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STABLE COEXISTENCE IN A FLUCTUATING ENVIRONMENT: AN EXPERIMENTAL DEMONSTRATION

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Abstract. Adding fluctuations to models of resource competition provides one solution to Hutchinson's paradox. Fundamental to fluctuation dependent mechanisms of coexistence are differential species' responses to environmental fluctuations through time. The covariance between the environment and competition this generates leads to compensatory population dynamics, now thought to be important for community stability. We tested this theory experimentally using freshwater diatoms. We measured the growth of *Cyclotella pseudostelligera* and *Fragilaria crotonensis* over a range of temperatures. *Cyclotella* showed a higher growth rate and higher silicate assimilation rate between 6°C and 18°C, while *Fragilaria* showed greater competitive ability above 18°C. These results were used to parameterize a resource competition model. Numerical simulation of this model predicted stable coexistence and compensatory dynamics under fluctuating but not constant temperature. A long-term competition experiment with *Cyclotella* and *Fragilaria* confirmed the predictions of the model. These results demonstrate the role abiotic fluctuations may play in maintaining the diversity of natural communities.

Key words: *competition; diatoms; stability; storage effect; temporal variation.*

INTRODUCTION

Ecological communities are exposed to environmental fluctuations across a range of temporal scales. Differential species responses to short- and long-term environmental variation ensure that communities are in a constant disequilibrium. The changes in abundance that accompany species responses to environmental variation can have profound effects upon community structure and functioning. Ecologists now recognize the dual role environmental variability plays in explaining community diversity and stability (Chesson et al. 2002). Despite significant advances in our theoretical understanding of this problem unambiguous experimental validation of fluctuation dependent (nonequilibrium) stable coexistence remains scarce. Here, by coexistence we mean that the densities of competing species in a fluctuating environment do not show long-term trends; if densities decline they tend to recover so that species are retained in the community over long times scales (Chesson 2000).

Under the hypothesis of a constant environment, the diversity of natural phytoplankton seems to contradict the competitive exclusion principle (Hardin 1960). Classical theory states that the number of coexisting species cannot exceed the number of limiting resources. As a solution to this paradoxical situation, Hutchinson (1961) suggested that environmental fluctuations of the right frequency might hold conditions away from equilibrium and reverse competitive exclusion, thereby allowing the persistence of a species rich community. Since then, much work has been devoted to explaining species diversity, and a considerable theoretical synthesis has been achieved. Chesson (2000) identified two major classes of explanations for the stable coexistence of many species: (1) fluctuation-independent, and (2) fluctuation-dependent mechanisms. The first class includes resource partitioning, frequency-dependent predation, and spatial heterogeneity (Tilman 1982, Grover 1997). Although these mechanisms can operate in the presence of environmental fluctuations, they do not require them. Here, our focus is on fluctuation-dependent mechanisms. This class includes two categories of effects: (1) relative nonlinearity of competition, and (2) the storage effect (Chesson 1994).

Relative nonlinearity of competition involves different species having different nonlinear responses to resource variation; per capita growth rates of a population are typically a nonlinear function of limiting factors. Armstrong and McGehee (1976, 1980) demonstrated that n species can coexist on $k < n$ resources or limiting factors when their consumption of these resources causes them to cycle. Each nonlinearity in the functions describing growth rates and resource availability creates resource fluctuations; the variance of which can be considered a resource (Levins 1979, Tilman 1982). So, in a constant and homogeneous environment, nonequilibrium conditions generated by the competition process itself may allow the coexistence

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of more species than limiting resources (Huisman and Weissing 1999, Abrams and Holt 2002). To date, no experiments have demonstrated that competition can induce resource oscillations and coexistence in the manner suggested by the relative nonlinearity model.

Whether ''relative nonlinearity'' is sufficient to explain the paradox of the plankton thus remains an open question (Anderies and Beisner 2000, Chesson 2000, Schippers et al. 2001, Abrams and Holt 2002). An alternative is that environmental fluctuations, external to the system, may provide temporal niche opportunities, permitting species differentiation with respect to specific environmental conditions that change through time (Ebenhöh 1988). Here ''species can coexist by having complementary relationships with the temporally varying environment'' (Chesson and Huntly 1997). This ''storage effect'' is the outcome of the interaction between the environment and competition (individuals produced during favourable periods are ''stored'' within the population during unfavorable periods). Three conditions are required: (1) that each competitor responds differently to temporal variation in the environment; (2) covariance between the environment and competition, i.e., the population responses to the environmental fluctuation alters the relative strengths of interspecific and intraspecific competition; and (3) both of these conditions need to be limited by buffered population growth, i.e., biological traits that limit the impact of competition during unfavorable periods.

To date, a number of experimental studies have tested the theory of fluctuation-dependent coexistence by directly varying some aspect of the environment. Many have employed plankton communities and have imposed fluctuations of consumable resources such as nutrient supply (Robinson and Sandgren 1983, Sommer 1985, Grover 1988, Spijkerman and Coesel 1996) or light (Van Gemerden 1974, Flöder et al. 2002, Litchman 1998, 2003). But, in these cases, it is difficult to distinguish the contribution of relative nonlinearity and the storage effect. Similarly, few have demonstrated that n species can coexist on $k < n$ resources in a variable abiotic environment (from here on we use the term ''abiotic'' to mean some environmental factor that is nonconsumable). In the laboratory, Eddison and Ollason (1978) showed that temperature fluctuations could sustain higher microfaunal diversity although whether the temperature fluctuations induced coexistence via the storage effect was not established. Thus, to the best of our knowledge, no previous demonstration of coexistence due to abiotic fluctuations (sensu the storage effect) exists in the literature.

Testing the role that abiotic fluctuations may play in ensuring long-term species coexistence, requires precise control of the physical environment, and good knowledge of the species responses to the time varying environmental factor (Loreau et al. 2002). Although there are many types of physical variability that may be important for phytoplankton communities, this study is concerned with temperature fluctuations. Temperature has long been considered a primary factor determining phytoplankton succession because of its effect on maximum growth rates (Richardson et al. 2000). Moreover, it is known to influence the physiology of resource utilization (Rhee and Gotham 1981, Tilman et al. 1982). Here we demonstrate that temperature fluctuations can ensure the stable coexistence of two species of phytoplankton indicated by the presence of compensatory dynamics that are not observed under constant temperature in the laboratory. Following Tilman et al. (1982), we used short-term Monod growth experiments to establish the growth response of each species over a range of temperatures. The results of these short-term experiments were used to calibrate a resource competition model. Numerical simulation provided predictions of the conditions under which fluctuating temperature could generate stable coexistence. We then tested these predictions and demonstrated in a long-term growth experiment that temperature fluctuations could induce stable coexistence. The experimental test of our theoretical predictions suggests that results from short-term growth experiments, in a laboratory setting, can be used to predict long-term nonequilibrium dynamics.

Methods

Single-species experiments

Two freshwater diatom species, *Cyclotella pseudostelligera* and *Fragilaria crotonensis*, were maintained in a temperature-controlled (15°C) and cool-white light (21.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 12 h light:12 h dark photoperiod) incubator with twice daily magnetic stirring for 15 min. The species were obtained from the Culture Collection of Algae and Protozoa (Windermere, UK) that had been previously isolated from two lakes, Esthwaite Water and Blelham Tarn, in the English Lake District (UK). The growth medium was Guillard's WC (Guillard 1975) prepared with reagent-grade chemicals and distilled deionized water and modified giving a SiO_2 concentration of 50 μmol/L. These cultures were not axenic, but were free of protozoa and fungi. Sterile technique was used throughout the experiments.

Two 500-mL polycarbonate flasks containing WC, modified to 25 μmol/L SiO_2, were inoculated with each species and kept at the experimental temperature in a temperature-controlled water bath for one week (50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ on a 12 h light:12 h dark photoperiod) and were manually shaken twice a day to ensure algal cell suspension. These cultures were then used as the inoculum for the single-species experiments. The temperature preconditioning period was required to induce all of the necessary temperature-dependent enzymes for metabolic activity (S. S. Kilham, *personal communication*). Since diatoms cannot store silicon in large quantities, starvation of cells was not necessary for

these Si-limitation experiments (Van Donk and Kilham 1990).

To determine the dependence of the growth rate on limiting concentrations of silicate and temperature, each species was grown singly at four different temperatures (6°C, 12°C, 18°C, and 24°C) along a silicate gradient (0.5, 1, 2, 4, 6, 10, 20, 35, and 50 μmol/L). Experiments at all the temperatures were conducted simultaneously for each species. Nine 250-mL polycarbonate flasks were prepared with WC medium adjusted to the silicate concentrations identified above. Each flask was inoculated with approximately 80 cells/mL and maintained at the experimental temperature and manually shaken twice a day to ensure algal cell suspension. Each experiment lasted nine days. Every three days, 60-mL samples were collected to determine cell densities and silicate concentrations and replaced with sterile medium, for a dilution rate of 0.08 d^{-1}. Densities were determined by an adapted sedimentation method. An aliquot (3 mL) of algal culture was placed in a sedimentation chamber, 3 mL of glutaraldehyde (4%) was added, and the cells allowed to settle for 24 h. Algal counts were performed microscopically with the use of a Whipple disk, which has a reliable lower detection limit of 100 cells/mL (Wetzel and Likens 1991). Twenty five 1-mL subsamples per flask were used to measure Si by the silico-molybdate method (Strickland and Parsons 1968). Absorbance was determined using a 10-mm path-length cell in a Milton Roy Spectronic 501 (Dosapro Milton Roy, Pont-Saint-Pierre, France) spectrophotometer with a detection limit of ± 0.25 μmol/L SiO_2.

Diatom growth rates were estimated by a linear least-squares regression of log-transformed data, and have dimensions of individuals per individual per day (i.e., d^{-1}). The data for the change in per capita rate of increase as a function of silicate concentration for each species at each temperature were fit to the Monod model:

$$\frac{dN}{Ndt} = \frac{rR}{K + R} \tag{1}$$

where N is population density (cells/mL), r is the maximal growth rate (d^{-1}), R is the extracellular concentration of the limiting nutrient (μmol/L), and K is the half-saturation constant (μmol/L). A nonlinear least squares procedure was used to fit the data; r, K, and R were determined by iteration. All analyses were conducted in SAS (SAS Institute 1999).

Resource-based model of competition in a fluctuating environment

The central element of models of resource competition is the functional relationship between resource availability and the per capita growth rate of each species. Numerous experiments with nutrient-limited algae have shown that the Michaelis-Menten, or Monod, model provides a good fit in most cases (Tilman and Kilham 1976, Tilman 1977, 1981, Tilman et al. 1981). In the model considered below, the two species are assumed to compete for a shared resource. The model of resource competition is

$$\frac{dN_i}{N_i dt} = \frac{r_i R}{K + R} - D \tag{2a}$$

$$\frac{dR}{dt} = D(S - R) - \sum_{i=1}^{n} Q_i \left(DN_i + \frac{dN_i}{dt} \right) \tag{2b}$$

where N_i is the population density of diatom species i (cells/mL, $i = 1$ to 2), r is the maximal growth rate (d^{-1}), R is the extracellular concentration of the limiting nutrient (μmol/L), K is the half-saturation constant (μmol/L), D is the dilution rate (nutrient-independent loss rate), S is the concentration of the limiting resource supplied in the inflow, and Q_i is the cell quota (amount of resource required to produce one individual of species i). Q_i was expressed as a function of the number of cells produced and as the amount necessary to make a cell and it was calculated as the ratio of the silicate remaining in the medium and the cells produced by day nine (Kilham 1975). If we consider that the species are limited by the same nutrient, these two equations predict that at equilibrium, the one with the lowest requirement should competitively exclude all other species (Tilman 1977, 1981). The equilibrium nutrient requirement of a species, defined by setting Eq. 2a equal to zero, is

$$R_i^* = \frac{D_i K_i}{r_i - D}. \tag{3}$$

The values for R^* provide a simple way of predicting the equilibrium outcome of competition for one resource.

Sinusoidal variation in environmental temperature was added to the resource model above:

$$\text{Temperature } (t) = a \sin(ft + \pi) + \bar{T} \tag{4}$$

where a is the amplitude, f is the frequency (units of cycles per day), t is the time (units of 1 d), and $\bar{T}$ is the mean. We determined the dynamics of the two species and of the resource by numerically integrating the system of differential equations 2a and b. The values for each of the parameters r, K, and Q estimated at each of the four temperatures (Table 1) were interpolated to obtain continuous functions. We used the function INTERP1 of Matlab (v. 5.3, The MathWorks, Natick, Massachusetts, USA), which performed a linear interpolation between these points. These functions thus define the temperature dependence for r, K, and Q. The values used for each parameter at each time step were therefore defined by the temperature value given by Eq. 4, or were fixed at the observed value for 18°C (the mean value of the sinusoidal fluctuations) under simulation of constant temperature. The simulations were implemented in Matlab (v.5.3) with a fixed time step ($dt = 0.001$) for 10 000 ''days'' (1×10^7

TABLE 1. Silicate growth constants for *Fragilaria* and *Cyclotella* as fit to the Monod model with a nutrient threshold.

Temperature (°C)	R (μmol/L)	K (μmol/L)	R^* (μmol/L)	Q (μmol/cell)
Fragilaria crotonensis				
24	0.42 (0.41–0.43)	0.25 (0.19–0.31)	0.06	2.02×10^{-6}
18	0.37 (0.36–0.37)	0.20 (0.14–0.26)	0.06	2.03×10^{-6}
12	0.40 (0.37–0.43)	0.30 (0.12–0.47)	0.08	1.44×10^{-6}
6	0.25 (0.24–0.27)	0.06 (0.01–0.14)	0.03	3.21×10^{-6}
Cyclotella pseudostelligera				
24	0.02 (0.01–0.08)	†	†	†
18	0.39 (0.37–0.40)	0.15 (0.07–0.23)	0.04	6.19×10^{-7}
12	0.36 (0.34–0.37)	0.13 (0.04–0.22)	0.04	8.62×10^{-7}
6	0.32 (0.31–0.33)	0.07 (0.01–0.12)	0.02	3.01×10^{-7}

Notes: Confidence intervals (95%) are given in parentheses below each value. R is the extracellular concentration of the limiting nutrient, R^* is the equilibrium nutrient requirement, K is the half-saturation constant, and Q is the cell quota (amount of resource required to produce one individual). R^* was calculated according to Eq. 3. A dagger indicates the results of silicate-limited growth experiments that could not be fitted to the Monod model. The maximal growth rate given is thus the average growth rate observed for all silicate concentrations.

iterations, which correspond to 400 density cycles) to eliminate transient behavior.

Competition experiment under fluctuating conditions

The initial conditions and the mean, amplitude, and period of the fluctuations assigned to the experiment were determined from the simulations of the model. Competition experiments were performed for 125 d in 250-mL polycarbonate flasks containing 210 mL of WC medium modified to a SiO_2 concentration of 35 μmol/L. Algae were kept in suspension by daily manual shaking of each flask. The inocula for these experiments were grown to stationary phase in WC medium at the experimental temperature for two weeks before use. Algal monocultures and competition cultures were maintained under semicontinuous culture (dilution = 0.1 d^{-1}, with sterile medium).

The two-species cultures were started with each species in approximately equal abundance (≈200 cells/mL). Each temperature treatment also included single-species control cultures, thereby allowing us to evaluate species growth under the culture conditions, and to ensure that the results observed in the two-species cultures were due to competition. To limit wall growth, or any cell adhesion to the walls of the culture flasks, cultures were transferred to sterile flasks on day 75 of the experiment. No visible wall growth was observed. The 54 replicate microcosms (treatments: three cultures [two single-species and one two-species culture] × three temperatures × six replicates) were distributed among six light and temperature controlled water baths (50 $\mu mol \cdot m^{-2} s^{-1}$ on a 12 h light:12 h dark photoperiod). The six baths were divided into two groups, A and B. Group A baths produced constant temperature (18°C and 24°C) and group B baths produced fluctuating temperature with a change in temperature every 12 h according to the sinusoidal temperature series assigned to the computer-controlled water bath (temperature control was extremely good and never departed from the required temperature by more than ±0.1°C). The mean, period, and amplitude of the temperature fluctuations used accorded to those of the numerical simulations that predicted the maximum number and amplitude of cycles in cell density (see *Experimental results: Fluctuating temperature*). Samples were collected every seven days for determination of population densities and silicate concentrations (methods described above for single-species experiments). This sampling frequency allowed us to detect density oscillations with a minimum period of 14 days (twice the sampling interval). Experiments for each temperature treatment were done simultaneously, except for the constant 24°C treatment, which was conducted two months after the fluctuating and the constant 18°C experiments.

A repeated measures analysis of variance was conducted within SAS (v.8.02) to test for trends in growth rates after the initial growth phase (approximately day 35) across all treatments.

EXPERIMENTAL RESULTS

Short-term growth experiments

These experiments were done to determine the temperature dependence of each of the species' parameters for the Monod function used in the resource competition model (Table 1). Fig. 1 shows the results of these short-term Monod experiments for *Cyclotella* and *Fragilaria* (in all cases $F_{2,8} > 1000$; $P < 0.0001$). From the figure, it can be seen that both species exhibited an increasing but saturating growth rate over the temperature range of 6–18°C used here. Only *Fragilaria* grew at 24°C. Because *Cyclotella* did not grow well at 24°C (it did not grow at all at silicate concentrations >6 μmol/L) a line with a slope of zero and an intercept of 0.02 d^{-1} (the average growth rate) is shown in Fig. 1. The estimated half saturation constant (K) of these

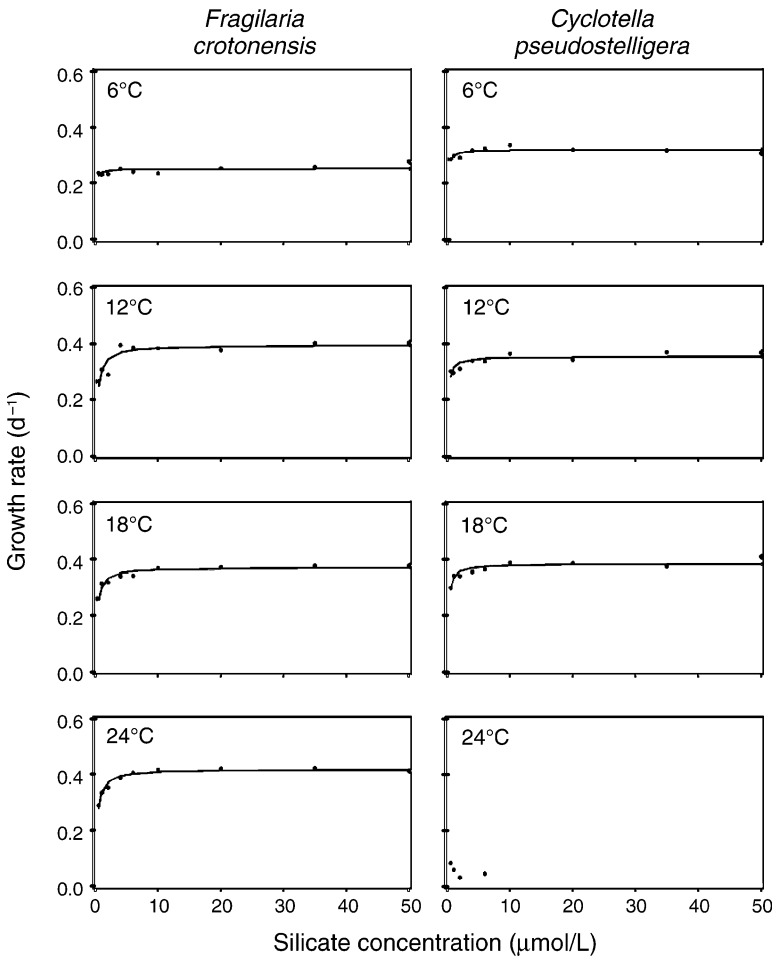

FIG. 1. Results of silicate-limited Monod growth experiments for *Cyclotella* and *Fragilaria* for 6°–24°C. All curves shown, except *Cyclotella* at 24°C, are least-squares nonlinear fits to the Monod model.

two species were at or below the limit of detection of silicate (0.25 μmol/L SiO_2), causing a large variance in the estimates of this parameter.

The R^* (Eq. 3) for each species was calculated to provide qualitative predictions for the outcome of resource competition (Table 1). The values represent the amount of silicate that each species needs, at equilibrium, to have a reproductive rate that exactly balances a loss rate of $D = 0.08\ d^{-1}$. To predict the outcome of competition between these species the R^* were compared and the dominant was identified as the species with the lowest R^*: *Cyclotella* is predicted to exclude *Fragilaria* from 12–18°C, while *Fragilaria* is predicted to out compete *Cyclotella* at 24°C. No prediction is possible at 6°C.

Simulation results

The aim of this analysis was to establish the conditions for fluctuation dependent coexistence of *Fragilaria* and *Cyclotella*. We varied three characteristics of the temperature fluctuations: mean, period, and amplitude. Fig. 2 shows the long-term outcome of competition between *Fragilaria* and *Cyclotella* for a range of amplitudes and periods at different mean temperatures.

Below 15°C and above 21°C, no coexistence was expected, whatever the periodicity and the amplitude of the temperature fluctuations. At 15°C, coexistence is predicted for an amplitude greater than 7.5°C and a periodicity range of 1–100 d. Compensatory dynamics (out-of-phase fluctuations of the two-species populations with inversions between the dominant species) are obtained for an amplitude of 9°C and a periodicity greater than 70 d. At 18°C, coexistence is predicted for amplitudes between 4°C and 6°C and a periodicity between 1 and 100 d. Compensatory dynamics at 18°C are observed for a range of parameter combinations: amplitude = 5°C, period = [10, 60] d; amplitude = 5.5°C, period = [10, 80] d; amplitude = 6°C, period = [30, 100] d. We obtain more than two successive cycles of compensatory dynamics at an amplitude of 6°C and for periods between 30 and 100 d. Initial densities (equal or unequal initial densities) have no effect on the outcome of competition.

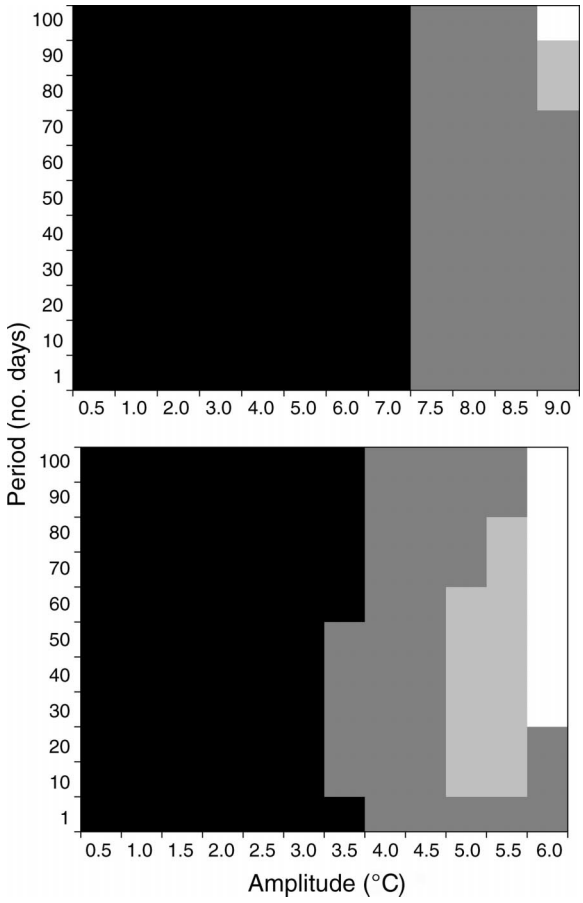

FIG. 2. Predicted outcomes of competition between *Cyclotella* and *Fragilaria* as a function of period and amplitude at a mean temperature of (A) 15°C and (B) 18°C. Amplitude (a, Eq. 4) is between 0.5° and 6°C and period ($2\pi/f$) is between 1 and 100 d. In the black zone of each panel, *Cyclotella* wins; in the dark gray zone, there is coexistence of *Fragilaria* and *Cyclotella* with no compensatory dynamics; in the light gray zone, there is coexistence with one cycle of compensatory dynamics; and in the white zone, there is coexistence with two cycles or more.

Any experimental test of this model is necessarily restricted to small subset of parameter combinations. To test the prediction of fluctuation dependent coexistence we selected parameter values that maximized the number and amplitude of cycles of population density: amplitude of 6°C, period of 60 d, and a mean temperature of 18°C.

Competition experiment

The single-species experiments ran for 105 d and the competition experiments for 125 d (and due to the rapid exclusion only 40 days at 24°C). The results (Fig. 3) for growth in monoculture and interspecific competition under constant and fluctuating temperatures are discussed in turn below. To keep the figures clear, one replicate is shown in Fig. 3; the results presented below are highly representative and figures for all other replicates are available in the Appendix.

Constant temperature

At 18°C, the monocultures grew well and we observed a good agreement between observed and predicted *Cyclotella* densities, although *Fragilaria* densities were greater (130 ± 15%, [mean ± SE]) than predicted. After 70 d (hatched zone in Fig. 4A) the *Cyclotella* densities declined and the concentration of silicate increased in the medium. This decline corresponded to the observation of an unidentified fungal contaminant which was found in both monocultures at this time, and which had not been observed prior to this point.

In competition at 18°C, *Cyclotella* began to displace *Fragilaria* (decreased by 67 ± 8%), as predicted by the model, and remained dominant (by 4000 ± 1040 cells/mL) up to day 70. Beyond this point, competitive strengths were reversed and *Fragilaria* densities exceeded those of *Cyclotella*. This change in behavior was associated with the same fungal contaminant observed in the monocultures, which apparently affected *Cyclotella* more than *Fragilaria*. This change was characterized by a significant inversion in the sign of the growth rates for both species (*Fragilaria*, $r = -0.01$ and $r = 0.02$, before and after infection, respectively; paired t test, $t = -1.73$, $df = 23$, $P = 0.09$; *Cyclotella*, $r = 0.002$ and $r = -0.09$, before and after infection, respectively; $t = 3.34$, $df = 22$, $P = 0.003$). During the period when *Cyclotella* was dominant, silicate concentrations were low (0.45 ± 0.09 μmol/L), they then increased briefly (day 70) and then decreased again as *Fragilaria* cell densities increased.

At 24°C, the monocultures of *Fragilaria* grew well and reached a steady state of 20 000 cells/mL. *Cyclotella* also grew, although slowly, and attained a lower steady state of 2000 cells/mL (with a growth rate of 0.09 ± 0.01 d^{-1}). Although it began to decline in some replicates, it never became extinct during the 40 days of the experiment. In competition, *Fragilaria* densities were greater (87 ± 24%) than predicted by the model, as were *Cyclotella* densities (by 16 ± 13%) up to day 23. As predicted by the model, *Fragilaria* remained dominant (by 29.5 ± 7.4%) throughout the experiment and succeeded in excluding *Cyclotella* after 33 days (Fig. 3G). No contamination was observed here.

Fluctuating temperature

Under the sinusoidal temperature variation, *Cyclotella* and *Fragilaria* densities fluctuated in monoculture (Fig. 3B and D), and silicate concentrations followed the density variations. Up to day 90, *Cyclotella* densities were 24 ± 7% lower than predicted, and thereafter were lower by 90 ± 4%; whereas *Fragilaria* densities were greater than predicted by the model (up to day 90 by 100 ± 20% and after day 97 by 21 ± 20%).

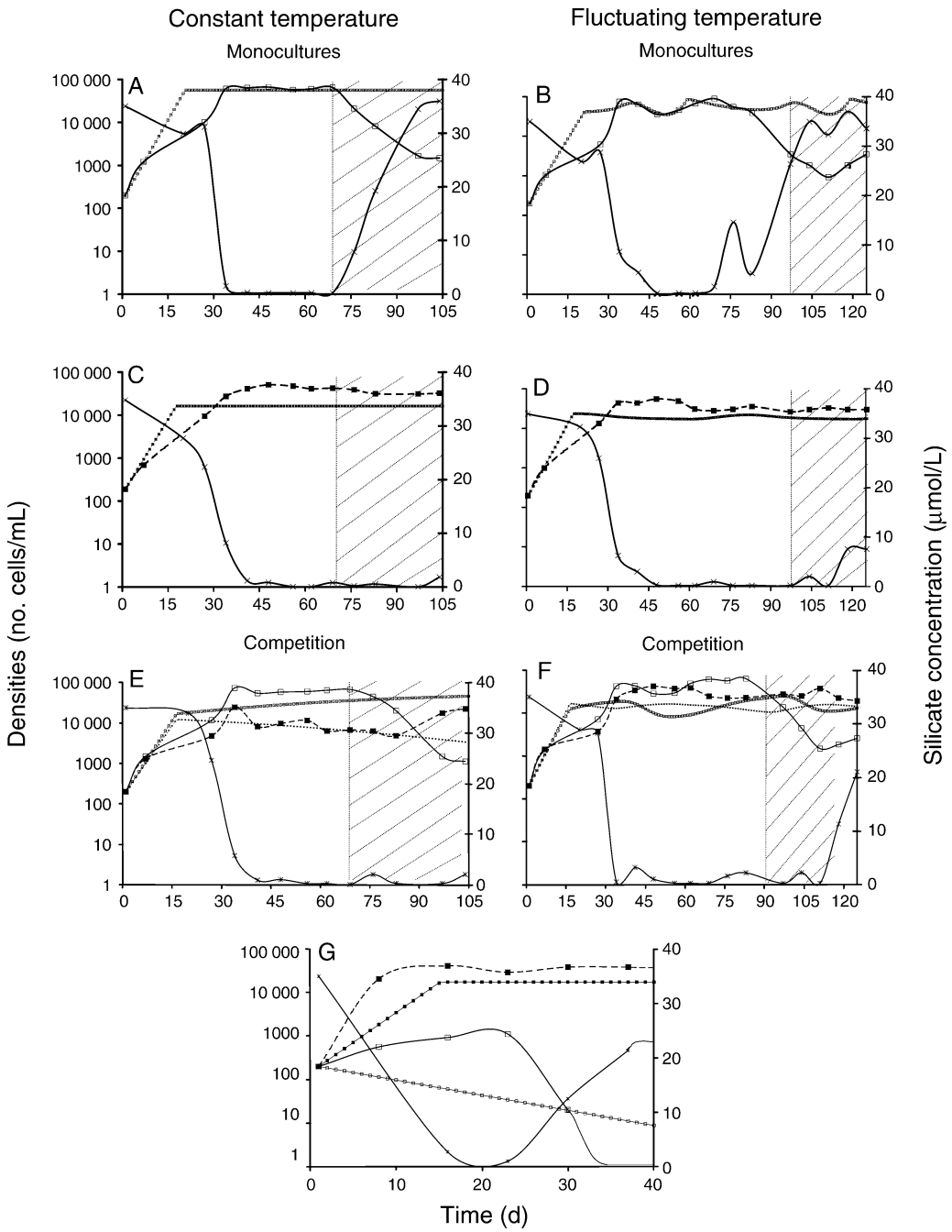

FIG. 3. Population and silicate dynamics for a single representative replicate during the competition experiment. Cultures are shown under constant temperature at 18°C (panels A, C, and E), under constant temperature at 24°C (G), and under fluctuating temperature (B. D. F). Monocultures of *Cyclotella* (A and B) and *Fragilaria* (C, D), and competition cultures (E, F, and G) are shown. Model predictions are indicated by open squares (*Cyclotella*) and dotted lines (*Fragilaria*). Experimental results are shown by: solid lines and crosses, silicate concentration; open squares and solid lines, *Cyclotella*; and dashed lines and solid squares, *Fragilaria*. The hatched zone indicates the period when contaminants were detected in the cultures (at day 70 for A, C, and E [bath A], at day 90 for B, D, and F [bath D]).

In competition, temperature fluctuations induced compensatory dynamics resulting in the coexistence of the two species that qualitatively corresponded to the model predictions up to day 90 (Fig. 3F). During this period, cell densities were greater (*Fragilaria* by 105%, *Cyclotella* by 69%) than predicted by the model, and the period of the density oscillations was slightly shorter (first period, 23.33 ± 1.91 d vs. 29 d; second period, 30.33 ± 1.91 d vs. 34 d predicted by the model). From day 90, an unidentified fungal contaminant was ob-

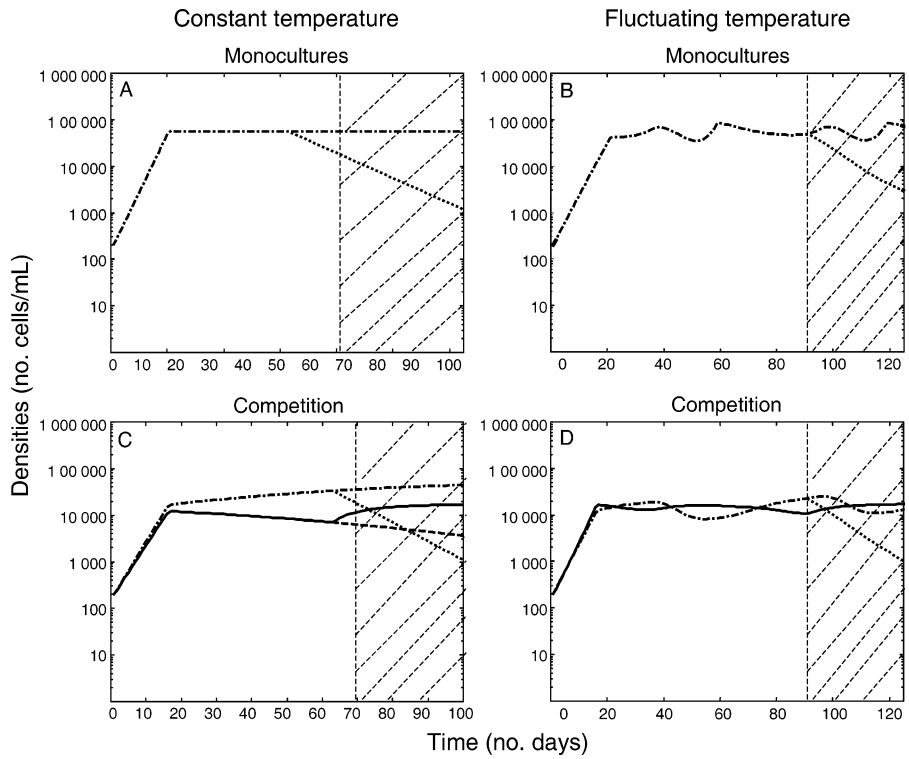

FIG. 4. Predicted population dynamics with and without an infection event. The hatched zone indicates the period when the contaminants were present in the model: at day 70 (A, C); at day 90 (B, D). Dynamics are shown under constant temperature of 18°C (A, D), and under fluctuating temperature (B, D). *Cyclotella* monocultures (A, B), and competition cultures (C, D) are shown: *Cyclotella* with an infection event (dotted line); *Fragilaria* with infection event (dashed line); *Cyclotella* without infection event (dot-dashed line); and *Fragilaria* without infection event (solid line).

served in the cultures and, from this point, *Cyclotella* densities declined (although they continued to fluctuate) and the compensatory dynamics between the two species ceased.

To detect whether exclusion was occurring we tested for trends in the population growth rates. We found notable differences between the constant (18°C) and the fluctuating treatments (mean 18°C). Under constant temperature, up to day 70, we observed a significant trend in *Cyclotella* growth rate (positive growth rate, $r = 0.032 \pm 0.016$ d^{-1}) and *Fragilaria* growth rate (negative growth rate, $r = -0.02 \pm 0.003$ d^{-1}; no time effect, $F_{4,48} = 1.08$, $P = 0.37$). Under fluctuating temperature, we could detect no trend in *Cyclotella* and *Fragilaria* growth rates (significant time effect, $F_{12,66} = 3.79$, $P = 0.003$). However, these series are too short to permit a more complete time series analysis.

DISCUSSION

These results confirm that environmental fluctuations may provide one solution to Hutchinson's paradox. We showed that a species may be a superior nutrient competitor through only a portion of the temperature range in which it can survive in the absence of competition (Tilman et al. 1982). Establishing the temperature dependence of the competitive abilities of the two diatom species was a necessary prerequisite. The combination of careful experimentation and modeling provided sufficient understanding for us to predict the conditions under which temperature variation could lead to coexistence.

Our main result is that temperature variation leads to the persistence of two species on one limiting resource. In theory, this could have been caused by endogenously generated resource fluctuations associated with the relative nonlinearity of the growth responses of each species (Armstrong and McGehee 1976, Abrams and Holt 2002). However, the absence of long-term coexistence under constant temperature (defined by a constant competitive advantage up to infection at 18°C and competitive exclusion at 24°C), and the lack of evidence for silicate fluctuations in either the single-species or two-species constant temperature cultures, suggests that endogenously generated resource variation was not responsible for our result. A clear empirical demonstration of the relative nonlinearity effect remains to be done.

The theoretical prediction that environmental variability may promote coexistence is known as the storage effect (Chesson 2000). The three requirements of the storage effect were observed here: the two species responded differently to environmental fluctuations,

the compensatory dynamics indicate the strong covariance between the environment and interspecific competition, and both species persisted when the environment was not at the growth optimum (buffered population growth). Our results confirm that stable coexistence requires appropriate differences between the growth and resource consumption functions of each species, and environmental variability at appropriate time scales. Here we demonstrated coexistence using sinusoidal variation with a 60-d period; however, the model simulations indicated that we could have obtained similar results for periodicities ranging from daily to seasonal temperature cycles (Fig. 2) that are typical for many temperate lakes.

The general agreement between the theoretical predictions and the experimental observations indicates the effectiveness of the resource competition model to predict the outcome of competition under fluctuating conditions. The conditions affording coexistence were defined by a parameter space including the mean, amplitude, and period of the environmental fluctuations. The model highlighted that coexistence was primarily dependent upon the mean temperature; fluctuations induced coexistence around the point of inversion of competitive strength (~18°C). Furthermore, fluctuations of amplitude greater than 5°C were required to generate compensatory population dynamics. Interestingly, given these conditions, coexistence was not strongly sensitive to the periodicity of the fluctuations and compensatory dynamics (of varying cycle duration) were obtained across the full range of periods explored (Fig. 2). These results correspond to previous intuitions linking coexistence to nonequilibrium environments.

The deviations we observed late in the experiment (after day 70) between the experimental dynamics and those predicted by the model were due to the presence of an unidentified fungal contaminant (Fig. 3A, B, E, and F). Contaminants are common in long-term cultures and these experiments were all longer than the great majority of those reported in the literature (e.g., Tilman et al. 1981). Diatoms cultured below their maximum growth rate exude a lot of organic matter on the cell surface, which favors the growth of bacteria, heterotrophic flagellates, and chytrid fungi (S. S. Kilham, *personal communication*). Infection can occur over a wide range of environmental conditions, even when conditions are favourable for growth of the host populations and their densities are low (Holfeld 1998, 2000). Of importance for the conclusions of this paper is that only *Cyclotella* appeared to suffer detectable mortality, both in the monocultures and in competition (Fig. 3). The fact that the infection reversed the competitive abilities of the two species could not have been predicted by our model, hence the divergence between model predictions and observed densities beyond day 70. Despite this, the diatoms still had sufficient time to go through two complete cycles of compensatory dynamics under fluctuating temperature. To further explore the hypothesis that the contaminants uniquely affected the growth rate of *Cyclotella*, we sought to simulate the experimental results by incorporating an infection event into the model, at day 70 in a constant temperature simulation and at day 90 in a fluctuating temperature simulation. We obtained an excellent agreement between the theoretical predictions and the experimental observations when *Cyclotella* growth rates were diminished by 95% in the constant treatment and by 90% in the fluctuating treatment (Fig. 4A, B, C, and D). These results reinforce the hypothesis that the fungal contaminants were responsible for the effects we observed late in the experiment and that the two diatoms had different sensitivities to the contaminants. These results, and the others presented above, are noteworthy because they confirm the utility of a coupled modeling and experimental approach.

We have demonstrated compensatory dynamics and long-term coexistence between two species on one resource in fluctuating environment and that this outcome was predictable through the parameterization of a resource competition model from short-term growth experiments. Grover (1997) wondered if the high degree of predictability characterizing resource theory would be robust to the addition of temporal variability, our results suggest that it is. Finally, it is worth noting that the compensatory dynamics we observed in the fluctuating environment are the basis of the ''insurance'' effects of species richness at the community level (Yachi and Loreau 1999, Lehman and Tilman 2000, Chesson et al. 2002). Further experimental work will consider the dual role environmental fluctuations play in maintaining the diversity and stability of ecological communities.

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APPENDIX

Figures showing population and silicate dynamics for each replicate during the competition experiment are available in ESA's Electronic Data Archive: *Ecological Archives* E086-152-A1.