I. SUMMARY

We used microcosm systems to test whether simple mathematical models can be valid descriptions of population and community dynamics. Our conclusion is that a priori mathematical formulations of interacting populations are unlikely to produce completely satisfying predictions because they tend to ignore important biological mechanisms. We employed the feedback between microcosm experiments and increasingly refined dynamic models to identify the critical biology governing population cycles in our system, a predator-prey (rotifer-algal) chemostat. Here we summarize the results from five years of work with this system, which confronted us with unexpected yet resolvable biological complexities. First, we found that age structure of the predator population is necessary to generate qualitatively correct predictions of population dynamics (stability versus cycles). Second, we identified rapid evolution of both the algae and rotifers (in separate instances) as critical processes occurring on the same time scale as the ecological dynamics. We have learned that microcosms may not just serve as a means to check model assumptions, but that the results of microcosm studies can lead to novel insights into the functioning of biological communities.
II. INTRODUCTION

Natural communities are complex associations of organisms that interact with each other and with the environment. Communities are intrinsically dynamic: both the number of populations comprising a community and the number of individuals within populations can vary over time (e.g., Tilman, 1996; Kendall et al., 1999). One central goal of ecology is to understand the temporal fluctuations in population numbers that occur in communities—the “abundance” half of one standard definition of ecology (Krebs, 2002). Although there are only a handful of dynamic states that populations may exhibit (stable equilibrium, deterministic extinction, stable population oscillations, and irregular fluctuations), there is a long list of factors that may interact to determine the community dynamics (e.g., competition, predation, parasitism, mutualism; age, stage and genetic structure, and evolution of populations; spatial structure of the habitat, climate, physical and chemical parameters). Thus, a key step in analyzing the community dynamics is to untangle this mixture of interacting factors and to identify those essential for the observed dynamics.

In this chapter we describe our (eventually successful) attempt to reconcile the dynamics of a simple laboratory community consisting of algae and rotifers with the predictions of an even simpler mechanistic model. Our initial goals were to establish a predator-prey system (planktonic algae and rotifers) in laboratory culture and to develop a tractable model that would adequately describe the range of dynamic behaviors. Following these “preparatory steps” our plan was to run the laboratory system under conditions producing sustained, regular predator-prey oscillations and then “force” it by periodic enrichment with nutrients. In theory (Kot et al., 1992), this treatment should drive the population dynamics into deterministic chaos (Fig. 1), a dynamic state frequently predicted by population models (May and Oster, 1976) but rarely observed in the field or the laboratory (Ellner and Turchin, 1995; Costantino et al., 1997). By doing so, we hoped to gain insight into the conflict between the apparent rarity of chaos in nature, despite its prevalence in models and the demonstration (in the Tribolium model system) that chaos is a biological possibility (Costantino et al., 1997). The ability to induce chaos “on demand” in a real predator-prey culture would allow us to investigate open questions about nonlinear population dynamics, such as “are noise and chaos distinguishable in population dynamics?” and “do organisms experiencing chaotic dynamics evolve life-history characteristics that reduce the likelihood of chaos?” However, our “simple” experimental system yielded some biological surprises that soon led our research into different but no less interesting avenues.
A quote attributed to Albert Einstein could serve as the motto for our theoretical and experimental explorations: “Everything should be made as simple as possible, but not simpler.” Following this guideline and starting with a model system made tractable by allowing only for trophic interactions among populations, cumulative experimental evidence (often surprising to ourselves) led us to re-evaluate repeatedly the set of mechanisms we considered essential for explaining the dynamics observed in the laboratory cultures. This is not to say that our study was a string of failures with a happy ending. We would rather understand it as a demonstration of how hypothesis-driven, experimental research can progressively advance ecological understanding (Turchin, 2003). Mathematical modeling enabled us to formulate hypotheses about the expected community dynamics. Experimentation sent us back to the drawing board to refine our hypotheses and to identify the most promising among the competing hypotheses. Repeating this cycle resulted in our identifying evolution as a mechanism essential for explaining the experimental dynamics. The most important lesson we learned is that even in an extremely simplified and heavily
controlled model system a multitude of essential biological processes can operate together, precluding the success of overly naïve modeling approaches. However, purposeful investigation can nonetheless identify the essential biological factors, which can lead to models with more predictive power and better understanding of the mechanisms underlying population dynamics.

We present this work in (nearly) historical order; beginning with Section III, our initial model formulation and the minor adjustments needed to obtain qualitative agreement with the dynamic patterns revealed by the experimental data. Section IV introduces theoretical evidence that the cyclical algal-rotifer dynamics we observed could not result from classical predator-prey interaction alone but that some additional biology was needed to explain the observed population oscillations. We mathematically tested several competing hypotheses about that missing biological mechanism and identified evolution of the algae as the most likely explanation. Section V describes a successful experimental test of this hypothesis showing that evolution (as cyclical clonal selection) does indeed play the role suggested by the models. Finally, Section VI provides evidence that rapid evolution of rotifers can also influence the population dynamics.

III. PREDATOR AND PREY IN THE CHEMOSTAT—A SIMPLE STORY?

Chemostats are laboratory flow-through microcosms used for the continuous culture of planktonic organisms (e.g., Boraas, 1980; Walz, 1993). Chemostats are very suitable for studying population dynamics because all basic processes occurring in the system can be easily translated into differential equations. We cultured two model organisms in the chemostats, the rotifer Brachionus calyciflorus and the unicellular green alga Chlorella vulgaris, to explore the dynamics of populations interacting in a predator-prey relationship. While the growth rate of Brachionus depends on the concentration of Chlorella, the growth rate of Chlorella depends on the availability of nitrogen, the limiting nutrient in our experiments. The “double Monod model” (DMM) (Jost et al., 1973a; Nisbet et al., 1983) is a simple mathematical formulation of this chemostat system. “Double Monod” refers to the fact that the uptake and per-capita recruitment rate of Chlorella (on nitrogen) and Brachionus (on Chlorella) are monotonically increasing but saturating (=Monod) functions ($F_B$ and $F_C$ in (4)) of the nutrient and prey concentrations, respectively (Monod, 1950; Holling, 1959), a more realistic assumption than the overly simplistic linear uptake functions of very early models.
\[
\frac{dN}{dt} = \delta (N_{in} - N) - F_C(N)C
\]
(1)

\[
\frac{dC}{dt} = F_C(N)C - \frac{1}{\epsilon} F_B(C)B - \delta C
\]
(2)

\[
\frac{dB}{dt} = F_B(C)B - \delta C
\]
(3)

with \( F_C(N) = \frac{b_C N}{K_C + N} \) and \( F_B(C) = \frac{b_B C}{K_B + C} \) (4)

In this chemostat DMM, \( N \) is the concentration of nitrogen, \( C \) the concentration of Chlorella, and \( B \) the concentration of Brachionus. \( \epsilon \) is the assimilation efficiency of Brachionus. Nitrogen is continuously added to the system at the dilution rate \( \delta \), all components are removed from the system at the same \( \delta \). \( N_{in} \) is the nitrogen concentration in the inflow medium, \( b_C \) and \( b_B \) are the maximum birth rates of Chlorella and Brachionus. \( K_C \) and \( K_B \) are the half saturation constants of Chlorella and Brachionus. The model equations are balanced, i.e., nitrogen removed from one trophic level is transferred to the next level up, only decremented by the conversion efficiency. Thus, \( \delta \) and \( N_{in} \) are the parameters available for experimental manipulation.

The DMM predicts washout of only Brachionus or Brachionus and Chlorella at high \( \delta \). Lowering \( \delta \) results in co-existence of algae and rotifers at either an equilibrium or at sustained, regular oscillations (stable limit cycles (Fig. 1a)), whose amplitudes increase with decreased \( \delta \). In the model, periodic input of nutrients at the right intensity and frequency forces otherwise regular oscillations into erratic, chaotic fluctuations (Fig. 1b). Chaos is likely to occur when the frequencies of intrinsic and extrinsic fluctuations are incommensurate, generating “dynamic friction” between the two oscillators. It is possible to imagine an experiment in which we attempt to produce chaos in an oscillating chemostat culture by pulsing the nutrient input. In order to minimize unintended side effects, we wanted to do this with the smallest possible amplitude of variation in the nutrient supply rate. We then asked several experts on nonlinear oscillations what ratio between the forcing and natural cycle frequencies would be most effective. We were surprised to find that our panel of experts gave very different answers. They had worked on different kinds of models, and the answer to our question appeared to be highly system-specific. So as a first step towards a forcing experiment, which was still our long-term goal, we needed a trustworthy model for our system.

A look at the results from our system (Figs. 2 and 3; Fussmann et al., 2000) reveals that the experimental dynamics are in partial agreement with
our model predictions. We found co-existence at equilibria when $\delta$ was high, and decreasing $\delta$ destabilized the system to oscillations (Fig. 3c and f). However, we also identified three major discrepancies with model predictions.

1. Decreasing $\delta$ to very low values did not produce high-amplitude oscillations, as the DMM predicts (Fig. 3a and d). Rather, the system re-stabilized to equilibrium dynamics (Fig. 3c and f).

2. Cycle periods were irregular and too long compared to prediction. The predicted period was $<10$ days; those observed were $>20$ days.

3. The predator and prey cycles were nearly exactly out of phase, meaning that maxima of algae and minima of rotifers (and vice versa) occurred at almost the same time.

These discrepancies between observed and predicted dynamics prevented us from pursuing our original plan to force the chemostat system into chaotic population dynamics. First, we needed to develop a better understanding of the dynamic behavior of this deceptively simple system.

One obviously unsatisfying assumption of the DMM is that the predators experience no losses other than washout from the chemostat vessel. At very low dilution rates (and, therefore, slow washout), this leads to extreme, sustained oscillations because even a miniscule nutrient input suffices to maintain positive net growth of algae and rotifers. Introducing a mortality term for the rotifers into the DMM produces stable dynamics at low dilution rates (Nisbet et al., 1983), a more realistic behavior that has also
been observed in other multispecies chemostat studies (Jost et al., 1973b; Dent et al., 1976). For our specific system it became evident (from counts of dead rotifers and eggs in the cultures) that the rotifers suffered two different kinds of loss in addition to washout: mortality in the chemostat and loss of fecundity as they senesce. To accommodate these two factors we partitioned (3) into two equations, one for the total concentration of Brachionus B and one for the concentration of reproducing Brachionus R (Fussmann et al., 2000):

\[
\frac{dN}{dt} = \delta(N_{in} - N) - F_{C}(N)C
\]

Figure 3 Stable and unstable dynamics in model simulations and experimental predator-prey cultures as a function of chemostat dilution rate \(\delta\). Model predictions (a, b, d, e; \(t = 1500\) to 2000 days) and experimental data (c, f) for minima, maxima (a to c) and coefficient of variation (CV = standard deviation/mean [%]; d to f) of time series. Thin lines or empty symbols: Chlorella. Thick lines or filled symbols: Brachionus. Headings refer to the model used for computation. Experimental data computed for 14 chemostat trials. Lines in (c, f) connect minima and maxima of one chemostat trial. Large differences between minima and maxima and high CV indicate unstable, oscillating dynamics, low differences and CV (noisy) equilibria. Because minima/maxima in long experimental time series are usually outliers, minima/maxima were computed from smoothed data (local polynomial regression). See Fussmann et al. (2000) for transformation from individual to molar concentrations in (c) and for computation of CV in (f). \(\lambda = 0.4\) d\(^{-1}\), \(m = 0.055\) d\(^{-1}\), other parameters as in Fig. 1. Reproduced with permission from AAAS/Science.
\[
\frac{dC}{dt} = F_C(N)C - \frac{1}{\epsilon} F_B(C)B - \delta C \tag{6}
\]
\[
\frac{dR}{dt} = F_B(C)R - (\delta + m + \lambda)R \tag{7}
\]
\[
\frac{dB}{dt} = F_B(C)R - (\delta + m)B, \tag{8}
\]

with \( F_C(N) \) and \( F_B(C) \) defined in (4), and \( m \) and \( \lambda \) as the rotifer instantaneous mortality and senescence rates, respectively. We used counts of dead rotifers from chemostat cultures to determine \( m \), counts of subitaneous eggs per rotifer provided estimates for fecundity and \( \lambda \).

With the addition of rotifer mortality and senescence, the model correctly predicted the transitions between different qualitative dynamic behaviors observed in the chemostat system: extinction, equilibria, and population cycles (Fig. 3b, c, e and f). Its successes demonstrated that a simple mechanistic model could capture many of the major features of complex multispecies dynamics. However, the model was still unable to account for the observed cycle period and phase relations. These failures indicated that the chemostat system held ecological complexity yet to be discovered.

IV. TESTING HYPOTHESES OF MECHANISM

Because the Fussmann et al. (2000) model was unable to account for some key features in the observed cycles, we inferred that the model neglected at least one biological mechanism crucial to the system—but which one?

A closer look at the predator’s per-capita recruitment rates provided a clue. To begin, we smoothed the observed time series of prey and predator densities with penalized regression splines (Simonoff, 1996). Such splines are continuously differentiable and thereby provide smoothed measures of population growth rates over time. To estimate the predator’s per-capita recruitment rates over time, we divided smoothed population growth rates by smoothed densities and adjusted for dilution and mortality rates. Plots of those recruitment rates against smoothed prey densities show a revealing pattern.

For any level of prey density, the Monod function (4) predicts a single level of predator recruitment rate. As populations cycle, theory would have the recruitment rate slide back and forth along the Monod curve. That did not happen. Instead, as our experimental populations cycled, recruitment rate itself cycled (e.g., Fig. 4), with more than one level of predator recruitment rate for any level of prey density. Furthermore, these rates were consistently lower after peaks in predator density and higher after troughs.
We initially suspected predator interference. However, predator interference alone could not account for the pattern, because there were many pairs of times \((t_1, t_2)\) when population densities were approximately equal \([C(t_1) = C(t_2), B(t_1) = B(t_2)]\), but recruitment rates were still quite different \([F_B(t_1) \neq F_B(t_2)]\) (Shertzer et al., 2002). Predator per-capita recruitment rates were consistently low after peaks in predator density and high after troughs. This suggested an explanation drawing increased attention in planktonic communities: organism quality (Nelson et al., 2001; Scheuerell et al., 2002).

Our first hypothesis was that algal size might be changing through the course of the population cycles, hence algal cell counts would not properly reflect the actual amount of food available to rotifers. To investigate this we used an automated particle counter to track changes in the algal size distribution through the prey cycle. We quickly discovered that there were no systematic trends in algal size that could account for the trends in rotifer recruitment seen in Fig. 4.
We next undertook a more systematic search for the missing mechanism. We developed four simple mechanistic models, each an extension to the original Fussmann et al. (2000) model, incorporating a single additional mechanism for which some empirical support existed (Shertzer et al., 2002). The first model posited that viability of rotifer eggs increases with food availability; the second that the nutritional value of algae increases with their cellular carbon:nitrogen content; the third that rotifer waste products limit population growth; and the fourth model assumed that the algae could evolve rapidly in response to predation. Abrams and Matsuda (1997) had shown that a model including prey evolution in response to varying predation pressure could produce out-of-phase predator-prey cycles qualitatively similar to those observed in our system, and our model (described in the following text) was similar to theirs in assuming a trade-off in prey between intrinsic growth rate and vulnerability to predation. The new models were treated as hypotheses to be tested. Each was fit to the time-series data using two separate methods: trajectory matching and probe matching (Kendall et al., 1999). The models were evaluated for the ability to fit prey and predator densities and to explain the key features in observed cycles that the original model could not. Those models (hypotheses) inconsistent with the chemostat data could be discarded.

After all four models were evaluated against the data, only one hypothesis was left standing—rapid prey evolution (Shertzer et al., 2002). In our model for that hypothesis, like the Abrams and Matsuda (1997) model, prey are able to evolve defense against predation, but only with a trade-off in their maximum population growth rate. Our model differed in assuming that the trade-off is mediated by a quantitative physiological trait \( z \), that determines both maximum growth rate and the vulnerability to predation and is under stabilizing selection in the absence of predation. The dynamics of \( z \) were assumed to depend on the partial gradient of the algal per-capita growth rate with respect to the trait value of an invading mutant, following the standard quantitative-trait model (Lynch and Walsh, 1998):

\[
\frac{dz}{dt} = V \frac{\partial}{\partial z_i} \left( \frac{1}{C_i} \frac{dC_i}{dt} \right),
\]

where \( V \) is the additive genetic variance, \( z_i, C_i \) are the trait value and density of a rare invading algal clone and the right-hand side is evaluated at \( z_i = z \), the current trait mean in the population. As the value of \( z \) increases, the model dictates that the vulnerability to predation decreases, but so does the population growth rate in the absence of predators. Thus, the quantitative-trait model explicitly assumes a nonlinear trade-off between algal vulnerability to predation and maximum population growth rate. Only the prey evolution model reproduced the long, out-of-phase predator-prey cycles we
observed in our chemostats. Our success with the quantitative trait model provided more than just a satisfying explanation of the observed cycles: it identified a plausible mechanism that could be tested directly with fresh experiments.

V. RAPID EVOLUTION: A CLONAL APPROACH

Given the success of the prey evolution model in producing a qualitative fit to our empirical observations, we then returned to the organisms themselves to test its core predictions: (1) a trade-off in algal phenotypes between defense against rotifer grazing and the cost of that defense, (2) the existence of multiple algal genotypes that differ in their position on the trade-off curve, and (3) prey evolution in the chemostats that produces long, out-of-phase predator-prey cycles. We found that chemostats containing clonally variable algae had dynamics that match closely to a model modified (see the following) to permit clonal evolution, whereas chemostats containing a single algal genotype had dynamics matching those of standard non-evolutionary predator-prey models.

To test for the existence of different Chlorella genotypes along the trade-off curve, algae from our stock cultures were exposed to either of two different selection conditions (rotifers present or rotifers absent), and then they were used to determine the effects of this selection under conditions common to both groups (Yoshida et al., 2004). We measured “algal food value” as a proxy for the strength of algal defense against predation. Algal food value was estimated as rotifer population growth rate when fed algae at a sufficiently high concentration. Algae cultivated under constant and intense rotifer grazing pressure became lower in food value relative to algae grown in the absence of rotifers but with a comparable mortality rate imposed by an elevated chemostat dilution rate (Table 1). The low-food-value algae were also slightly smaller, denser in terms of C and N content, but lacked obvious structural differences under transmission electron microscopy (T. Yoshida, 2003, unpublished data). This heritable response to rotifer predation shows that the selected low food-value algal genotypes are better able to survive rotifer grazing, although the mechanism for this remains to be determined. However, in contrast to assumptions made in our quantitative trait model and the Abrams-Matsuda (1997) model (Section IV), no significant difference was found in algal maximum population growth rate under nutrient rich conditions (Table 1). Instead, algae selected in the presence of rotifers were competitively inferior in the sense that their growth rate was lower in nutrient deficient conditions, compared to algae selected in the absence of rotifers (Table 1). Thus, the cost of algal defense only becomes apparent when nutrients are scarce. In essence, the
low-food-value algae have a higher half-saturation constant for growth response to N in Monod growth kinetics. Contrary to the assumptions of the quantitative trait model, the results demonstrated the existence of different genotypes in the algal population and a trade-off between algal food value and competitive ability among clones.

These findings required us to change the evolutionary model and re-examine whether it could still account for observed cycle properties. At the same time we abandoned the simplifying assumption of quantitative trait dynamics, which had been imposed so that the four contending models would have similar numbers of parameters, in favor of explicitly modeling clonal selection. Clonal selection is appropriate because *C. vulgaris* is obligately asexual (Pickett-Heaps, 1975), and because we demonstrated the presence of multiple clones in our laboratory populations (Table 1: Yoshida *et al.*, 2004). Just as importantly, a clonal model allows genetic variation to be a dynamic variable in our model whereas variation is fixed in the quantitative trait model. It turns out that allowing clonal variation to respond to selection yields a much better fit to the dynamics we observe.

Thus, we replaced the single algal variable with a set of competing clones specified in terms of their food value, $p$, and related competitive ability. Here, $p$ represents relative value as food for rotifers, and ranges between 1 (“good”) and $\approx 0$ (“poor”), with lower $p$ clones having a reduced predation risk. Thus, low food value comes at the cost of reduced fitness when nutrients are scarce. The relationship between food value and fitness is conveniently defined by a trade-off curve. In our experiments (noted above), the cost of defense was only evident when nutrients are scarce, so we assumed a trade-off between algal food value, $p$, and the half-saturation constant for uptake of the limiting nutrient, $K_c$. A two-parameter trade-off curve (shape parameter $\alpha_1$, and scale or “cost” parameter $\alpha_2$) was used:

### Table 1  Evolutionary trade-off between algal food value and competitive ability for *Chlorella vulgaris*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Algal food value ($d^{-1}$)</th>
<th>$80 \ \mu$mol nitrate $1^{-1}$</th>
<th>$1 \ \mu$mol nitrate $1^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotifers present</td>
<td>$1.07 \pm 0.05$</td>
<td>$2.00 \pm 0.05$</td>
<td>$1.25 \pm 0.02$</td>
</tr>
<tr>
<td>Rotifers absent</td>
<td>$1.58 \pm 0.05$</td>
<td>$2.02 \pm 0.02$</td>
<td>$1.73 \pm 0.03$</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. Algal food value was measured by rotifer population growth rate when feeding on algae at sufficiently high concentration. Difference in algal growth rate at a nutrient-deficient condition ($1 \ \mu$mol nitrate $1^{-1}$) but not at a nutrient-rich condition ($80 \ \mu$mol nitrate $1^{-1}$) indicates that algae selected under rotifer grazing pressure are poorer competitors for scarce nutrients but have the same maximum population growth rate when nutrients are plentiful.
\[ K_c(p) = K_{c,\text{min}} + \alpha_2(1 - p^{z_1})^{1/z_1}. \]  

Note that \( K_c \) attains its minimum value at \( p = 1 \), so reducing a clone’s food value also effectively reduces its ability to compete for nutrients. Examples of trade-off curves are found in Fig. 5a and c. In the simulations we set a minimum food value, \( p_{\text{min}} \), above which food values were randomly chosen, since a food value too close to 0 would cause rotifer populations to collapse immediately (Table 1), contrary to our observations.

Figure 5  Model-predicted effects of prey clonal diversity on population dynamics (from Yoshida et al., 2003). (a, c) Trade-off curves between algal food value and competitive ability (where competitive fitness is represented by half-saturation, \( K_c \), for nutrient uptake by algae), with \( z_1 > 1 \) (a) and \( z_1 < 1 \) (c). (b, d) Effects of clonal diversity on predator-prey cycle length for the two trade-off curves between given \( z_1 > 1 \) (b) and \( z_1 < 1 \) (d). Cycle periods in (b) and (d) result from 700 simulation runs each for one, two, three, five, or seven clones initially present. Cycle periods are plotted against the number of clones surviving after the model is run long enough for competitively inferior clones to be gone extinct. ‘Degenerate’ cases (in which some of the clones initially present were eliminated) are plotted immediately to the right of non-degenerate cases in panels (b) and (d). (e, f) Cycles predicted by the model for the Brachionus predator (solid line) and Chlorella prey (dotted line): (e) single-clone system; (f) multiple-clone system.
Where the original model (5) to (8) assumes that rotifer feeding rate is a function of total algal density, our clonal model allows rotifers to respond to the relative abundance of clones with different food values. The equation for rotifer feeding rate is based on the “clearance rate,” which is the volume of water per unit time that a rotifer clears of prey. Feeding trials with our study organisms (T. Yoshi da, 2003, unpublished data) showed that clearance rate increases as algal density falls, but eventually reaches a maximum value at some critical algal density \( C^* \). Further decreases in algal density below \( C^* \) are not compensated by increases in clearance rate. Above \( C^* \), the total feeding rate is described well by a Monod equation, as in (4). This led us to use the following equation for the rotifer clearance rate:

\[
g(\bar{C}) = G/(K_b + \max(\bar{C}, C^*))
\]

(11)

Here \( G \) is a parameter determining the maximum clearance rate, and \( p_i \) is the food value of the \( i \)th clone; therefore, \( \bar{C} \) is the total food value of the algal population. An alternate form of (11) was also tried, in which clearance rate depended on total algal density irrespective of food value, but our experimental data from cycling populations could be fitted better using (11). The rate at which rotifers consume algae with food value \( p_i \) is therefore

\[
F_{b,i} = C_i p_i G/(K_b + \max(\bar{C}, C^*))
\]

(12)

and consequently the total feeding rate on the entire \textit{Chlorella} population is

\[
F_b(\bar{C}) = \bar{C} G/(K_b + \max(\bar{C}, C^*))
\]

(13)

In addition, we expressed the clonal model in terms of cell counts rather than nitrogen concentration, because this made it easier to do parameter estimation by calibrating the model against experimental cell-count data. The model then becomes (Yoshida et al., 2003, online supplement):

\[
\frac{dn}{dt} = \delta(VN_{in} - n) - \sum_{i=1}^{q} F_{c,i}(n/V)c_i
\]

\[
\frac{dc_i}{dt} = \chi_c F_{c,i}(n/V)c_i - F_{b,i}b - \delta c_i, \quad i = 1, 2, \ldots, q
\]

\[
\frac{db}{dt} = \chi_b F_b(\bar{C})r - (\delta + m)b
\]

\[
\frac{dr}{dt} = \chi_b F_b(\bar{C})r - (\delta + m + \lambda)r
\]

(14)

Here \( V \) is the chemostat volume, \( n = NV \) is the concentration of nitrogen (\( \mu \)mol per chemostat), \( c_i \) is the cell count for \textit{Chlorella} clone \( i \) (\( 10^9 \) cells per chemostat), \( q \) is the number of clones, and \( b \) and \( r \) are the total population and the fertile population of \textit{Brachionus} (individuals per chemostat),
respectively. The $\chi$ parameters are conversions between consumption and recruitment and were not present in (5) to (8), because all state variables there were expressed in terms of total nitrogen.

Bringing the evolutionary model into line with our subsequent experiments did not change its fundamental prediction: evolution of algal food value substantially alters population cycles. Model simulations with randomly generated sets of 1, 2, 3, 5, or 7 clones show that when multiple clones are present, long cycles are possible (Fig. 5b and d) irrespective of the trade-off curve specified between food value and competitive ability (Fig. 5a and c). If the trade-off curve is concave down (Fig. 5a), it is more likely that the surviving co-existing clones are two extremes in food value. However, where the trade-off curve is concave up (Fig. 5c), intermediate (in terms of food value) algal types are most likely to persist.

Cycle lengths for two or more co-existing clones varied in period. Short cycles could occur if the algal population only contains two very similar clones, but with more than two clones short cycles become rare. In contrast, a single-clone system always produces relatively short cycles with the classic phase relation: peaks in predator abundance follow peaks in prey abundance by a quarter cycle (Fig. 5e). However, multi-clone cycles with two or more phenotypically divergent clones have phase relations resembling those observed, with algae and rotifers almost exactly out of phase (Fig. 5f), as a result of ongoing changes in clonal frequency driven by rotifer predation and competition for nutrients.

These cycle properties result from low food-value clones becoming dominant while the algal population is being reduced by grazing. Then, once the rotifer population has crashed due to food shortage, the algal density again increases but the rotifers do not respond immediately. Rotifer populations cannot increase until the algae return to high density, at which point the higher food-value clones (which are better competitors) increase in abundance. The rotifers can then increase, eventually becoming abundant enough to start grazing the algae down and start another round of the cycle.

To test these model predictions we returned to the laboratory, manipulating prey evolution by altering clonal diversity (i.e., genetic variability) in the prey population. We initiated replicated chemostat trials either with a single clone as an evolutionarily “stagnant” population or with multiple clones as an evolutionarily “active” one, at dilution rates and nutrient concentrations that would produce population cycles. We then compared cycle periods and phase relations between the treatments (Yoshida et al., 2003).

Exactly as predicted, the population cycles in the single-clone, evolutionarily stagnant treatment were much shorter and of smaller variance than those in the multiple-clone treatment. In addition, the single-clone treatments had a much shorter phase delay, just slightly longer than the classical quarter cycle (Figs. 6 and 7). Furthermore, the clonal model was able to...
match the experimentally determined bifurcation diagram of the system (the transition points between stable and cyclic dynamics as the dilution rate is varied) more accurately than the non-evolutionary model (Fig. 8).

Because the algal taxon that we used, *C. vulgaris*, is obligately asexual, it is perhaps necessary to explain why we consider the process of cyclical clonal replacement to be algal evolution rather than algal community
We do not wish to review here the long-standing debate over what constitutes a species, particularly under conditions of asexual reproduction. Rather, we simply state that we are satisfied that the dynamics are driven by a change in genotypes through time and that this satisfies fundamental definitions of evolution that refer to heritable change in form over many generations. To get the pattern we observe empirically, our model tells us that the genetic mean and variance of our algal population must have changed over the course of many cell divisions through the process of natural selection and response. Additionally, it is worth noting that the documented impact of rapid selection response on predator-prey dynamics does not depend upon the clonal nature of the prey in our system. Both our quantitative trait model (Shertzer et al., 2002) and that of Abrams and

Figure 8  Bifurcation diagram comparing experimental results with model predictions for stability versus cycles in the chemostat system, as a function of the dilution rate $\delta$ at $N_{in} = 80 \mu$mol nitrate $l^{-1}$. Vertical lines show the predicted locations of Hopf bifurcations, yielding stability at low and high dilution rates and cycles at intermediate rates. Solid lines are the predictions of the clonal selection model, dashed lines the predictions of the single-clone model. Plotted values (circles and squares) are the coefficient of variation (CV) of predator and prey populations, corrected for effects of sampling error; these are re-drawn using the same values as in Fig. 3 of Fussmann et al. (2000). Two of the seven high-CV experiments plotted in this figure had only one cycle of data, and therefore were not used for estimates of cycle period and phase lag (Fig. 7). Reproduced with permission from AAAS/Science.
Matsuda (1997) produce qualitatively similar results without specifying the nature of inheritance. A similar argument applies to our interpretation of rotifer evolution in the next section.

VI. RAPIDLY EVOLVING ROTIFERS

Predators can also evolve rapidly, either separately or through co-evolution with their prey (Abrams, 2000; Bohannan and Lenski, 2000). We have not yet investigated whether the predators in our experimental system display evolutionary changes in response to the cyclically varying food value of their prey, but it is significant that the experimental results previously described made sense in the light of prey evolution alone. We conclude this review of our work on algal-rotifer chemostats with an example demonstrating the importance of predator evolution in a non-trophic context (Fussmann et al., 2003).

Monogonont rotifers like Brachionus exhibit cyclical parthenogenesis, that is, a population persists through time with phases of amictic (ameiotic, asexual) and mictic (sexual) reproduction (e.g., Gilbert, 2003). In B. calyciflorus, crowding induces amictic females to produce mictic daughters (Gilbert, 1963) but there is significant clonal variation in the propensity to produce mictic females in response to an (unknown) crowding stimulus (Gilbert, 2002). We found that this genetic variance, which is usually present in naturally occurring rotifer populations, may be subject to rapid clonal selection, with serious implications for the population dynamics of the algal-rotifer system (Fussmann et al., 2003).

We ran replicated chemostat experiments that differed in two important ways from the experiments described in the previous sections. (1) We started our cultures with rotifers directly sampled from the field, that is, our inoculum presumably comprised a multitude of clones. (2) We ran the experiments at higher nutrient concentrations of 514 μmol nitrate l⁻¹ which, due to enrichment, resulted in the extinction of the rotifer population after one predator-prey cycle (Fig. 9). What we observed in all four replicate chemostats were not the simple “boom-and-crash dynamics” that our standard model (Fussmann et al., 2000) predicted. Instead, the experimental rotifer dynamics showed two distinct local maxima of abundance before going extinct (Fig. 9). Again we found ourselves in a situation where our basic mathematical model was just a bit too simple and where we needed to invoke some additional biology to reconcile experimental observation with mathematical prediction. Daily inspection of our cultures gave us a strong indication of what that additional biology might consist: our cultures were rife with mictic rotifer females and, consequently, males (Fussmann et al., 2003) (historically, the experiments described in this section were the earliest we
performed; the propensity to reproduce sexually faded quickly away under permanent chemostat culture and did not interfere with experimentation reported in Sections III to V. It is important to realize that initiating sexuality in the chemostats could not result in reproduction, because experimental conditions (constant bubbling) prevented mating, and even if not, sexually produced eggs undergo diapause and would thus wash out of the chemostat vessel long before hatching. Therefore, investing in reproduction in the chemostat means, for a rotifer, investing in an evolutionary dead-end.

Given the enormous number of sexually produced animals, we concluded that mixis had to be the key to explaining the peculiar dynamics we observed.

Using successive amendments to the basic model (Fussmann et al., 2000) we found that both mixis and evolution against mixis were necessary to produce the bimodal pattern of abundance of *Brachionus* (Fig. 9). Evolution was incorporated into the mathematical model as directional selection for the quantitative trait “propensity to reproduce sexually” (which evolves toward asexual reproduction; see Fussmann et al., 2003, for details). Incidentally, invoking the evolutionary process is not just a mathematical gimmick that does the trick but is corroborated by biological facts: (1) our initial sample of rotifers was very likely to be clonally diverse and, therefore, provided the genetic variance on which selection could act; and (2) in long-term chemostat cultures of *Brachionus* we observed an exponential decay of the propensity to reproduce sexually (see Fig. 1 in Fussmann et al., 2003).

![Figure 9](image.png)

**Figure 9** Predator dynamics of *Brachionus* subject to evolution against mictic reproduction. Comparison of predictions by three different model versions with real, experimental data (empty circles). “Basic model” indicates the time series predicted by model (5) to (8) (Fussmann et al., 2000) which does neither account for mixis nor selection against mixis. The best fit of a model version including “mixis only” provides a quantitatively better prediction but fails to match the bimodal abundance pattern of the experimental data that only a model version including selection is able to produce. Corresponding data of the prey (*Chlorella*) are not shown.
Interaction and feedback between theory and experiment allowed us to learn much about our system, especially from conflicts between models and data: stability at low dilution rate rather than large-amplitude cycles, out-of-phase cycles in persistent populations, and the double-hump in rotifer abundance during the unstable boom-crash cycle under highly enriched conditions. Working with short-lifespan organisms in microcosms allowed rapid feedback between theory and experiment, with our experiments providing real challenges to theory due to tight experimental controls and good data. Instead of being free to explain the discrepancies by invoking unmeasured or uncontrolled variables (or misinformation from our allies), we had to clarify them by erecting and then testing new hypotheses and the corresponding new models.

Some ecologists have complained that microcosms are not real ecological systems, so that microcosm studies, at best, have limited relevance (Carpenter, 1996; see Huston, 1999, for a different opinion). However, framing the issue that way is a red herring: the real question is whether microcosms help us to understand real ecological systems. One does not ask if a novel is real, one asks if it provides real insights. One important way that microcosms can enhance our insight, illustrated by our studies, is that the necessity to fully explain our data forces us to invent new hypotheses which may carry over to the field. Our studies showed that evolutionary change could be an important “engine” driving ecological dynamics, even though the evolutionary trade-offs are so subtle that experiments under extreme conditions (Table 1) were required to reveal the costs associated with the benefit of reduced predation risk. The implication for the field is clear: even cryptic evolutionary changes may have large impacts on ecological interactions and dynamics. Another way microcosm studies can enhance our insight into real systems is by providing “quality control” on ecological theory, so that the models and hypotheses we use as the basis for field experiments or for interpreting data on macroscopic ecosystems have survived rigorous scrutiny using real organisms.

We believe that these potential payoffs are amply validated by past successes. Approaches to stage-structured population modeling that were originally developed to explain contrasting patterns of cyclicity in laboratory insect populations (Gurney and Nisbet, 1985) are now providing important insights into natural population regulation and cycles (Murdoch et al., 2002, 2003), and chemostat studies provided the first validation for the $R^*$ rule, now one of the basic principles of resource competition theory (Tilman, 1982). We hope to emulate these examples, initially with planktonic systems in small temperate lakes, where large-amplitude oscillations in
phytoplankton and zooplankton abundance are prominent and regular features of seasonal succession (e.g., the annual “clear water phase”). In addition, the pervasive importance of rapid evolution in our system brings us back full circle to the question that originally motivated our study: if we readily find complex dynamics in models and the laboratory, why do we mostly see cycles and stability in nature (Kendall et al., 1999)? Theoretical work to date including our own (Schliekelman and Ellner, 2001; Shertzer and Ellner, 2002) has assumed “slow” evolution in which organisms’ life histories are fixed adaptations to long-term patterns of variability, and has failed to resolve the issue of why evolution does not emerge as a strong or consistent force for population stability (Mueller and Joshi, 2000). So instead of testing current models directly (as we had planned), we have done so indirectly by calling into doubt their basic premise about evolutionary dynamics, thus setting the stage for fresh theoretical approaches to the macroecology of population stability.

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