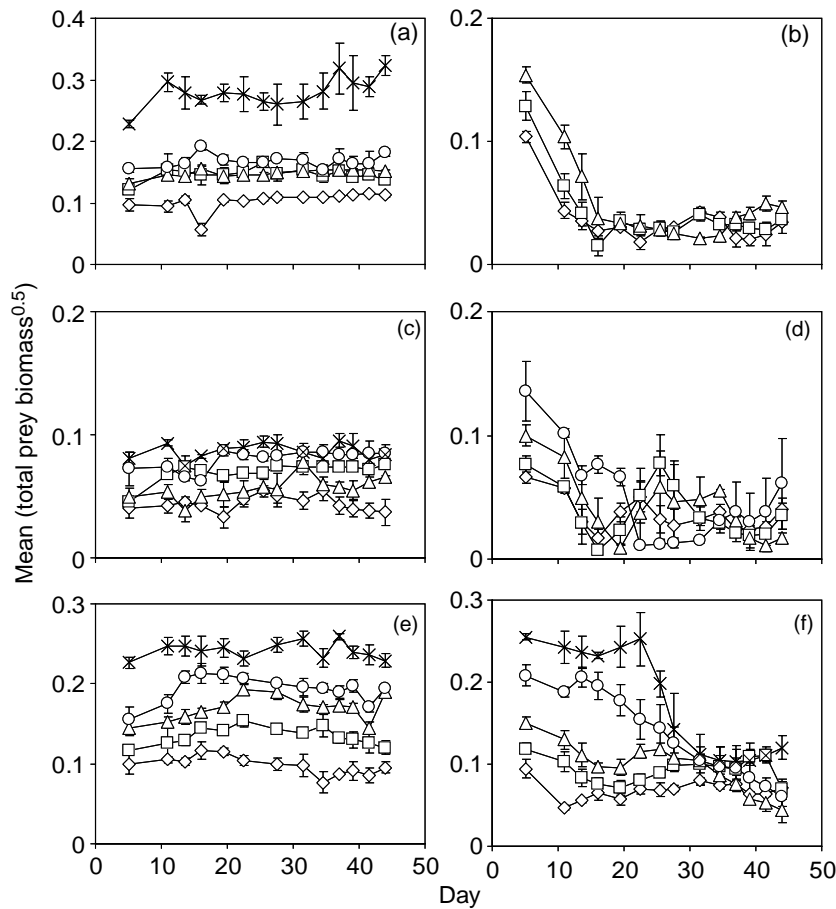


Fig. 3. Mean square root-transformed total prey biomass ( $\text{mm}^3 \text{ml}^{-1}$ ,  $\pm 1$  SE) in each treatment on each sampling day. Symbols indicate different productivity levels: diamonds,  $0.14 \text{ g PP l}^{-1}$ ; squares,  $0.28$ ; triangles,  $0.42$ ; circles,  $0.56$ ; crosses,  $0.84$ . Different panels give different predator and prey treatments: (a–b) *Colpidium*, (c–d) *Tetrahymena*, (e–f) four prey species, (a, c, e) no predators, (b, d, f) with predators. Note that vertical scales vary among panels. Only those sampling days included in the statistical analyses of prey biomass are shown.

predators in food webs may have arisen in part from apparent competition between the highly-edible *Colpidium* and *Tetrahymena* early in the experiment, before *Tetrahymena* went extinct. Apparent competition may also have occurred between the highly-edible prey and the less-edible *P. tetraurelia* in those replicates where predators were large enough (at least for a time) to consume *P. tetraurelia* (Fig. 1f, 1h). Apparent competition should increase predator density, and the effect might become stronger with increasing productivity since apparent competition should increase with productivity (Holt 1977). This argument assumes (as is the case in this system) that prey do not compete so strongly for resources that they cannot coexist in the absence of the predator (Fox 2002, Fox and Barreto, in press). Very strong exploitative competition among prey would weaken apparent competition.

Top-down effects of predators should increase with productivity in depauperate food chains but decrease with productivity in diverse food webs because prey biomass in the presence of predators should increase with productivity only in diverse food webs (prediction 2). However, predators did not reduce the slope of the positive relationship between productivity and total prey biomass in either food chains or webs, except perhaps in *Colpidium* food chains. Failure of predators to prevent time-averaged total prey biomass from increasing with productivity in food chains contrasts with results of a previous microcosm experiment with a different top predator (Kaunzinger and Morin 1998). The reason for the contrast with my results is unknown.

The strength of top-down effects should depend on prey composition as well as prey diversity (prediction 3). Time-averaged predator effect size on total prey



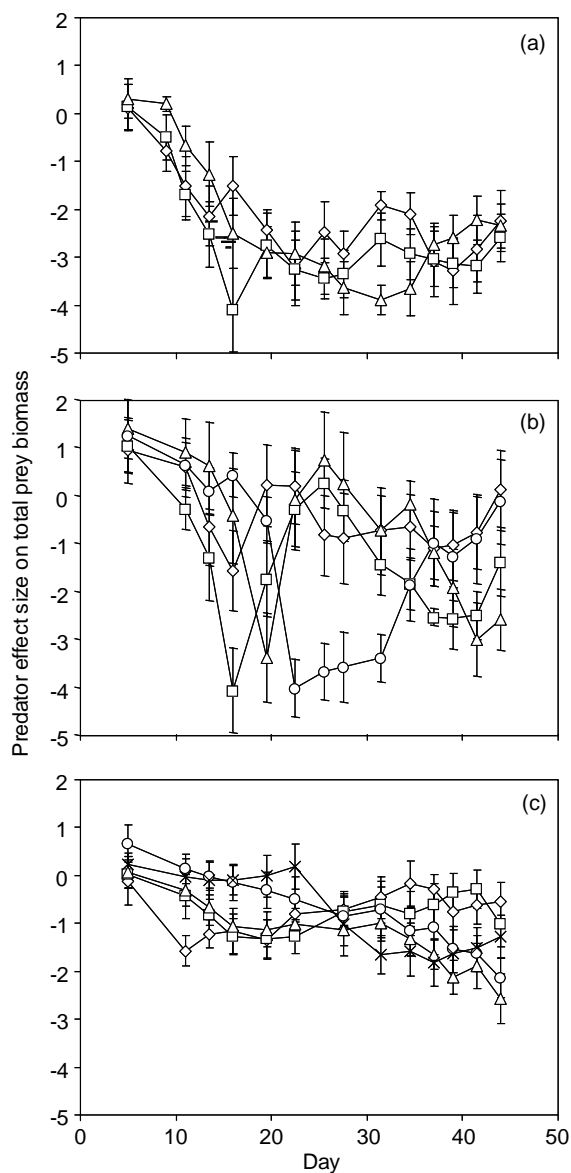


Fig. 4. Predator effect size on total prey biomass ( $\pm 1$  SE) in each treatment on each sampling day. Symbols indicate different productivity levels: diamonds, 0.14 g PP l<sup>-1</sup>; squares, 0.28; triangles, 0.42; circles, 0.56; crosses, 0.84. Different panels give different prey treatments: (a) *Colpidium*, (b) *Tetrahymena*, (c) four prey species. Note that vertical scales are the same for all panels. Only those sampling days included in the statistical analyses of prey biomass are shown.

biomass was weaker in *Tetrahymena* food chains than in *Colpidium* food chains. This result is consistent with equilibrium theory, which predicts stronger trophic cascades in linear food chains with competitively-dominant prey able to attain high biomass in the absence of predators (Leibold 1996). However, relative predator effect sizes in food chains with *Colpidium* vs

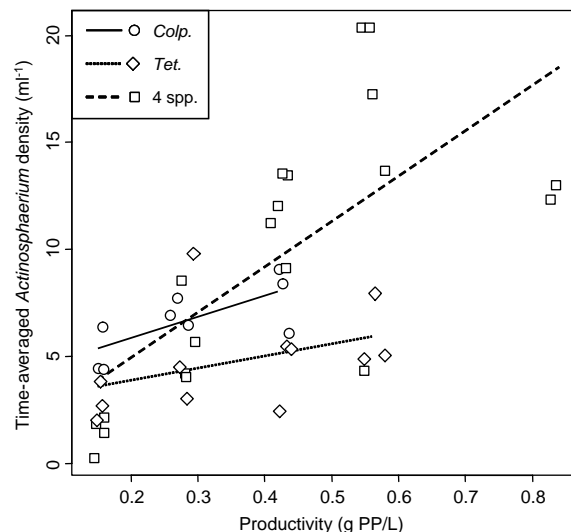


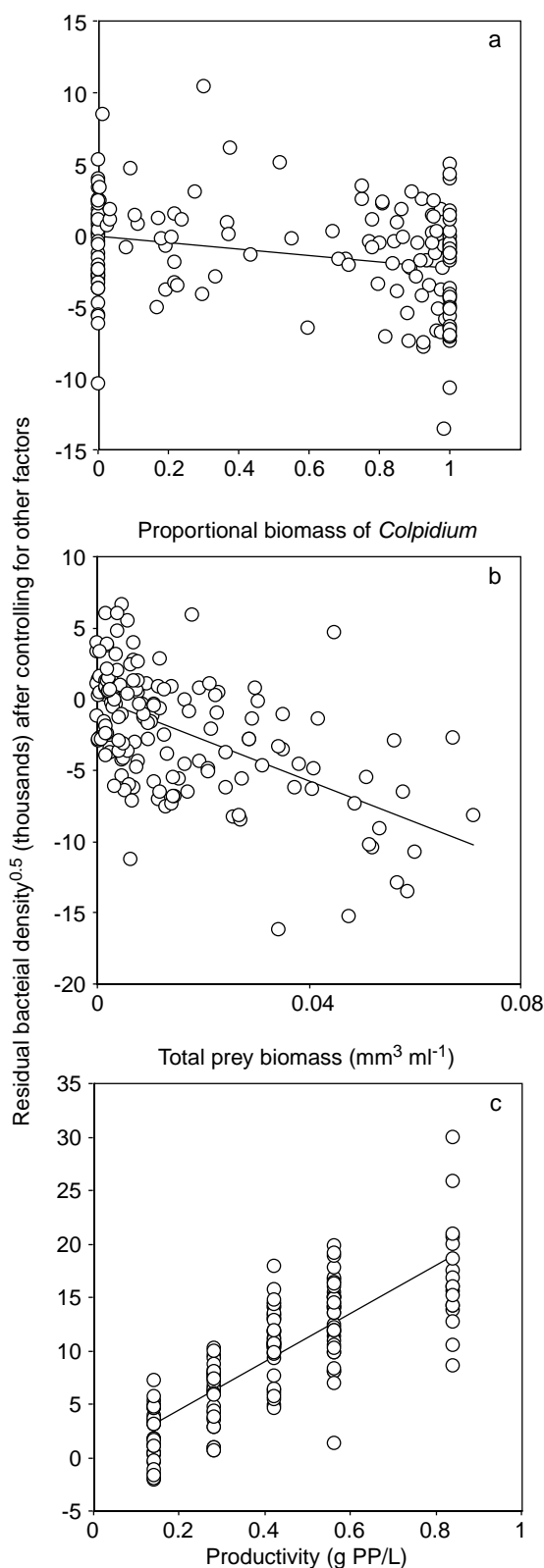
Fig. 5. Time-averaged predator density as a function of productivity. Each point gives data from a different replicate. Symbols indicate different prey treatments, as shown in the legend; lines are linear regressions.

*Tetrahymena* varied greatly over time. The match between the time-averaged data and equilibrium theory therefore should be interpreted cautiously.

Mismatches between time-averaged data and equilibrium predictions may arise from the combination of temporal variability and nonlinear density dependence (Abrams and Roth 1994). Time-averaged densities in a fluctuating, nonlinear system do not generally equal equilibrium densities (Abrams and Roth 1994). In particular, *Actinosphaerium* should have a nonlinear functional response. *Actinosphaerium* must regrow axopods that have been used to capture prey, so that per-capita predation rates at high prey densities should be limited by the rate of axopod regrowth (Suzaki et al. 1980). However, previous work suggests that nonlinearities and temporal variation do not inevitably frustrate equilibrium predictions. The results of Kaunzinger and Morin (1998) accord with equilibrium theory even though their data comprise time averages of a fluctuating system with a predator known to have a nonlinear functional response (C. Kaunzinger, pers. comm., Hewett 1980). Fitting population dynamic models to the time series data could determine the strength of nonlinearities in species' per-capita growth rates, allowing deeper insight into why time-averaged densities sometimes behave as predicted by equilibrium theory, but sometimes do not.

## Bacterial densities

Bacterial density responds more strongly to bottom-up than top-down effects over the range of productivity



levels used here, in the sense that variation in productivity explains more of the variation in bacterial density than variation in total prey biomass or the proportional biomass of the dominant grazer. Of course, total prey biomass increased with productivity, so the multiple regression results must be treated with some caution due to the correlation between these two independent variables. Prey composition appears to have minor effects on bacterial density compared to other factors, a somewhat surprising result given that *Colpidium* is a far-superior bacterial grazer to the other species, especially at low productivity levels (Fox 2002). The weak effect of prey composition on bacterial density suggests that prediction 1 (increased prey diversity will weaken top-down and bottom up effects due to density compensation) was at best weakly satisfied for bacteria.

Failure of prey to suppress bacteria to a constant, productivity-independent density in the absence of predators is not expected from the simplest equilibrium theory, which assumes bacteria comprise a single homogeneous resource (Fox 2002). Failure of prey to suppress bacteria to a constant, productivity-independent density in the absence of predators may well reflect the presence of less-edible or inedible bacteria. Liess and Diehl (2006) found that the relative abundance of (presumably less-edible) bacterial aggregates increases with productivity in this system. Theory predicts that variation in edibility among bacteria should allow total bacterial density to increase with productivity (Leibold 1989, 1996, Abrams 1993).

The multiple regression on bacterial density does not directly test for temporal variation in treatment effects on bacteria. However, total prey biomass and proportional biomass of *Colpidium* vary strongly over time, and variation in these variables apparently generates variation in bacterial density. This provides indirect evidence that predators can generate instability in prey dynamics that cascades to lower trophic levels. In a review of field studies, Halpern et al. (2005) also found that predators destabilize the dynamics of their prey. However, in contrast to my results, field studies find that indirect cascading effects of predators on stability of plant dynamics are near-zero on average (Halpern et al. 2005). This contrast may reflect common features of plant biology (e.g. many plants have seed banks) that stabilize plant dynamics, and which bacteria lack.

Fig. 6. Partial regressions of square root-transformed bacterial density against (a) proportional biomass of *Colpidium*, (b) total prey biomass, and (c) productivity. Each panel shows partial regressions of bacterial density against one factor after controlling for the other two factors.

## Are microcosm trophic cascades unusually strong?

Species interactions in microcosms might be thought to be unusually strong, due to spatial homogeneity, low diversity, and benign, constant abiotic conditions. However, the overall mean effect size (unweighted) of *Actinosphaerium* on total prey biomass ( $-1.28$ , averaged over all 12 sampling days in all treatments) is very similar to the overall unweighted mean effect size of predators on their prey ( $\sim -1.2$ ) in a recent meta-analysis of 102 trophic cascade experiments conducted in a variety of systems (Shurin et al. 2002). Even the largest effect sizes of *Actinosphaerium* observed at any time in any treatment ( $\sim -4$ ) are well within the range of variability for natural aquatic systems (Shurin et al. 2002). While a formal calculation of the indirect cascading effect of *Actinosphaerium* on bacteria is not possible, due to the irregular schedule on which bacteria were sampled, the log of the ratio of the largest to smallest bacterial densities observed in the 162 bacterial samples is 4.53. This value sets a rough upper bound on how large the predator effect size on bacteria could possibly be at any time point. Since 4.53 is within a factor of  $\sim 2$  of the mean predator effect size on plants observed in some natural systems (Shurin et al. 2002, Borer et al. 2005), it seems likely that predator effect sizes on bacteria in this experiment fall within the natural range. Overall, top-down effect sizes apparently are broadly similar in protist microcosms and other systems, although it is unclear whether similarity of effect sizes reflects similarity of underlying ecological mechanisms determining effect size. The mechanisms that determine effect size in nature are largely unknown: a recent meta-analysis found that only 31% of the variation in effect size of predators on plants in natural systems could be explained by commonly-considered biological and methodological factors (Borer et al. 2005).

## Future directions

Ecologists still have much to learn about the factors controlling the strength of trophic cascades (Borer et al. 2005). The “conventional wisdom” that high within-trophic-level diversity reduces the strength of trophic cascades has received only mixed empirical support, even from experiments that directly manipulate diversity while controlling for other factors (this study, Balčiūnas and Lawler 1995, Mikola 1998, Cochran-Stafira and von Ende 1998, Bohannan and Lenski 1999, 2000, Steiner 2001, Shurin et al. 2002, Borer et al. 2005). Further manipulations of diversity and composition on intermediate trophic levels are required to reveal the conditions under which diverse food webs

can be expected to behave as homogeneous food chains and exhibit strong trophic cascades. These manipulations should run as long as possible and incorporate collection of detailed time series data, as the apparent strength of trophic cascades likely will vary over time.

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