COMPLEX POPULATION DYNAMICS IN MICROBIAL SYSTEMS

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“Einst vor langer Zeit waren die Spiegelwelt und Menschenwelt noch nicht getrennt. Zu jener Zeit konnte man auch durch den Spiegel hindurch kommen und gehen. Eines Nachts jedoch drangen die Spiegelwesen ohne Warnung in unsere Welt ein, und es brach Chaos aus.

Die Menschenwesen stellten schnell fest, dass die Spiegelwesen das Chaos selbst darstellten. ... Dank der magischen Fähigkeiten des Gelben Kaisers gelang es, sie durch einen mächtigen Zauber zu besiegen und in ihren Spiegel zurückzutreiben.

Eines Tages wird der Zauber aber so schwach werden, dass sich in unserem Spiegel turbulente Gestalten zu regen beginnen. ... Und plötzlich wird die lange eingekerkerte Welt des Chaos in unsere eigene Welt hinein überkochen.“

Ist es schon da?

“Once before long time the mirror world and the people world were not yet separated. To that time one could come and go by the mirror through. One night, however, the mirror natures penetrated into our world without warning, and it broke chaos off.

The people stated fast that the mirror natures represented the chaos themselves. … Owing to the magic abilities of the yellow emperor, one succeeded to defeat by driving them back into the mirror by a powerful charm.

One day, however, the charm will become so weak that in our mirror turbulent shapes begin to move. … And the long incarcerated world of the chaos suddenly will cook over into our own world. “

Is it already there?

From “Complete works of Chuang-tzu, 1968“

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CONTENTS

INTRODUCTION

Population dynamics is the study of how and why population numbers change with time and space. Studies of population dynamics have a long history and go back to the early decades of 1900 (Elton 1924, Volterra 1926). Intrinsic mechanisms - processes that pertain a focal population - and extrinsic mechanisms interact by determining population dynamics and lead to different dynamic patterns. On one hand, extrinsic processes are often stochastic and may lead to dynamics without any pattern. On the other hand, extrinsic forces may have a strong seasonal character leading to seasonal population fluctuations. Deterministic intrinsic processes can generate also different patterns of dynamic behaviours such as damped oscillations, stable limit cycles, or chaotic dynamics. Even though chaotic dynamics appear to be without any pattern like the stochastic fluctuations there is a clear distinction between the two processes. For chaotic dynamics there is dependence between the states of the system over time, while pure stochastic dynamics are lacking any dependence between states. The dependence can easily be shown by means of time delay reconstructions (Fig. 1). The chaotic systems show a clear pattern (Fig. 1a) but no pattern is detectable for the stochastic dynamics (Fig. 1b). Furthermore, chaos is characterised by a sensitivity to initial conditions, which means infinitesimal differences in the starting values of the system amplify and lead to complete different fluctuations over time. Sensitivity to initial conditions is indicated by a positive Lyapunov exponent λ. In contrast, differences in initial conditions are damped over time in stochastic systems. A third important attribute of chaotic patterns is that they have upper and lower boundaries (state value never over- or respectively undershoot certain values), because data (system states) stay inside an attractor. Hence, chaotic dynamics are predictable on a short time-scale. Stochastic dynamics have no boundaries and thus lack predictability on any time scale. While the first two attributes are mainly used to distinguish chaos from stochasticity, the second and latter one may be used for applied methods (Hastings *et al.* 1993). However, the interactions of intrinsic and extrinsic processes make the understanding and prediction of population dynamics difficult, but fascinating.

The focus on intrinsic driven patterns and chaos was introduced to population ecology by the works of Robert May (1974) who showed that a simple mathematical model reveals different intrinsic driven dynamics by

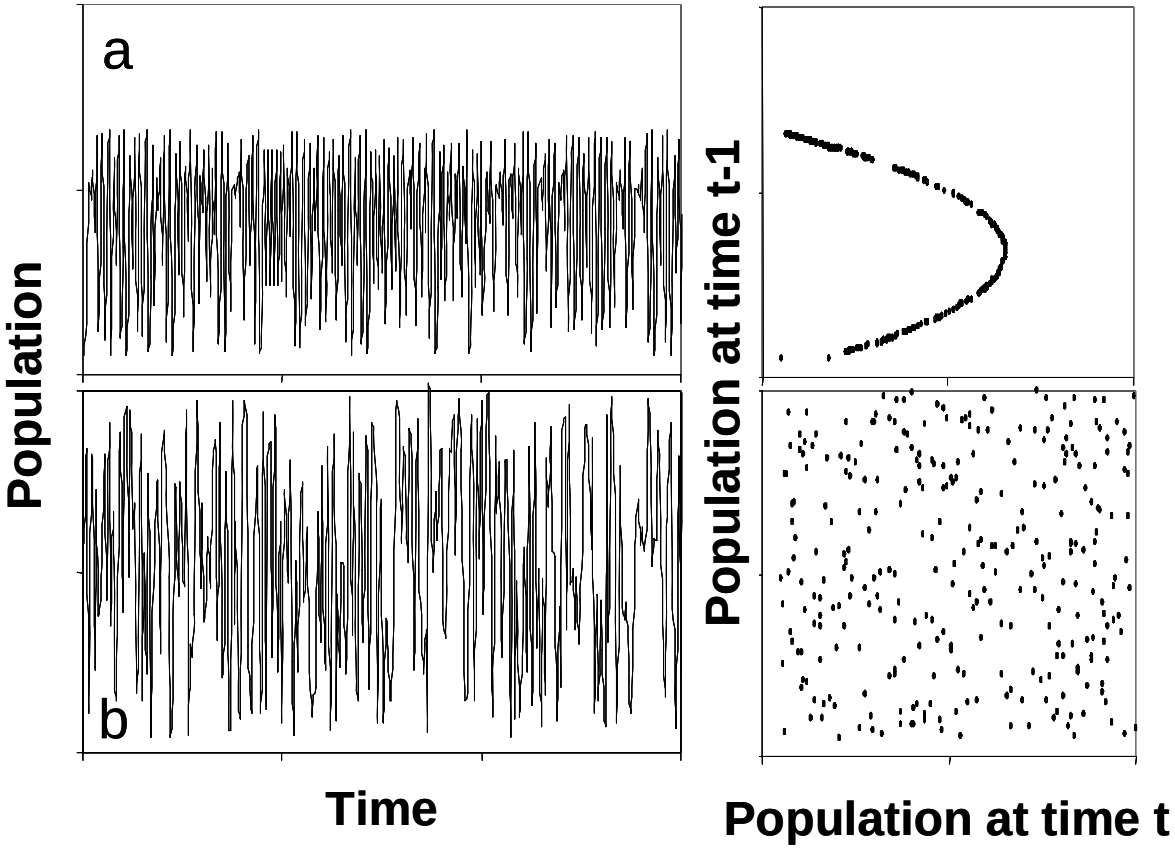

Fig. 1. Comparison of chaotic (a) and stochastic (b) fluctuations shown as time series and the corresponding time delay reconstruction where the population densities are plotted against the population density the day before. Chaotic time series is generated by the logistic growth equation with r = 2.9, stochastic time series are random numbers between 0.1 and 2.

changing one control parameter. He showed that the logistic growth equation exhibits complex dynamic behaviour in dependence on the growth rate r, and for high r values even chaos. Therewith chaos became a subject of ecology and an ongoing intensive and controversial debate about the occurrence and meaning of chaos in population dynamics began.

The occurrence of complex intrinsic driven dynamics and chaos was found in many deterministic mathematical models, from one-species to multi-species systems involving more and more ecological mechanisms discovered over time (e g. Gilpin 1979, Hastings & Powell 1991, Fussmann *et al.* 2005). These theoretical studies conveyed that chaos might be a common pattern in population dynamics for certain parameter settings. An

empirical support for the occurrence of chaos in population dynamics - from experiments or field data - is rare, which leads some studies to conclude that chaos does not occur or is negligible (Chattopadhyay & Sarkar 2003). Potential reasons for the discrepancy between theoretical and empirical studies are miscellaneous. One problem is that signals of deterministic processes, thus also chaos, of empirical data are woven together with stochasticity, making field data samples insufficient for the analysis of the meaning of chaos, even combined with mathematical modelling (Ellner & Turchin 1995). Controlled laboratory experiments with a reduced and manageable population system have been shown as a useful tool to study population dynamics regarding intrinsic mechanisms (Costantino *et al.* 1997, Fussmann *et al.* 2000). Complex dynamics, from damped oscillations, stable limit cycles to chaos, was shown for the one-species system of the flour beetle *Tribolium castaneum*. Damped oscillations and stable limit cycles were shown for a two-species system of an algae-rotifer system. Mathematical models pointed out that the length and the linkages in a food web are relevant for the occurrence of chaos. Long food chains with no or less linkages between the trophic levels tend to show a high potential for chaotic dynamics (in the sense of a large parameter range). More links between the trophic levels are predicted by mathematical models to reduce the potential of chaos in the food web (Fussmann & Herber 2002, Gross *et al.* 2005). Chaos was only shown in a one-species system (Costantino *et al.* 1997) and empirical studies on chaos in multi-species systems may shed more light on the question if chaos occurs in population dynamics.

The question if chaotic dynamics are exhibited in the real world is not restricted to population ecology. Other nonlinear biological systems are thought to exhibit chaotic dynamics, but most of them are lacking possibilities to study the chaotic behaviour experimentally. For instance, mathematical models were shown to describe the behaviour of measles dynamics over the last years. But it is impossible to test the model hypothesis that measles dynamics are chaotic (Earn *et al.* 2000). Other systems like dynamics in human brain (Tirsch *et al.* 2004), heart dynamics, or the interplay between glucose and insulin in the blood (Kroll 1999) show also indications for chaotic dynamics. But again, manipulations to study these system behaviours are restricted.

The present study should find answers to the question of relevance of chaos in population dynamics by means of an experimental multi-species system. Experiments with complex population dynamics of a three-species system should be conducted to study the persistence and the transitional behaviour of the dynamics. The persistence of complex behaviour and especially chaotic dynamics in response to disturbance are important for the understanding of chaos in nature and for the spatial and temporal extent of chaotic dynamics. Therefore a conceptional model of a two-prey-one-predator system should be implemented into a microbial laboratory system. A clear defined species composition by the use of axenic cultures should enable the study of complex population dynamics. The possible persistence of deterministically driven population dynamics should be analysed: First, by a change in one experimental parameter and second by coupling of different populations with different intrinsic dynamics.

References:

Chattopadhyay, J. & Sarkar, R.R. Chaos to order: preliminary experiments with a population dynamics model of three trophic levels. *Ecol. Model.* **163**, 45-50 (2003).

Costantino, R.F., Desharnais, R.A., Cushing, J.M. & Dennis, B. Chaotic dynamics in an insect population. *Science* **275**, 389-391 (1997).

Earn, D.J.D., Rohani, P., Bolker, B.M. & Grenfell, B.T. A simple model for complex dynamical transition in epidemics. *Science* **287**, 667-670 (2000).

Ellner, S. & Turchin, P. Chaos in a noisy world: new methods and evidence from time series analysis. *Am. Nat.* **145**, 343-375 (1995).

Elton, C.S. Periodic fluctuations in the number of animals: Their causes and effects. *British Jour. of Exp. Biol.* **11**, 215-244 (1924).

Fussmann, G. F., Ellner, S. P., Shertzer, K.W. & Hairston, N.G. Jr. Crossing the Hopf bifurcation in a live predator-prey system. *Science* **290,** 1358- 1360 (2000).

Fussmann, G. F. & Heber, G. Food web complexity and chaotic population dynamics. *Ecol. Lett.* **5**, 394-401 (2002).

Fussmann, G.F., Ellner, S., Hairston, N.G.Jr., Jones, L.E., Shertzer, K.W. & Yoshida, T. Ecological and evolutionary dynamics of experimental plankton communities. *Advances in Ecological Research* **37**, 221-244 (2005).

Gilpin, M.E. Spiral chaos in a predator-prey model. *Am. Nat.* **113**, 306-308 (1979).

Gross, T., Ebenhöh, W. & Feudel, U. Long food chains are in general chaotic. *Oikos* **105**, 135-144 (2005).

Hastings, A. & Powell, T. Chaos in a three-species food chain. *Ecology* **72**, 896-903 (1991).

Hastings, A., Hom, C.L., Ellner S., Turchin, P. & Godfray, H.C.J. Chaos in ecology: Is Mother Nature a strange attractor? *Annu. Rev. Ecol. Syst.* **24**, 1-33 (1993).

Kroll, M.H. Biological variation of glucose and insulin includes a deterministic chaotic component. *BioSystems* **50**, 189-201 (1999).

May, R. M. Biological populations with nonoverlapping generations: stable points, stable cycles, and chaos. *Science* **186,** 645-647 (1974).

Tirsch, W.S., Stude, P., Scherb, H. & Keidel, M. Temporal order of nonlinear dynamics in human brain. *Brain Research Reviews* **45**, 79-95 (2004).

Volterra, V. Fluctuations in the abundance of a species considered mathematically. *Nature* **118,** 558-600 (1926).

CHAPTER I

THEORETICAL FRAME-WORK OF ANALYSES OF THE DYNAMIC BEHAVIOURS IN THREE-SPECIES FOOD WEBS

Since Robert May showed in the 1970´s that populations exhibit different complex dynamics for the simple discrete logistic growth equation - like stable equilibrium, stable limit cycles and chaos - different approaches have evolved to understand and analyse population dynamics. One approach following the idea of May is to find general patterns and mechanisms in mathematical population models. Furthermore, time series analyses of field data or experimental results are a tool to understand population dynamics. Combining mathematical modelling with time series analyses of experimental data is probably most efficient. Field data have the great disadvantage that deterministic and stochastic processes interact, which makes a detection of a general mechanism in pattern formation difficult (Turchin 2003). Laboratory experiments under constant controlled conditions minimize the stochastic behaviour to a tradable degree. Due to short generation times (sometimes less than one day), microorganisms like bacteria and protozoa have a long history in experimental population studies (Gause 1934, Jessup *et al.* 2004, Luckinbill & Fenton 1978). One can observe many generations in a relatively short time period. Furthermore the use of microorganisms or small metazoan enables the possibility to conduct experiments under clearly defined conditions which is necessary to understand mechanisms in population dynamics (Cadotte *et al.* 2005). Microorganisms are mainly cultured under batch or flow through conditions. One of the main advantages of flow through conditions like in a chemostat are that the dilution rate of the system can be used as a control parameter of the system and changes in the dilution rate may lead to different dynamic behaviours (e.g. Vayenas & Pavlou 1999, Fussmann *et al.* 2000, Kooi & Boer 2003).

To include the most relevant mechanism for population communities, competition and consumption (Chase *et al.* 2002), one of the simplest three species systems is a two-prey-one-predator system. Coexistence for the three species is possible if the preferred prey of the predator is assumed to be the superior competitor. A conceptual model of a two-prey-one-predator system is described in Fig. 1. Considering one randomly chosen starting situation (a in Fig. 1). The preferred prey and superior competitor are highly abundant, while the less preferred prey (and inferior competitor) and predator are at low densities. Due to the high supply of the preferred food, the predator population increases. But at the same time the grazing pressure on

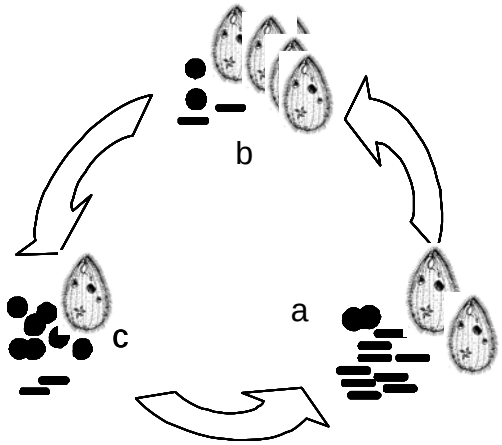

Fig. 1: Schematic two-prey-one-predator system. Ciliate drawing represents the predator, rods the preferred prey and superior competitor, and circles the less preferred prey and inferior competitor.

the preferred prey and superior competitor increases and their population abundance decreases simultaneously (b in Fig.1). In this situation the grazing pressure is still high on the superior competitor and the inferior competitor can overgrow the superior competitor. In turn, the predator abundance decreases because the preferred prey is low abundant (c in Fig. 1). Now the grazing pressure is decreasing on the preferred prey and the superior competitor population increases, while the less preferred prey and inferior competitor populations decrease (a in Fig.1). Four different types of dynamic behaviour can be revealed by this simple three species system. Figure 2 summarizes the typical population dynamics as time series and Poincaré plots where the population abundances for each population are plotted against each other. The Poincaré plots show the typical attractors corresponding to the underlying dynamic behaviour. First, one of the species can go extinct due to high grazing activity by the predator and a low growth rate, while the two other species coexist at equilibrium (Fig. 2a). Second, all three species coexist at equilibrium due to the same rates of loss and growth (Fig. 2b). Third, if the cycle in Fig. 1 is always exactly repeated, the system shows stable limit cycles (Fig. 2c). Fourth, it is possible that the same cycle is never repeated and the system exhibit chaotic dynamics (Fig. 2d). The corresponding attractors are point attractors for coexistence at equilibrium (Fig. 2a, b left column), ring attractors for stable limit cycles (Fig. 2c left column) and strange attractors for chaos (Fig. 2d left column).

The aim of this work was to find a mathematical model of a two-prey-one-predator system under controlled chemostat conditions that can be tested experimentally. Control parameters should be found that trigger the system to exhibit different dynamic behaviours such as coexistence at equilibrium of the three species, stable limit cycles and chaotic dynamics.

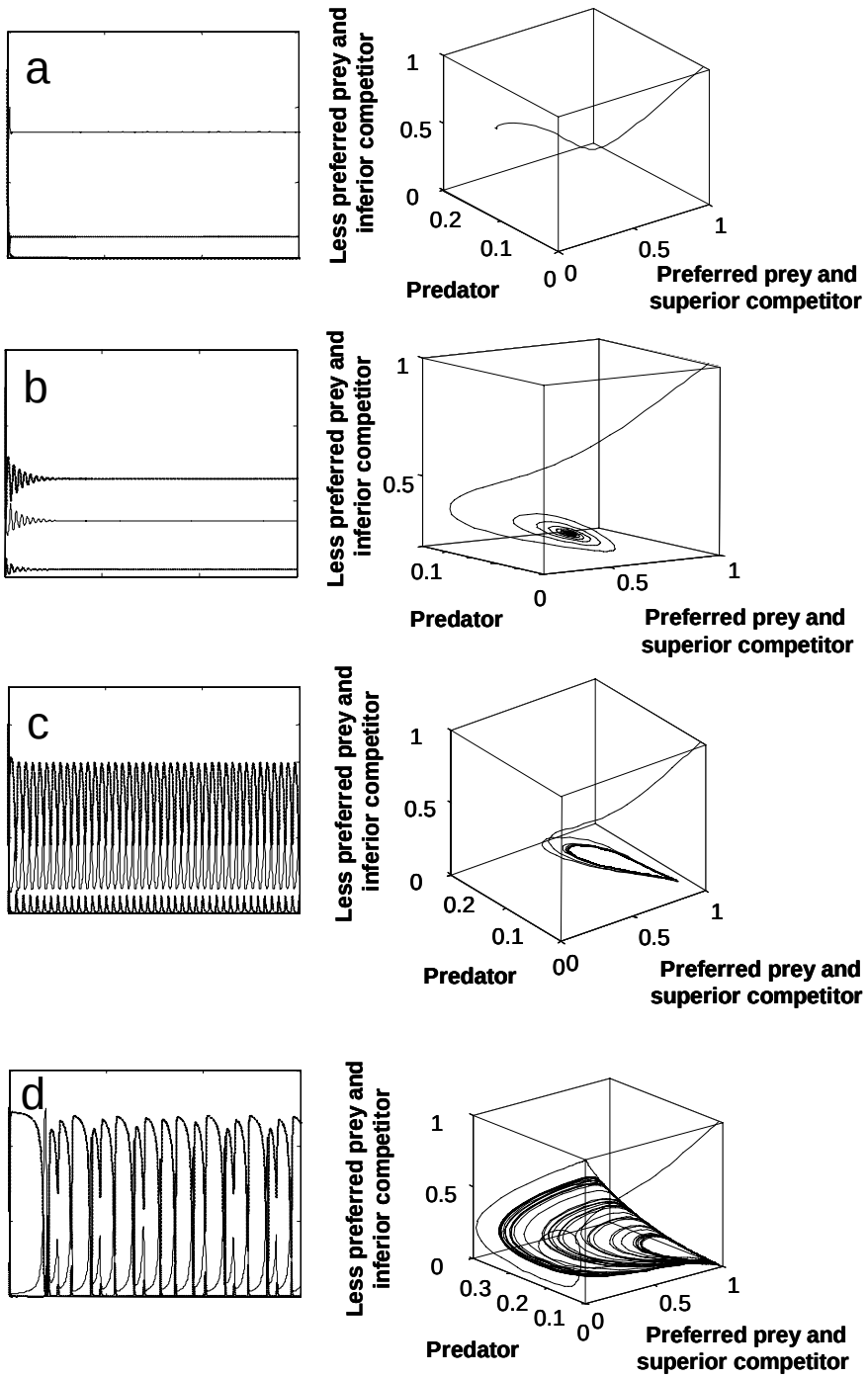

Fig. 2: Typical dynamic pattern occurring in mathematical models of a two-prey-one-predator system as time series (left columns) and Poincaré plots, where population densities are plotted against each other (right columns). (a) Extinction of one population and stable equilibria for the other two populations and a resulting point attractor in the Poincaré plot, (b) stable equilibria for all three populations and a corresponding point attractor, (c) stable limit cycles forming a characteristic ring attractor in the Poincaré plot, and (d) chaos with a corresponding strange attractor (after Takeuchi & Adachi, 1983; model parameterised with $b_1 = b_2 = b_3 = 1.0$, $\alpha = 1.0$, $\beta = 1.5$, $\mu = 1.0$, $d = 0.5$, (a) $e = 3$, (b) $e = 5$, (c) $e = 6$, and (d) $= 9$; initial values 1.0 for the prey population and 0.01 for the predator population).

Mathematical Model

The dynamic behaviour of mathematical models of two-prey-one-predator systems has been analysed through the last decades showing that complex dynamics occur. Most models use continuous time systems to describe the population changes over time regarding changes in the parameter setting. Fujii (1977) showed that a two-prey-one-predator system reveals limit cycles for a Lotka-Volterra model with a linear type of functional response. Vance (1978) and later Gilpin (1979) discovered spiral chaos for this system. Takeuchi and Adachi (1983) analysed a Lotka-Volterra model and could show that the occurrence of chaotic dynamics depends on the greater competitive abilities of one prey population compared to the strength of the prey preference of the predator. The complex dynamics of the three populations occurs also in models of microbial two-prey-one-predator systems under chemostat conditions for different dilution rates of the chemostat system (Vayenas & Pavlou 1999, Kooi & Boer 2003, Vayenas *et al.* 2005). The dilution rates that reveal complex dynamics like stable limit cycles or chaos were for all models low, with $D < 0.1\ d^{-1}$ and intervals of $0.01\ d^{-1}$. An experimental implementation of such low dilution rates in chemostats is hardly realisable. Even though microorganisms reproduce fast the experimental conditions must be constant for at least 30 days to see the asymptotic or long-term behaviour of the populations. To find a mathematical model that shows complex dynamics for dilution rates that are realisable in experiments with microbial two-prey-one-predator systems, a model after Takeuchi & Adachi (1983) was adapted to chemostat conditions by introducing the dilution rate D. The intrinsic death rate b_3 of the predator was replaced by the dilution rate D.

$$\begin{aligned} dx_1/dt &= x_1 * (b_1 - x_1 - \alpha * x_2 - e * z - D) \\ dx_2/dt &= x_2 * (b_2 - \beta * x_1 - x_2 - \mu * z - D) \\ dz/dt &= z * (d * e * z - d * \mu * z - D) \end{aligned} \qquad ①$$

x_1 and x_2 denote the densities of the two prey and z the density of the predator population. b_1 and b_2 are the intrinsic rates of increase, $\alpha > 0$ and $\beta > 0$ are parameters, that describe the competitive effect between the two prey species, $e > 0$ and $\mu > 0$ are coefficients of decrease of the prey species

due to predation, d > 0 is the transformation rate of the predator describing the amount of energy that can be used for growth, D is the dilution rate of the chemostat system per day. The model was parameterised with $b_1 = b_2 = 1.0$ per day; $\alpha = 1.0$, $\beta = 1.5$, $\mu = 1.0$, $d = 0.5$, and $x_1(0) = 1.0$, $x_2(0) = 1.0$, and $z(0) = 0.01$ as initial values for the population. The prey preference e was used as a bifurcation parameter and varied between 0 and 8 ($0 < e < 8$). The dilution was varied between 0 and 1 ($0 < D < 1$) to analyse the dynamic behaviour of the system regarding the dependence on the dilution rate. Model analyses were done using Matlab 7.0 Release 14 (Mathworks Inc.).

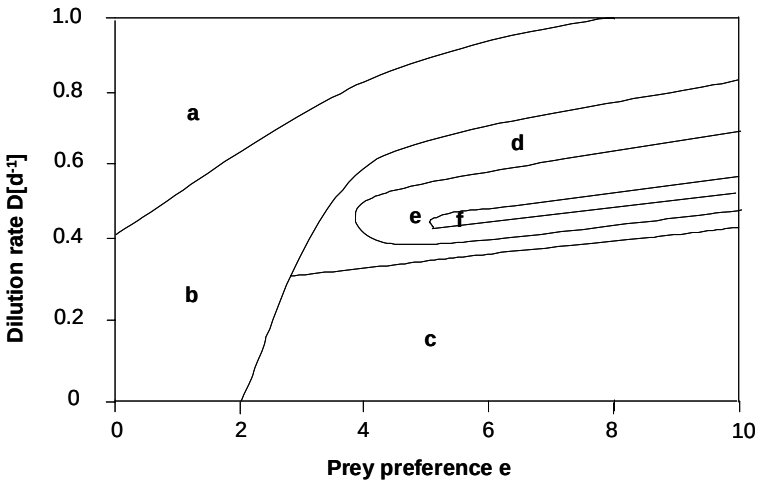

Fig. 3: Operating diagram for the mathematical two-prey-one-predator model ① under chemostat conditions summarizing the dynamic behaviour of the population depending on the prey preference e and the dilution rate D. Region a: Extinction of the less preferred prey x_2 and the predator z. Region b: Extinction of the less preferred prey x_2. Region c: Extinction of the preferred prey b_1 and the predator z. Region d: Coexistence of the two prey x_1, x_2, and the predator z at an equilibrium. Region e: Coexistence of the two prey x_1, x_2, and the predator z at stable limit cycle. Region f: Coexistence of the two prey x_1, x_2, and the predator z showing irregular fluctuations, probably chaos.

The introduction of the dilution rate D did not change the general dynamic behaviour of the two-prey-one-predator model compared to the results of Takeuchi and Adachi (1983). Fluctuating dynamics occurred for dilution rates $0.3 < D < 0.8$ d^{-1} for $e > 2$ with a shift to higher dilution rates for increasing predation rates e. Irregular dynamics - likely chaos - were found for a small parameter set of e and D (f in Fig. 3).

Experimental system

A microbial community was chosen to analyse the dynamic behaviours in a two-prey-one-predator system predicted by model ①. We used a defined community from axenic cultures to ensure a constant species composition in the experimental system. The system consisted of the bacterivorous ciliate *Tetrahymena pyriformis* (average length 85µm * 22µm; from CCAP 1630/1W) as the predator and the two bacteria *Pedobacter* spec. (Cytophaga Flexibacter group, 2 * 1 µm) and *Brevundimonas* spec. (α-Proteobacter, 2,5 µm * 2,5µm) isolated by K. Beck from Lake Schöhsee, Germany, and kindly provided by Klaus Jürgens, Rostock-Warnemünde) as prey organisms. Coexistence of two competing species is possible by predator mediated coexistence (Hairston *et al.* 1960, HilleRisLambers & Dieckmann 2003). Coexistence is ensured if the predator preys preferentially on one prey species and the preferred prey is the superior competitor at the same time. Prey preference of the predator *Tetrahymena* was determined in grazing experiments and competition abilities were analysed in chemostat experiments without predation.

Grazing experiments

Grazing experiments were carried out in 50-ml-tissue culture flasks (Sarstedt) to determine the grazing rates of *Tetrahymena* on *Pedobacter* and *Brevundimonas*. Five times 7.5 ml of both bacterial liquid cultures (in PPY100: 0.2 g/l proteose peptone, 0.025 g/l yeast extract) with an equal density of about $4*10^6$ were added to 5 ml of a *Tetrahymena* culture. Since specific antibody staining worked only until the first three minutes after vacuole formation, grazing experiments were terminated by adding buffered paraformaldehyde (Eisenmann *et al.* 1998; 4.4% paraformaldehyde, 150 µl/l 1 M NaOH, 7.6 g/l NaCl, 1.57 g/l Na_2HPO_4 * 2 H_20, 0.47 g/l NaH_2PO_4) after three minutes. The fixed samples were collected on black 0.8 µm pore-size polycarbonate membrane filters and washed 2 times with 5 x PBS buffer (Phosphate Buffered Saline: 40g/ml NaCl, 1 g/l KCl, 5.76 g/l $Na_2HPO_4*2H_2O$, 1 g/l KH_2PO_4; pH 7.3). The ingested bacteria were determined in the food vacuoles by using immunofluorescence and DAPI (4',6-diamino-2-phenylindole, Porter & Feig 1980) labelling. The following protocol was used:

Samples were treated with Triton X-100 (Merck Chemicals; final concentration 8%) for permeabilization of the ciliate membranes to ensure a better labelling result in the food vacuoles (Christoffersen *et al.* 1997). Triton X-100 was added to membrane filters and incubated for 20 minutes. The filter was washed three times for five minutes with PBS buffer. The strain specific antibodies were diluted 1:200 in 5% BSA (bovine serum albumin/ PBS (volume 3.0 ml)) and added to the samples which were incubated for five minutes. After washing three times with PBS, the filters were incubated with the second, Cy3-labelled antibodies (1:200 diluted with 5% BSA/PBS, volume 3.0 ml) and DAPI solution (final concentration 5 µg/ml) for 5 minutes and washed 3 times with PBS. Membrane filters were mounted on glass slides with an oil droplet (Zeiss 128 F) and stored at -20°C until microscopic analysis. Always care was taken to exclude bacterial contaminations. Ingested bacteria were counted using the epifluorescence microscope (Zeiss Axioskop, filterset 14 for the detection of Cy3-labelled antibodies and filterset 01 to detect DAPI staining). Since both specific primary antibodies were detected by the same second, Cy3 labelled antibodies the staining could only be performed for one bacteria strain in one staining process. The bacteria abundance of the other bacteria was calculated from the differences between the total number of bacteria (enumerated from the unspecific DAPI staining) and the specific stained bacteria. Results from the first enumeration were verified, using a second staining process with the other specific antibodies.

Grazing experiments revealed that both bacteria were grazed and detectable by the use of specific antibody staining and unspecific staining with DAPI (Figs. 4 and 5). Experiments showed that *Pedobacter* could be found four times more in food vacuoles than *Brevundimonas* after three minutes grazing on an equal dens mixture of bacteria (Fig. 4). Thus, we can assume that *Pedobacter* is the preferred prey species of the predator *Tetrahymena* by a factor of four. Both staining procedures showed the same abundances of ingested bacteria. No differences occurred in the relation of ingested bacteria from the two strains when either stained with specific antibodies against *Pedobacter* (Fig. 4a) or against *Brevundimonas* (Fig 4b).

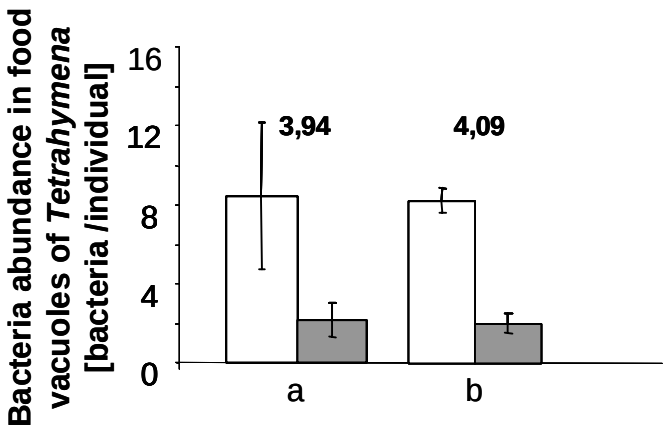

Fig. 4: Bacteria abundance in food vacuoles of *Tetrahymena* after three minutes grazing, determined after staining with specific Cy3 labelled antibodies and unspecific 4', 6-diamino-2-phenylindole (DAPI) staining. (a) *Pedobacter* abundance determined by specific staining while *Brevundimonas* abundance was calculated from the difference between all bacteria stained with DAPI and the specific stained *Pedobacter*. (b) *Brevundimonas* abundance was determined by specific antibody labelling and *Pedobacter* abundance was calculated. Numbers are the relation of ingested *Pedobacter* to *Brevundimonas*.

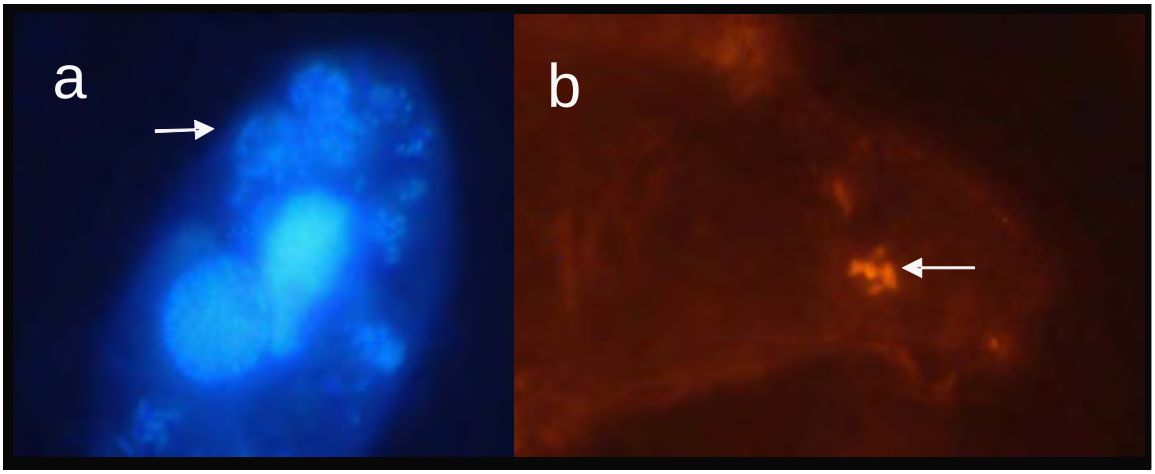

Fig. 5: Results from grazing experiments showing ingested bacteria in food vacuoles of Tetrahymena after staining with (a) unspecific 4', 6-diamino-2-phenylindole (DAPI) and (b) specific Cy3 labelled antibodies against *Pedobacter*. Arrows indicates the food vacuoles with the bacteria.

Competition experiments

Competition experiments were run in one-stage chemostat systems (Fig. 6) at different dilution rates (D = 0.2, 0.45, 0.5, 0.75, 0.9 d^{-1}) with *Pedobacter* and *Brevundimonas*. Chemostat experiments were conducted under sterile and constant conditions (temperature 20 ± 1°C). Chemostats (glass vessels with a volume of 185 ml) were constantly fed with organic medium (PPY100: 0.2 g/l proteose peptone, 0.025 g/l yeast extract; pumps: Watson Marlow 205S) and mixed by gentle aeration.

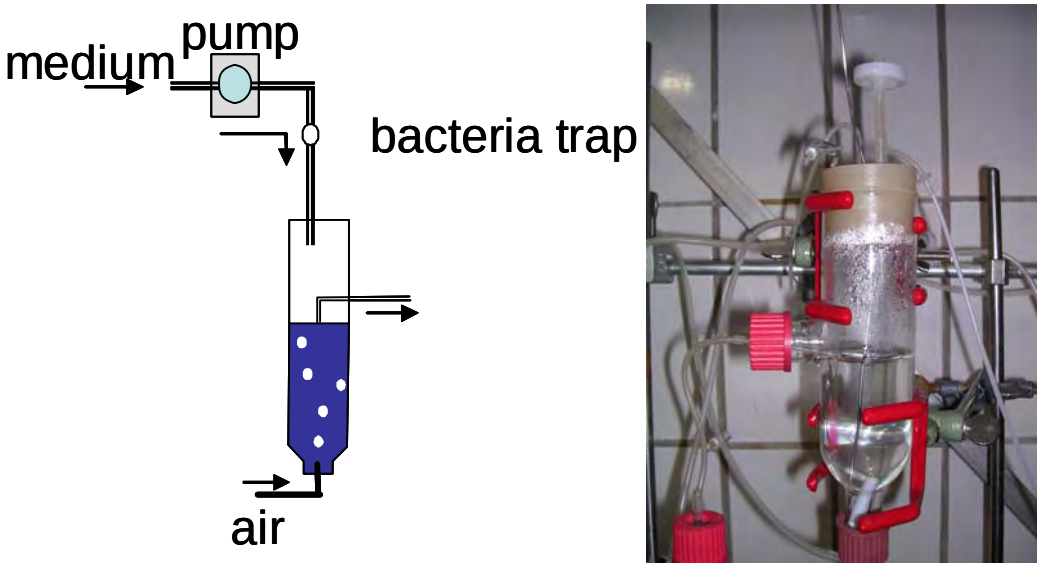

Fig. 6: Chemostat system used for competition experiments and all other chemostat experiments described in chapter II - IV. Left column show a simplified scheme of a one-stage-chemostat system. Right column shows a picture of an one-stage-chemostat reactor.

Chemostats were inoculated from overnight cultures in LB-medium (Trypton: 10 g/l, yeast extract 5 g/l, NaCl 10 g/100 ml; bacteria taken from a deep frozen stock (-80°C)) with initial densities of $1*10^5$ cells per ml. Triplicate samples were taken daily from the middle of the chemostats (fixation with formaldehyde, final concentration 3%) followed by staining with DAPI (4', 6-diamino-2-phenylindole) on black 0.2 µm pore-size polycarbonate membrane filters (Porter & Feig 1980) and enumeration under an epifluorescence microscope (Zeiss Axioskop with filter set 01). At least 300 bacteria of each strain were counted per filter.

All time series recorded from the chemostat experiments revealed that *Brevundimonas* was outcompeted while *Pedobacter* existed at stable equilibrium (Fig. 7).

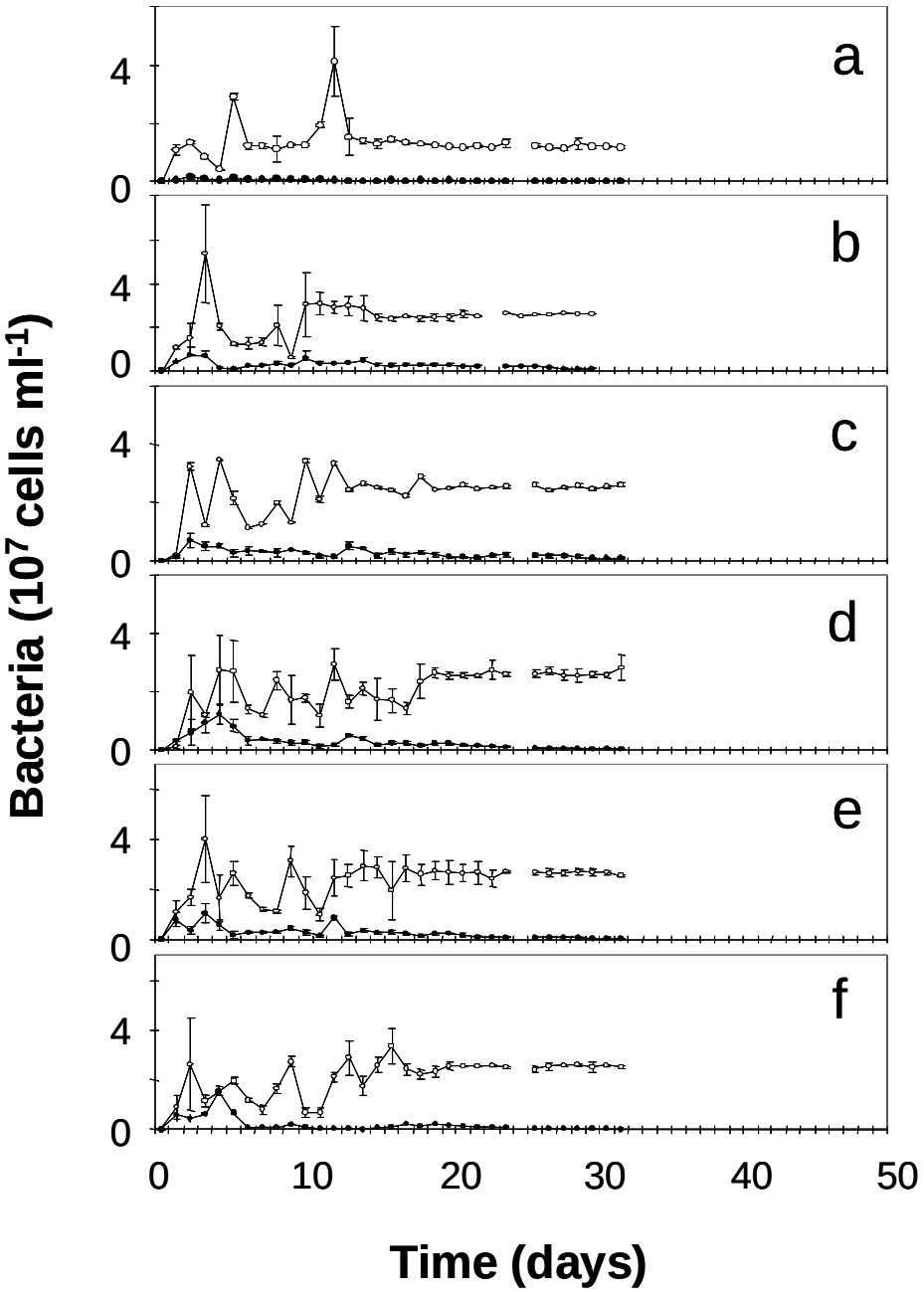

Fig. 7: Experimental results showing the population dynamics of the two bacteria *Pedobacter* (open circles) and *Brevundimonas* (filled circles) in chemostat systems at the different dilution rates D. (a): 0.2 d^{-1}, (b): 0.45 d^{-1}, (c): 0.5 d^{-1}, (d): 0.75 d^{-1}, (e): 0.9 d^{-1}. Vertical bars represent standard deviation of triplicates taken separately form one chemostat. No sampling took place on day 24.

In conclusion, grazing and competition experiments revealed that the experimental system allows the analysis of complex population dynamics as described in the mathematical model. Grazing experiments showed that the prey preference of *Tetrahymena* on *Pedobacter* was e = 4 when the prey preference on *Brevundimonas* was set to μ = 1. The model predicted limit

cycles and coexistence at equilibrium for all three species for a dilution rate of $0.3 < D < 0.6$ d^{-1} with e = 4 (Fig. 3). Extinction of one population was found for a dilution rate D smaller than 0.3 $d^{-1}$and for $0.6 < D < 0.8$ d^{-1}. For a dilution rate greater than D = 0.8 d^{-1} extinction of the less preferred prey b_2 and the predator p were recorded from the mathematical model. Dilution rates D > 0.1 d^{-1} and experimental intervals of 0.05 are realisable for experiments with microorganisms. The use of axenic cultures give the possibility of analyses without any dynamics from contaminations with other microorganisms, as are found in other systems used for studies of intrinsically driven population dynamics. We can assume different dynamic behaviours in the two-prey-one-predator system with *Tetrahymena*, *Pedobacter*, and Brevundimonas for dilution rate of $0.1 < D < 0.9$ d^{-1}.

References:

Becks, L., Hilker, F.M., Malchow, H., Jürgens, K. & Arndt, H. Experimental demonstration of chaos in a microbial food web. *Nature* **435**, 1226-1229 (2005).

Cadotte, M.W., Drake, J.A. & Fukami, T. Constructing nature: laboratory models as necessary tools for investigating complex ecological communities. *Advances in Ecological Research* **37**, 335-353 (2005).

Chase, R.D., *et al.* The interactions between predation and competition: a review and synthesis. *Ecol. Lett.* **5**, 302-315 (2002).

Christoffersen, K., Nybroe, O., Jürgens, K. & Hansen, M. Measurement of bacterivory by heterotrophic nanoflagellates using immunofluorescence labelling of ingested cells. *Aquat. Microb. Ecol.* **13**, 127-134 (1997).

Eisenmann, H., Harms, H., Meckenstock, R., Meyer, E. I. & Zehnder, A. J. B. Grazing of a *Tetrahymena* sp. on adhered bacteria in percolated columns monitored by in situ hybridization with fluorescent oligonucleotide probes. *Appl. Environ. Microbiol.* **64**, 1264-1269 (1998).

Fuji, K. Relationship of two-prey, one-predator species model: local and global stability. *J. Theor. Biol.* **69**, 613-623 (1977).

Fussmann, G. F, Ellner, S. P., Shertzer, K.W. & Hairston, N.G. Jr. Crossing the Hopf bifurcation in a live predator-prey system. *Science* **290**, 1358-1360 (2000).

Gause, G. F. Experimental analysis of Vito Volterra's mathematical theory of the struggle for existence. *Science* **79**, 16-17 (1934).

Gilpin, M.E. Spiral chaos in a predator-prey model. *Am. Nat.* **113**, 306-308 (1979).

Hairston, N.G., Smith, F.E., Slobodkin, L.B. Community structure, population control, and competition. *Am. Nat*. **94**, 421-425 (1960).

HilleRisLambers, R. & Dieckmann, U. Competition and predation in simple food webs: intermediately strong trade-offs maximize coexistence. *Proc. R. Soc. Lond. B.* **270**, 2591-2598 (2003).

Jessup, C.M. *et al.* Big questions, small worlds: microbial model systems in ecology. *Trends Ecol. Evol.* **19**, 189-197 (2004).

Kooi, B.W. & Boer, M.P. Chaotic behaviour of a predator-prey system in the chemostat. *Dynamics of Continuous Discrete and Impulsive Systems Series B: Applications and Algorithms* **10**, 259-272 (2003).

Luckinbill, L.S.& Fenton, M.M. Regulation and environmental variability in experimental populations of protozoa. *Ecology* **59**, 1271-1276 (1978).

May, R. M. Biological populations with nonoverlapping generations: stable points, stable cycles, and chaos. *Science* **186**, 645-647 (1974).

Porter, K.G. & Feig, Y.S. The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceangr.* **25**, 943-948 (1980).

Takeuchi, Y. & Adachi, N. Existence and bifurcation of stable equilibrium in two-prey, one predator communities. *Bull. Math. Biol.* **45**, 877-900 (1983).

Turchin, P. (2003). *Complex Population Dynamics: A Theoretical /Empirical Synthesis*. Princton Univ. Pres, Princton.

Vance, R.R. Predation and resource partioning in one predator, two prey model communities. *Am. Nat.* **112**, 797-813 (1978).

Vayenas, D.V. & Pavlou, S. Chaotic dynamics of a food web in a chemostat. *Math. Biosci.* **162**, 69-84 (1999).

Vayenas, D.V., Aggelis, G., Tsagou, V., Pavlou, S. Dynamics of a two-prey-one-predator system with predator switching regulated by a catabolic repression control-like mode. *Ecol. Model.* **186**, 345-357 (2005).

Chapter II

Experimental demonstration of chaos in a microbial food web

LETTERS

Experimental demonstration of chaos in a microbial food web

Lutz Becks[1]*, Frank M. Hilker[2], Horst Malchow[2], Klaus Jürgens[3,4] & Hartmut Arndt[1]*

Discovering why natural population densities change over time and vary with location is a central goal of ecological and evolutional disciplines. The recognition that even simple ecological systems can undergo chaotic behaviour has made chaos a topic of considerable interest among theoretical ecologists[1–4]. However, there is still a lack of experimental evidence that chaotic behaviour occurs in the real world of coexisting populations in multi-species systems. Here we study the dynamics of a defined predator–prey system consisting of a bacterivorous ciliate and two bacterial prey species. The bacterial species preferred by the ciliate was the superior competitor. Experimental conditions were kept constant with continuous cultivation in a one-stage chemostat. We show that the dynamic behaviour of such a two-prey, one-predator system includes chaotic behaviour, as well as stable limit cycles and coexistence at equilibrium. Changes in the population dynamics were triggered by changes in the dilution rates of the chemostat. The observed dynamics were verified by estimating the corresponding Lyapunov exponents. Such a defined microbial food web offers a new possibility for the experimental study of deterministic chaos in real biological systems.

Apart from the intuitive understanding that external (extrinsic) stimuli influence the variability of abundances, mathematical models have made it apparent that the internal (intrinsic) qualities of a population give rise to population dynamics with large and (at certain parameter ranges) even chaotic fluctuations of abundances, even under wholly constant and predictable conditions[5]. Predator–prey interactions have been considered as a possible driving force of population dynamics since the beginning of ecological studies[6,7]. In his analysis of mathematical models, May[1] found that even simple processes of population growth can show (for a certain range of parameters) an unpredictable behaviour driven by intrinsic mechanisms. May's studies marked the beginning of an intensive debate on the question of whether or not natural systems are characterized by chaotic behaviour. In this context, the term 'deterministic chaos' can be defined as bounded aperiodic fluctuations with sensitive dependence on initial conditions[4]. Under chaotic conditions, population abundances never show a precisely repeated pattern over time; such patterns are only observable in populations at equilibrium or at stable limit cycles. Theoreticians can clearly define parameter ranges of mathematical models that create chaotic behaviour in idealized biological systems[3,8–10]. However, only a very few experiments indicating that bifurcations of dynamic behaviour might occur in the real world have been conducted (for example, ciliate–bacteria interactions[11], flour beetle (*Tribolium castaneum*) dynamics[12,13] and rotifer–algae interactions[14]). Indications of chaotic dynamics under controlled conditions have so far been reported for one-species systems only[13]. A robust tool to verify observed dynamics is estimations of Lyapunov exponents from time series, which test for the exponential divergence of nearby trajectories. Mathematically, stable (convergent) systems show negative Lyapunov exponents, whereas chaotic (divergent) systems have at least one positive Lyapunov exponent[4].

The aim of the present study was to verify the biological relevance of chaotic behaviour in a real multi-species system. The long generation durations of most organisms and the complexity of natural environments have generally made the explanation of underlying ecological mechanisms difficult[15]. However, experiments using microbial populations propagated in controlled environments reduce ecosystem complexity to the point at which understanding simple processes in isolation becomes possible. The rapid reproduction of bacteria and protists is one of the main advantages of working with microorganisms as model organisms[7,16,17]. In addition, the community structure can be exactly defined; for example, single strains of bacteria and protists can be selected. Microorganisms can also be cultured under chemostat conditions. This has the great advantage that extrinsic factors are negligible and changes in population dynamics can be attributed to intrinsic factors. In terms of predation and interspecific competition, one of the simplest systems imaginable is a three-species system with two prey organisms and one predator. Several theoretical studies have been made of such model systems[8–10,18]. Generally, different patterns of population dynamics are predicted by models; for example, the extinction of one or two species and the coexistence of all three species. Assuming that the two prey populations compete with each other and assuming that the better competitor is the preferred prey, three patterns may occur: coexistence at equilibrium, coexistence at stable limit cycles, and coexistence at chaos[8–10,18].

Our study was aimed at identifying these different patterns of coexistence in controlled experiments in a chemostat. We used the dilution rate as the bifurcation parameter in the experiments, because the dynamical behaviour of chemostat models can change with dilution rate[9,10,14]. We constructed one-stage chemostat systems consisting of axenic cultures of three species: a predator (the ciliate *Tetrahymena pyriformis*) and two coexisting prey bacteria, the rod-shaped *Pedobacter* and the coccus *Brevundimonas*. The effective consumption of these bacteria by the ciliate and its food preference was analysed by immunofluorescence techniques. The ciliate can establish stable populations when feeding on either bacterium, but it dies off in the highly diluted organic medium when bacteria are absent. The growth conditions for the bacteria and the mortality of the ciliate are determined by the dilution rates (controlled by peristaltic pumps). *Brevundimonas* was always outcompeted in chemostat experiments containing both bacterial strains without a predator. Thus, *Pedobacter* was considered to have a better fitness. In

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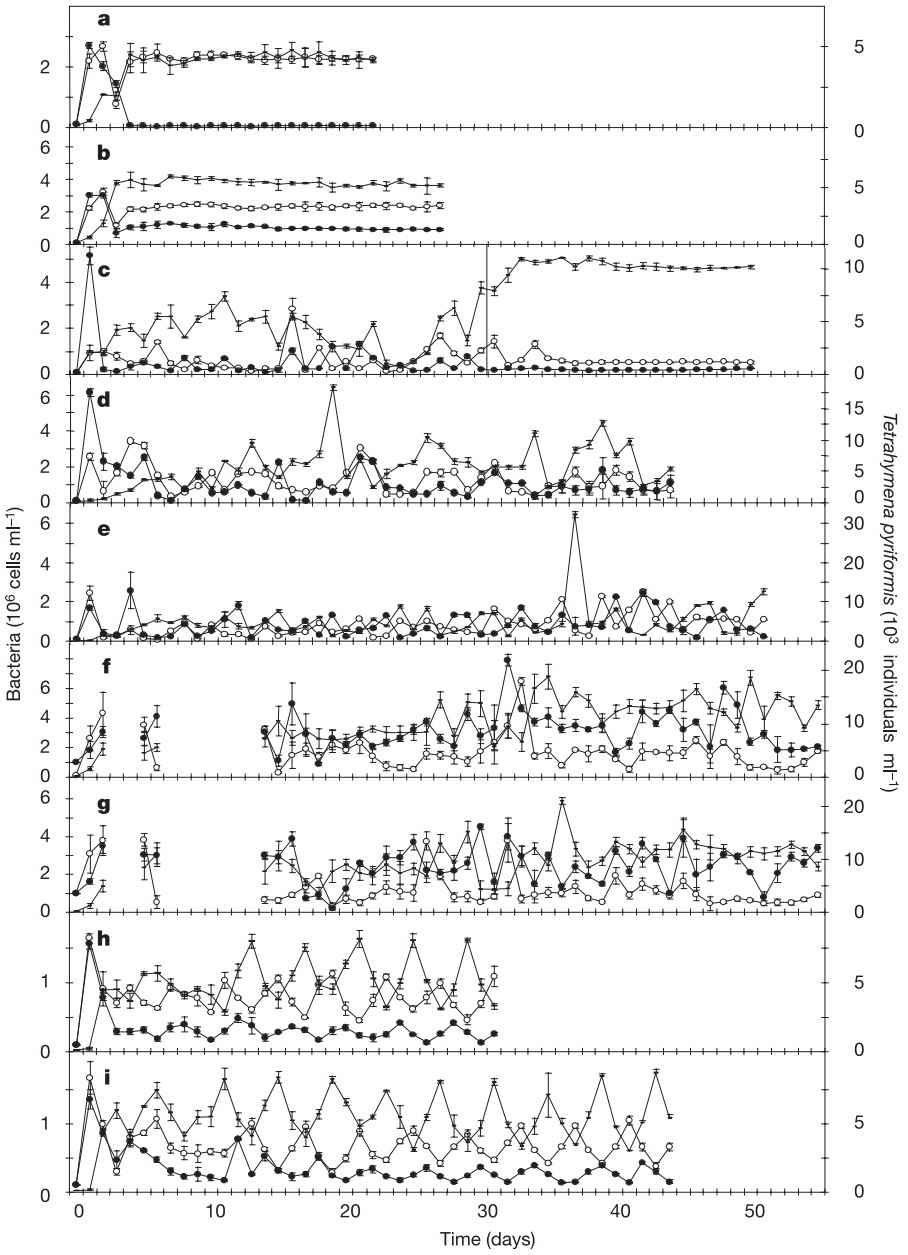

Figure 1 | Experimental results showing the population dynamics of bacteria–ciliate chemostat systems. Dilution rates D were as follows: **a**, 0.90 d^{-1}, **b**, 0.75 d^{-1}; **c**, 0.50 d^{-1} (the line indicates the change to 0.75 d^{-1} at day 30); **d**–**g**, 0.50 d^{-1} (replicate experiments; no sampling took place on days 3, 4 and 7–13 in **f** and **g**); **h**, **i**, 0.45 d^{-1} (replicate experiments). Open circles, *Pedobacter* (preferred prey); filled circles, *Brevundimonas* (less-preferred prey); horizontal bar, *Tetrahymena* (predator). Vertical bars represent the s.d. of triplicate samples taken separately from one chemostat.

contrast, our grazing experiments revealed that *Pedobacter* is preferred as prey by the ciliate over *Brevundimonas* by a factor of four (see Supplementary Information). Experiments were performed with dilution rates of 0.90, 0.75, 0.50 and 0.45 d^{-1}. These dilution rates were selected on the basis of preceding model calculations.

The results revealed a different dynamic behaviour of the experimental system, depending on the applied dilution rate (Fig. 1). At the highest dilution rate ($D = 0.90$ d^{-1}), *Brevundimonas* had died off by the sixth day; the remaining species existed in stable coexistence at equilibrium (Fig. 1a). The establishment of constant, equilibrium population densities of all species was achieved after about 5 days at $D = 0.75$ d^{-1} (Fig. 1b). To check the robustness of the stable equilibrium, we repeated this experiment with a preceding 30-day period of aperiodic dynamics at $D = 0.50$ d^{-1} (Fig. 1c). Although similar dynamic behaviour was observed after a transition period of 5 days, the abundances reached by the three species were different from those of the previous experiment. One possible explanation for these differences in abundances of the rapidly reproducing microbes (30 days represents about 240 generations in the experiments) might be a potential evolutionary shift in population structure[19]. Obviously stable limit cycles were established in the two parallel chemostat systems after a period of about 8 days at a dilution rate of 0.45 d^{-1} (Fig. 1h, i). Maxima and minima for all three species recurred during the whole observation period. Slight differences can be attributed to the sampling interval, which was kept constant at about 24 h. The cycles started with a maximum abundance of the preferred bacterium, followed by a peak of the less-preferred bacterium and the predator. Aperiodic oscillations were always obtained when dilution rates were set to 0.50 d^{-1} (Fig. 1d–g). All four trials showed different patterns in their dynamics. The observed aperiodic oscillations of the chemostat populations were analysed for possible chaotic behaviour by using estimates of corresponding Lyapunov exponents (Fig. 2; see

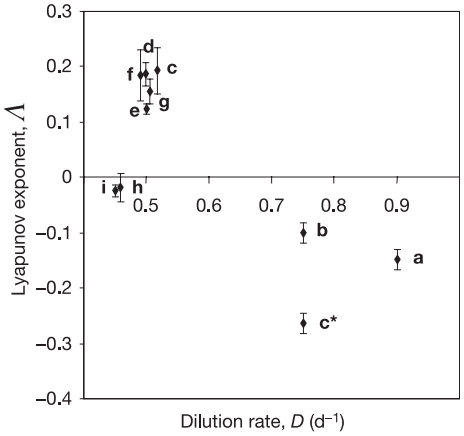

Figure 2 | Relationship between trajectory stability of data sets from Fig. 1 and the corresponding dilution rates. Lyapunov exponents were determined by the method of Rosenstein *et al.*[20] by fitting the rate of exponential separation of initially close trajectories. The error bars correspond to the asymptotic errors in the fit. Letters correspond to panels in Fig. 1 (**c*** is the Lyapunov exponent calculated for the second part of the time series in Fig. 1c; $t = 31$–50 days). Note that for dilution rates of 0.45 d^{-1} and 0.5 d^{-1}, data points were spread slightly along the *x* axis for visual clarity.

Supplementary Information). They were determined from each time series with a previously published algorithm[20], which tests directly for the exponential divergence from nearby trajectories and provides a very robust method for also dealing with small data sets (see Methods). According to general theoretical expectations, the data sets with extinction of the less-preferred prey species (Fig. 1a) and with coexistence at stable equilibrium (Fig. 1b) revealed negative Lyapunov exponents. This also holds true for the second part of the time series in Fig. 1c (after a change in the dilution rate to 0.75 d^{-1}). All experiments with $D = 0.5\ d^{-1}$ (Fig. 1c, left of the vertical line, and Fig. 1d–g) have positive Lyapunov exponents. Thus, we obtained strong experimental evidence for the existence of chaos in a real multi-species system. Note that for small data sets the range of the confidence interval generally increases. The stable sustained oscillations (Fig. 1h, i) have Lyapunov exponents close to zero (Fig. 2). Their absolute value is at least one order of magnitude smaller than all the other exponents. The exponential divergences of nearby trajectories show strong, sustained oscillations as well. There is a large asymptotic standard error in the fit of the Lyapunov exponents because of the strong oscillations. We conclude that the underlying dynamics are stable limit cycles. The observed dynamics in the experiments changed as predicted by a model[10] (stable coexistence at high dilution rates, chaos at intermediate dilution rates, and stable limit cycles at low dilution rates).

There are two important conclusions to be drawn. First, chaotic dynamics of small, rapidly growing organisms can occur in all microbiotopes. Second, because of the low generation times of microbes (only a few hours), such dynamics may be established before perturbations by external stimuli are effective. Examples of such communities are the tiny, fragmented populations of protists and bacteria that can occur on each grain of sand in a sediment, as well as on each small detritus particle in the pelagic zone of the open ocean or lakes[21,22]. The defined microbial food web that we established under chemostat conditions offers a completely new possibility for the experimental study of deterministic chaos in real biological systems. It is now possible to address many questions previously posed by theoreticians. We have provided a biological system that allows the investigation of the transition between different dynamical states, the analysis of interactions of fragmented populations showing either similar or different dynamic behaviours, the study of resilience and the importance of perturbations under varying dynamical states, and the interplay between complex dynamics and biodiversity[1–4,8,16,18,23]. When combined with molecular techniques, this system would also allow the evolutionary consequences of different dynamic behaviours to be analysed[19].

METHODS

Chemostat experiments. We established cultures of the ciliate *Tetrahymena pyriformis* (axenic culture from CCAP 1630/1W, average length and width 85 μm × 22 μm), the bacterium *Pedobacter* sp. (Cytophaga Flexibacter group, 2 μm × 1 μm) and *Brevundimonas* sp. (α-Proteobacteria, 2.5 μm × 2.5 μm) in 185 ml glass chemostats at 20 ± 1 °C in the dark. Both bacterial species were isolated by K. Beck from Lake Schöhsee, Germany; bacteria were always inoculated from deep-frozen stock cultures. The one-stage chemostat systems were fed continuously with sterile medium (0.2 $g\,l^{-1}$ proteose peptone, 0.025 $g\,l^{-1}$ yeast extract) at different dilution rates and mixed by continuous gentle aeration to ensure an even distribution of organisms. Chemostats were always started with the same inoculum. Sterile syringes were used to take samples daily at about 11:00 from the centre of the chemostats. Living ciliate samples were counted under a phase-contrast microscope immediately after sampling (more than 150 individuals were counted). Samples of bacteria were fixed with formaldehyde and stained with 4′,6-diamidino-2-phenylindole (DAPI)[24] for subsequent counting on membrane filters (pore size 0.2 μm) under an epifluorescence microscope (Zeiss Axioskop) with Zeiss filter set 01. At least 300 bacteria were counted on each filter. Organism abundances were the average of triplicates taken separately from one chemostat. The total volume of water taken from the chemostats during one sampling was 3 ml. Chemostats were checked regularly for the appearance of contaminant bacteria by using strain-specific antibodies against *Pedobacter* and *Brevundimonas* and by non-specific staining of the bacterial community with DAPI. With our present apparatus, the maximum number of samplings possible before contamination or any other technical problem hindered further experimentation was 50–55 days.

Grazing experiments. Experiments were performed to determine the food preference of *Tetrahymena*. A bacterial mixture (1:1) of *Pedobacter* and *Brevundimonas* (each strain at 4×10^6 cells ml^{-1}) was offered as prey in 50-ml vessels at 20 °C. The contents of the vessels were fixed with a buffered paraformaldehyde solution 3 min after inoculation of *Tetrahymena*[25]. The abundances of *Pedobacter* and *Brevundimonas* in the food vacuoles of *Tetrahymena* were determined by immunofluorescence[26] after hybridization with specific Cy3-labelled antibodies (permeabilization was performed with 8% Triton X-100).

Calculation of Lyapunov exponents. The calculations of the Lyapunov exponents by using the algorithm of Rosenstein *et al.*[20] were performed with the TISEAN package[27] (see Supplementary Information). Similarly to the independently published algorithm of Kantz[28], it directly tests the presence of exponential divergence and thus permits a decision on whether it makes sense to compute a Lyapunov exponent for given data. In contrast, the first published and widely used algorithm of Wolf *et al.*[29] makes the *a priori* assumption that there is an exponential divergence of nearby trajectories and is therefore prone to yield finite positive Lyapunov exponents also for stochastic data. This has been criticized in the ecological literature[4,30], and alternative approaches have been proposed that rely on approximating the equations of the underlying dynamics. The exponents are calculated from the jacobian, which resembles the linear part of the dynamics. This method is efficient if the data permit a good reconstruction of the dynamics. However, one has to be careful, because a good approximation of the dynamics does not guarantee well-approximated partial derivatives in the jacobian. However, because the present data stem from constant experimental conditions in a chemostat environment, the algorithm of Rosenstein *et al.*[28] should reveal more reliable estimates. The exponents were calculated by reconstructing the attractor dynamics from the time series of the predator's abundances with appropriate embedding dimensions and reconstruction delays, which robustly exhibited exponential divergence. The Lyapunov exponent was then fitted as the slope of the linear increase in the log-transformed divergence by using the least-squares method.

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1. May, R. M. Biological populations with nonoverlapping generations: stable points, stable cycles, and chaos. *Science* **186,** 645–647 (1974).
2. Hastings, A. & Powell, T. Chaos in a three-species food chain. *Ecology* **72,** 896–903 (1991).
3. Huisman, J. & Weissing, F. J. Biodiversity of plankton by species oscillations and chaos. *Nature* **402,** 407–411 (1999).
4. Turchin, P. *Complex Population Dynamics: A Theoretical/Empirical Synthesis* (Princeton Univ. Press, Princeton, 2003).

5. Begon, M., Harper, J. L. & Townsend, C. R. *Ecology* (Blackwell Science, Oxford, 2002).
6. Volterra, V. Fluctuations in the abundance of a species considered mathematically. *Nature* **118,** 558–600 (1926).
7. Luckenbill, L. S. & Fenton, M. M. Regulation and environmental variability in experimental populations of protozoa. *Ecology* **59,** 1271–1276 (1978).
8. Takeuchi, Y. & Adachi, N. Existence and bifurcation of stable equilibrium in two-prey-one-predator communities. *Bull. Math. Biol.* **45,** 877–900 (1983).
9. Vayenas, D. V. & Pavlou, S. Chaotic dynamics of a food web in a chemostat. *Math. Biosci.* **162,** 69–84 (1999).
10. Kooi, B. W. & Boer, M. P. Chaotic behaviour of a predator–prey system in the chemostat. *Dynam. Contin. Discrete Impulsive Syst. B* **10,** 259–272 (2003).
11. Jost, J. L., Drake, J. F., Frederickson, A. E. & Tsuchiya, H. M. Interactions of *Tetrahymena pyriformis*, *Escherichia coli*, *Azotobacter vinelandii*, and glucose in a mineral medium. *J. Bacteriol.* **113,** 834–840 (1973).
12. Costantino, R. F., Desharnais, R. A., Cushing, J. M. & Dennis, B. Chaotic dynamics in an insect population. *Science* **275,** 389–391 (1997).
13. Dennis, B., Desharnais, R. A., Cushing, J. M. & Costantino, R. F. Transitions in population dynamics: equilibra to periodic cycles to aperiodic cycles. *J. Anim. Ecol.* **66,** 704–729 (1997).
14. Fussmann, G. F., Ellner, S. P., Shertzer, K. W. & Hairston, N. G. Jr Crossing the Hopf bifurcation in a live predator–prey system. *Science* **290,** 1358–1360 (2000).
15. Heerkloss, R. & Klinkenberg, G. Chaotic dynamics of a plankton community in a species-depleted mesocosmos. *Verh. Int. Verein. Limnol.* **25,** 995–1000 (1993).
16. Rainey, P. B., Buckling, A., Kassen, R. & Travisano, M. The emergence and maintenance of diversity: insights from experimental bacterial populations. *Trends Ecol. Evol.* **15,** 243–247 (2000).
17. Jessup, C. M. *et al.* Big questions, small worlds: microbial model systems in ecology. *Trends Ecol. Evol.* **19,** 189–197 (2004).
18. Gilpin, M. E. Spiral chaos in a predator–prey model. *Am. Nat.* **113,** 306–308 (1979).
19. Yoshida, T. *et al.* Rapid evolution drives ecological dynamics in a predator–prey system. *Nature* **424,** 303–306 (2003).
20. Rosenstein, M. T., Collins, J. J. & De Luca, C. J. A practical method for calculating largest Lyapunov exponent from small data sets. *Physica D* **65,** 117–134 (1993).
21. Arndt, H. *et al.* in *The Flagellates* (eds Leadbeater, B. S. C. & Green, J. C.) 240–268 (Taylor & Francis, London, 2000).
22. Caron, D., Davis, P. G., Madin, L. P. & Sieburth, J. M. Heterotrophic bacteria and bacterivorous protozoa in oceanic macroaggregates. *Science* **218,** 795–797 (1982).
23. Nelson, W. A., McCauley, E. & Wrona, F. J. Stage-structured cycles promote genetic diversity in a predator–prey system of *Daphnia* and algae. *Nature* **433,** 413–417 (2005).
24. Porter, K. G. & Feig, Y. S. The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.* **25,** 943–948 (1980).
25. Eisenmann, H., Harms, H., Meckenstock, R., Meyer, E. I. & Zehnder, A. J. B. Grazing of a *Tetrahymena* sp. on adhered bacteria in percolated columns monitored by in situ hybridization with fluorescent oligonucleotide probes. *Appl. Environ. Microbiol.* **64,** 1264–1269 (1998).
26. Christofferson, K., Nybroe, O., Jürgens, K. & Hansen, M. Measurement of bacterivory by heterotrophic nanoflagellates using immunofluorescence labelling of ingested cells. *Aquat. Microb. Ecol.* **13,** 127–134 (1997).
27. Hegger, R., Kantz, H. & Schreiber, T. Practical implementation of nonlinear time series methods: The TISEAN package. *Chaos* **9,** 413–439 (1999).
28. Kantz, H. A robust method to estimate the maximal Lyapunov exponent of a time series. *Phys. Lett. A* **185,** 77–87 (1994).
29. Wolf, A., Swift, J. B., Swinney, H. L. & Vastano, J. A. Determining Lyapunov exponents from a time series. *Physica D* **16,** 285–317 (1985).
30. Ellner, S. P. & Turchin, P. Chaos in a noisy world: New methods and evidence from time-series analysis. *Am. Nat.* **145,** 343–375 (1995).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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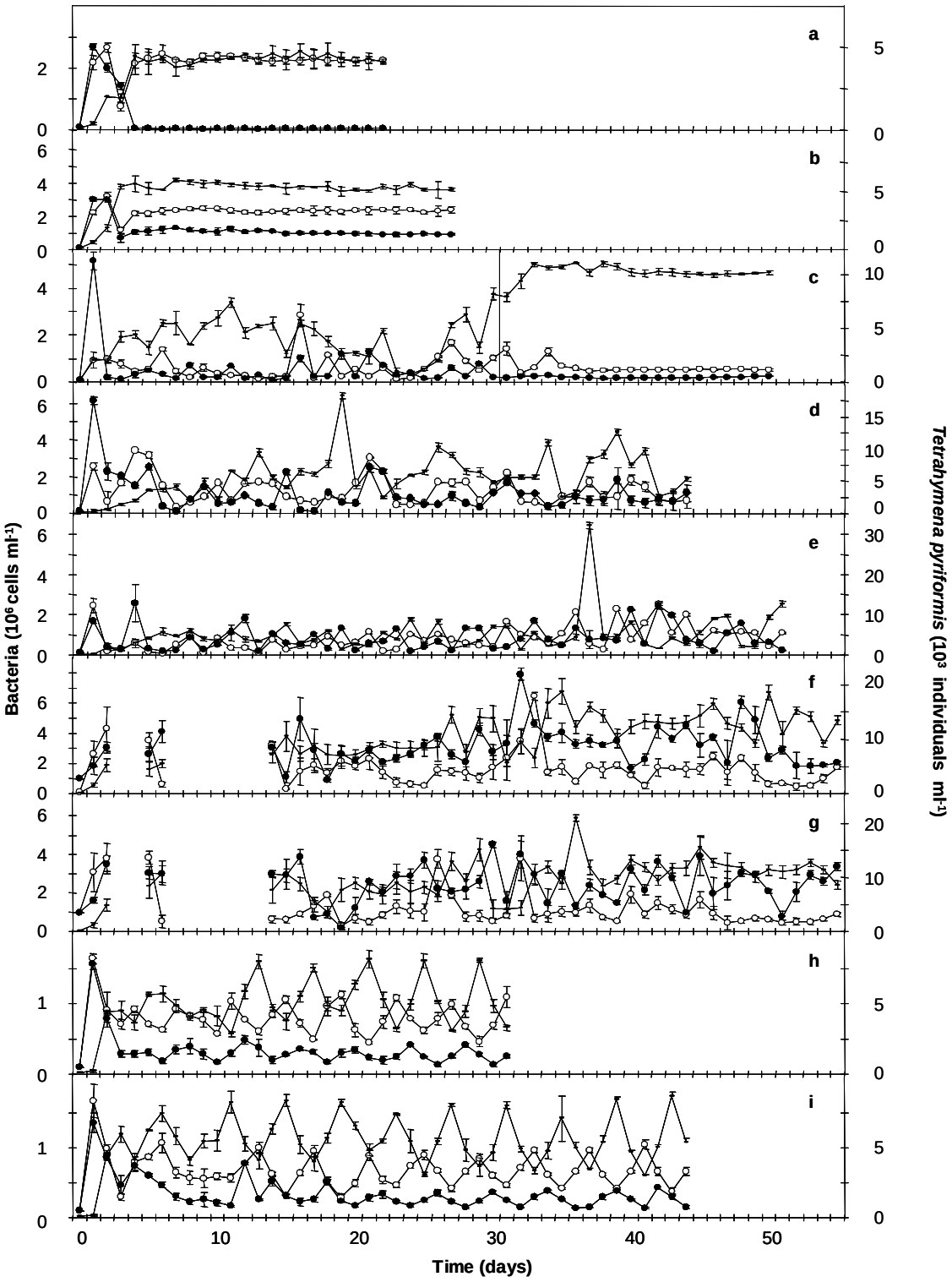


Figure 1: Experimental results showing the population dynamics of bacteria-ciliate chemostat systems at different dilution rates D. a: 0.90 d^{-1}, b: 0.75 d^{-1}, c: 0.50 d^{-1}, line indicates the switch to 0.75 d^{-1} at day 30; d-g: 0.50 d^{-1}, no sampling took place on day 3,4 and 7-13 in f and g, h, i: 0.45 d^{-1}; open circles: *Pedobacter* (preferred prey), filled circles: *Brevundimonas* (less-preferred prey), horizontal bar: *Tetrahymena* (predator). Vertical bars represent SD of triplicates taken separately from one chemostat.

Supplementary Information: Lutz Becks et al., Experimental demonstration of chaos in a microbial food web. Nature (2005).

Growth characteristics of the three species

The food preference of the ciliate *Tetrahymena pyriformis* was determined by grazing experiments (see Methods). *Tetrahymena* grazed 163 ± 6 cells* h^{-1} * individual^{-1} of the bacterium *Pedobacter* and 41 ± 2 cells* h^{-1} * individual^{-1} of the bacterium *Brevundimonas* in a food suspension containing equal numbers of both bacteria. Mean growth rates in the chemostat experiments were 0.54 ± 0.11 d^{-1} for *Tetrahymena*, 2.98 ± 0.47 d^{-1} for *Pedobacter* and 1.93 ± 0.86 d^{-1} for *Brevundimonas*.

Attractor reconstruction and divergence plots

The first step in determining the largest Lyapunov exponent involves reconstructing the attractor dynamics from a single time series. Here, we have chosen the predator abundances. The embedding theorem of Takens (1981) guarantees that the reconstructed trajectory portrays the dynamics in the higher dimensional state space.

Reconstruction delay: The reconstruction delay (also called lag) has been set to 1 d. This choice is recommended for biological systems with continuous reproduction and generation times less than the unit time interval (Turchin 2003). Moreover, we have also checked the autocorrelation function (ACF) and the mutual information. For a delay of 1 d, the ACF drops below 1/2 as suggested by Turchin (2003) and thus below 1-1/e as suggested by Rosenstein et al. (1993), except for the stationary systems (Fig. 1 a,b and the second part of c), where the ACF still remains below 0.7. Another choice can be the time at which the first local minimum of the mutual information is reached (Yamamoto, 1999). In the majority of the considered time series, this again yields a delay of 1 d or close to this delay (2 d for Fig. 1 d,g and 3 d for Fig. 1 a, b, d).

Embedding dimension: A first impression about the embedding dimension *m* can be obtained by considering the dimension for which the fraction of false nearest

neighbours drops substantially to zero (Hegger et al. 1999, Yamamoto 1999). This has been the case for an embedding dimension of 3 or 4; only for the time series in Fig. 1 first part of c, f and g, there was no clear decline in the fraction of false nearest neighbours. For the calculation of Lyapunov exponents, the (minimum) embedding dimension is required for which the slopes of the exponential divergence do not change anymore. The rate of exponential separation has been computed for $m = 2,\ldots, 6$. Robust results have been obtained for embedding dimensions larger than 2 or 3. Hence, Lyapunov exponents are throughout given for $m = 4$.

Divergence plot: The Lyapunov exponent quantifies the exponential divergence/convergence of initially close trajectories. Chaotic systems typically show an initial linear increase in the separation of trajectories, followed by a constant plateau since chaotic attractors are bounded. The Lyapunov exponent can be estimated by the slope of a straight line that is fitted to the linear part of the ln-transformed divergence. By way of example, this is shown in Supplementary Figure 1. Fitting is done by least squares. For the time series with the switch in the dilution rate (Fig. 1 c) and with the sustained oscillations (Fig. 1 h and i), respectively 4 and 10 data points have been omitted in order to avoid transient effects.

References

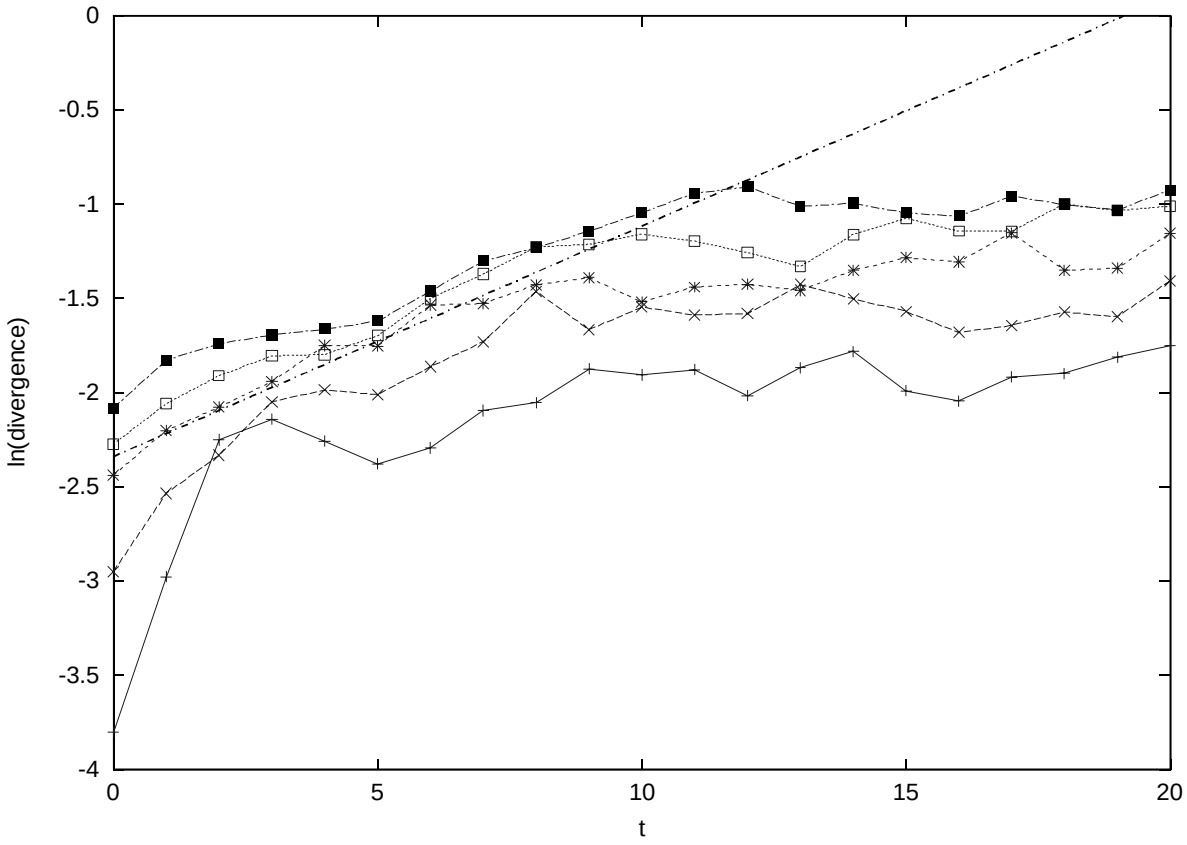
S1. Hegger, R., Kantz, H. & Schreiber, T. Practical implementation of nonlinear time series methods: The TISEAN package. *Chaos* **9**, 413-439 (1999).

S2. Rosenstein, M.T., Collins, J.J. & De Luca, C.J. A practical method for calculating largest Lyapunov exponent from small data sets. *Physica D* **65**, 117-134 (1993).

S3. Takens, F. Detecting strange attractors in turbulence. *Lecture Notes in Mathematics* **898**, Springer-Verlag, New York (1981)

S4. Turchin, P. *Complex Population Dynamics: A Theoretical/Empirical Synthesis* (Princeton Univ. Press, 2003).

S5. Yamamoto, Y. Detection of chaos and fractals from experimental time series. In: Windhorst, U. & Johansson, H. (eds.) *Modern Techniques in Neuroscience Research*. Springer-Verlag, Berlin Heidelberg, 669-687 (1999).



Supplementary Figure 1: Estimation of the Lyapunov exponent by fitting a straight line (without points) to the linear part of the ln-transformed divergence of nearby trajectories. The lines with points correspond to the embedding dimensions m = 2,…, 6 (in increasing order at t=0).

Contribution of co-authors:

Frank M. Hilker and Horst Malchow helped with data analyses and calculations of the Lyapunov exponents.

Klaus Jürgens provided the bacteria and specific antibodies.

CHAPTER III
SHORT TRANSITION OF DYNAMIC BEHAVIOUR IN EXPERIMENTS WITH CHAOTIC FOOD WEBS

Understanding temporal fluctuations in natural systems is of fundamental interest in natural sciences. Deterministic models predict that shifts in ecological parameters may lead to a transition between deterministic chaos and stable equilibria or limit cycles. For the first time, we experimentally document short-term transitions between these different dynamics. After manipulation of one external stimulus (chemostat dilution rate) in a multi-species food web of two bacteria and a bacterivorous ciliate, experiments showed that switching between different dynamic behaviours may occur surprisingly fast in microbial populations (4-7 days). Thus chaotic dynamics may be easily overlooked in field observations.

Intrinsic and extrinsic forces and the interplay between them drive system dynamics and result in temporal and spatial population fluctuations (May 1974, Ellner & Turchin 1995, Hastings 2001, Henson *et al.* 2003) as well as fluctuations in other complex systems (e.g. measles dynamics (Olsen & Schaffer 1990), hydrodynamics and oscillatory chemical reactions (Gaspard *et al.* 1999), neural activity in the brain (Martinerie *et al.* 1998)). Due to the strength of intrinsic mechanisms, populations can show different asymptotic or long-term behaviour such as stable equilibria (point attractors) or cyclic or chaotic behaviour (May 1976). During the last decade, the importance of intrinsically driven transitions between different kinds of dynamics became evident (Hastings & Higgins 1994). It was found that transitions in dynamic behaviour may support the coexistences of a large number of species using the same food sources (Huisman & Weissing, 1999, 2001). Intrinsically driven dynamics may also be superimposed by external forces (Higgins *et al.* 1997, Kaitala *et al.* 1997, Bjørnstad & Grenfell 2001, Clouson *et al.* 2004); the necessity to change the focus of ecological thinking towards dynamical changes of population densities within shorter timescales has become evident (Hastings 2004, Earn *et al.* 2000, Noonburg & Abrams 2005). According to model analyses, a transition in dynamic behaviour can be induced by shifts in biotic and abiotic parameters changing in space and time (May 1974, Hastings 2001, Earn *et al.* 2000). Models predict that ecosystems of small spatial and/or temporal scales lead to the dominance of transient dynamics (De Angelis & Waterhouse 1987, Chen & Cohen 2001).

Experimental evidence of transitions between different kinds of dynamic behaviour of real populations is very rare. In an experimental one-species system of the floor beetle Tribolium castaneum, manipulations of a demographic parameter (i.e. rates of between-stage cannibalism) lead to a transition from stable to cyclic and chaotic behaviour within a few generations (Costantino *et al.* 1995, Dennis *et al.* 1997). And the only available two-species example of a rotifer-algae chemostat system showed transitions from equilibrium to limit cycles after manipulation of dilution rates (Fussmann *et al.* 2000, Chapter I, compare to Becks *et al.* 2005). The key question is: How fast do transitions occur and how long do they last? If transitions occur within a few days, such processes may be easily overlooked and may have resulted in underestimations of chaotic processes in natural habitats in the past.

Most models predict that transitions should last several hundred generations (Hastings & Higgins 1994, Earn *et al.* 2000, DeAngelis & Waterhouse 1987), while a few empirical studies (Costantino *et al.* 1995, Dennis *et al.* 1997, Fussmann *et al.* 2000, Becks *et al.* 2005) indicate a possible transition within only about 5-7 generations. If the latter is true, short-term persisting, intrinsically triggered chaos may often be miss-interpreted as stochastic fluctuations (noise) leading to a completely different conclusion regarding the causal interrelationships. Similar problems are typical for other complex systems studied in medicine and economy. Experimental testing in these disciplines is often impossible, and biological experiments may provide a tool to analyze and understand transitions in general.

A unique microbial chemostat system allows us to address the question regarding the occurrence and duration of transitions between different dynamic behaviours in a multi-species system for the first time (Becks *et al.* 2005). In a two-prey-one predator system we demonstrate how the behaviour of the system shifts due to manipulations of the dilution rate of a chemostat system. The high reproduction rate of microorganisms and the resultant high number of generations observed in short-term experiments make microorganisms good model organisms for the study of population dynamics. By reducing ecosystem complexity to a degree at which dynamics are comprehensible and by minimizing external perturbation it is possible to analyze deterministic population behaviours (c.f Chapter II, compare to

Becks *et al.* 2005, Jessup *et al.* 2004). Dynamic behaviour can be visualized by phase space graphs and estimated by calculations of corresponding Lyapunov exponents from observed time series (Hastings *et al.* 1993, Turchin 2003). We were able to drive the chemostat system from one dynamic state to the other by establishing dilution rates of either 0.9 d-1, 0.75 d-1, 0.5 d-1, 0.45 d-1 or 0.2 d-1. The one-stage chemostat system consisted of the predator *Tetrahymena pyriformis*, the preferred prey and superior competitor *Pedobacter* sp. (Cytophaga/Flavobacter-group) and the less preferred prey and inferior competitor *Brevundimonas* sp. (α-proteobacteria).The data are presented as time-dependent changes in abundances (Fig. 1 a-e) as well as in the time delay reconstruction (Fig. 2 a-e). A shift in dynamic behaviour is obvious in all five experiments, and detectable transitions between the types of dynamic behaviour occurred in four experiments (Figs. 1a-d, 2 a-d). The duration of transition after the shift of the dilution rate was determined graphically by counting the days before the trajectories settle on a different type of attractor. For a dilution rate shifted from D = 0.5 d-1 to D = 0.2 d-1, a change from chaotic behaviour (Lyapunov exponent Λ positive) to equilibrium of *Tetrahymena* and *Pedobacter* occurred within four days (Figs. 1a, 2a). For a shift in dilution rate from D = 0.5 d-1 to D = 0.45 d-1, a change from chaos (Λ positive) to stable limit cycles of *Tetrahymena*, *Pedobacter*, and *Brevundimonas* was observed within four days (Figs. 1b, 2b). Changing the dilution rate from D = 0.5 d-1 to D = 0.75 d-1 revealed a switch from chaos (Λ positive) to stable equilibrium of all three species within six days (Figs. 1c, 2c). For a shift in dilution rate from D = 0.5 d-1 to D = 0.9 d-1, a change from chaotic behaviour (Λ positive) to equilibrium of *Tetrahymena* and *Pedobacter* was evident after seven days (Figs. 1d, 2d). Changing the dilution rate from D = 0.75 d-1 to D = 0.5 d-1 lead to a change from stable equilibrium of all three species to irregular fluctuations (Λ could not be calculated due to a lack of sufficient data). The duration of the transition could not be estimated in this case, but seems to be similar to the other experiments (Figs. 1e, 2e).

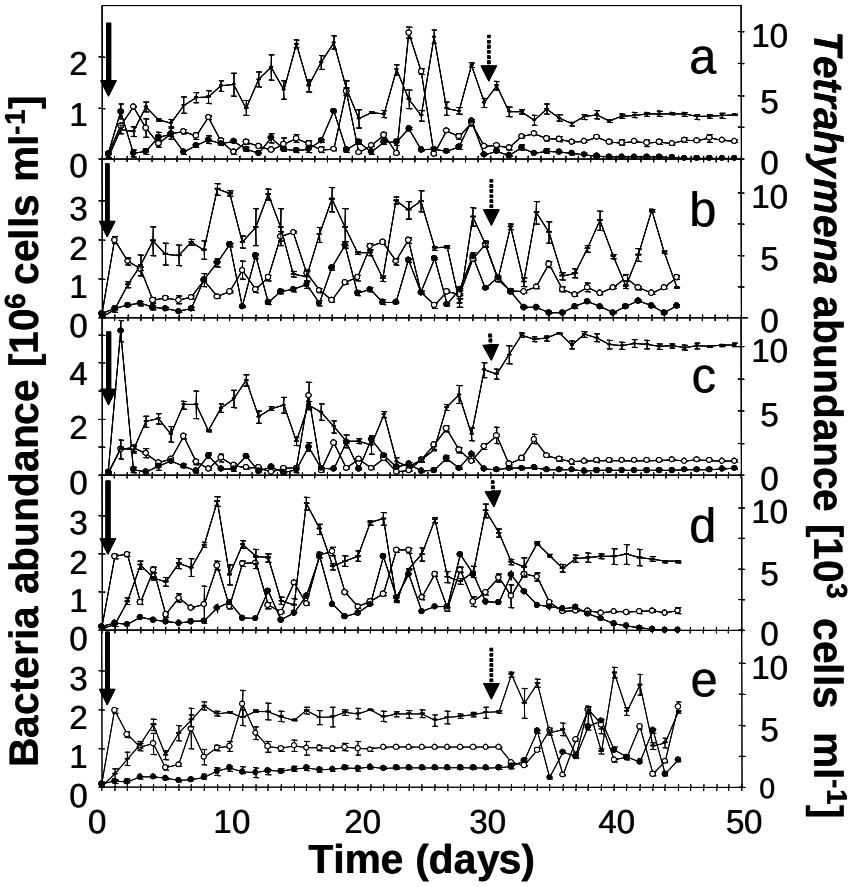


Fig. 1: Time series data of an experimental two-prey-one-predator system of a bacterivorous ciliate and two prey bacteria species cultured in chemostats at different dilution rates (185 ml vessels supplied with organic medium PPY 100, at 20 ±1°C). Open circles, abundances of the rod-shaped bacterium *Pedobacter* spec. (preferred prey and better competitor; 2 µm x 1 µm); filled circles, abundances of the coccus *Brevundimonas* spec. (less preferred prey and inferior competitor; 2 µm x 2 µm; both bacteria were isolated from Lake Schöhsee, Germany by K. Beck); horizontal bar, abundances of the ciliate *Tetrahymena pyriformis* (predator, culture from CCAP 1630/1W). Vertical bars represent the standard deviation of separately taken triplicate samples. Continuous arrows indicate the start of the experiment, dashed arrows mark the change in the dilution rate after 30 days. Dilution rates were set to (a) D = 0.5 d-1 and 0.2 d-1 after 30 days, (b) D = 0.5 d-1 and 0.45 d-1 after 30 days, (c) D = 0.5 d-1 and 0.75 d-1 after 30 days (taken from Chapter II, compare to Becks *et al.* 2005 Fig, 1c), (d) D = 0.5 d-1 and 0.9 d-1 after 30 days, and (e) D = 0.75 d-1 and 0.5 d-1 after 30 days.

We could show that a transition between different complex dynamics of a three-species system was realized through a manipulation of one experimental parameter. Point attractors were observed for equilibrium dynamics (Figs. 2a, c, d, and e) and are consistent with general theory (Turchin 2003). For stable limit cycles (Fig. 2b; after the shift in dilution rate), data points forming a ring attractor do not cumulate as strictly on a ring as expected based on theory.

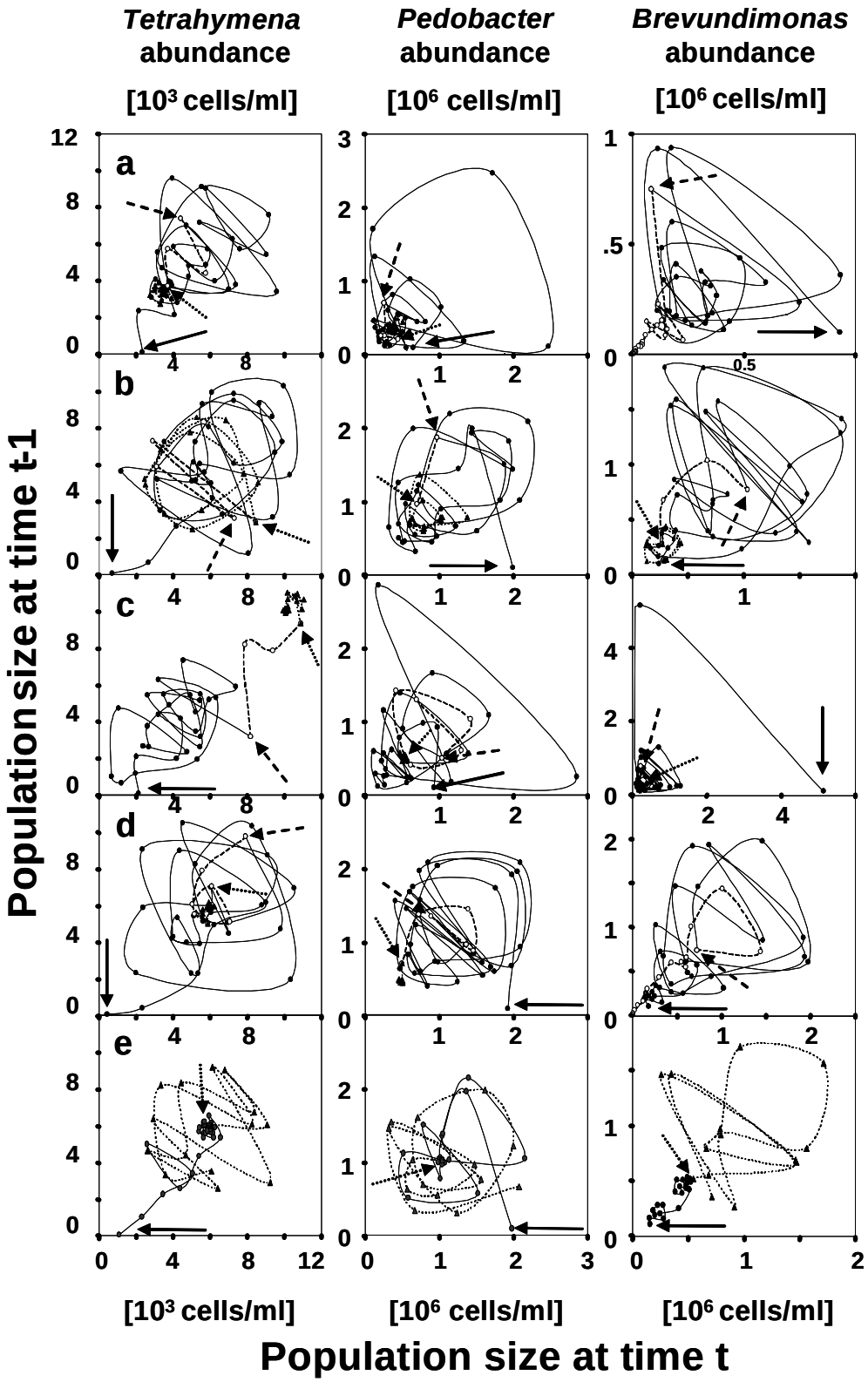


Fig. 2: Time delay reconstruction graphs for the three coexisting species *Tetrahymena* (left column), *Pedobacter* (middle column) and *Brevundimonas* (right column) from chemostat experiments. The dynamic behaviour is indicated before the shift in the dilution rate (days 0-30, solid line and filled circles); the transition time (dashed line and open circles) and the dynamic behaviour after the transition (dotted line and filled triangles). The population sizes of organisms at time t are plotted against population sizes the day before (t-1). Letters a-e correspond to panels in Fig. 1. Continuous arrows indicate the start of the experiment, dashed arrows mark the data point at the shift of the dilution rate, and dotted arrows indicate the settlement of trajectories on the new attractor (determined graphically).

This is probably due to the small number of data points after the shift (14 days). Attractors projected from time series for a dilution rate of D = 0.5 d-1 always showed a cyclic and bounded pattern. The trajectories behave as if influenced by some attraction. Even though experiments were conducted under constant laboratory conditions, process noise could induce some stochasticity.

In addition, reproduction of organisms is not a “rigid schedule” (Dennis *et al.* 2001), so that at least some demographic stochasticity leading to fuzzy attractors is also present. From time delay reconstruction graphs it is obvious that all attractors had a lower and upper boundary (Fig. 2a-e). This result emphasizes that even if population dynamics are chaotic there is a measure of predictability, since all data points remain between the boundaries (Hastings *et al.* 1993).

The following important conclusions can be drawn from our studies: Population dynamics of microbes seem to switch easily between the different types of dynamic behaviour. This supports the idea that small rapidly growing organisms may live under chaotic dynamics (c.f. Chapter II, compare to Becks *et al.* 2005). Chaotic dynamics may occur in the field only as a part of a more complex behaviour with transitions and asymptotic dynamics due to shifts in parameters affecting population dynamics (Hastings 2004). Model predictions indicate that ecosystems of small spatial scale, spatial dynamics and time delays may lead to the dominance of transient dynamics (De Angelis & Waterhouse 1987, Chen & Cohen 2001). Recent theoretical work has shown that transient dynamics enhance the chance of coexistence of competing species (Huisman & Weissing 1999, 2001). Knowing that transitions between the different types of dynamic behaviour in the microbial world can occur within a few days makes it very probable that short-term periods of chaos are often overlooked due the fact that complex dynamics are woven with environmental stochasticity. This might also be true for other complex systems (e.g. epidemics, chemical reactions, cell-cell communications). The major part of the biosphere (deep sea and groundwater) is characterized by constant environmental parameters which support intrinsically driven microbial population dynamics. Thus, chaos may be much more common in nature than currently assumed.

Materials and Methods

We conducted one stage-chemostat experiments under constant conditions, as described by Chapter II (compare to Becks *et al.* 2005). Chemostat communities contained populations of two bacterial strains *Pedobacter* spec. and *Brevundimonas* spec. and the bacterivorous ciliate *Tetrahymena pyriformis* from axenic cultures (bacteria isolated by K. Beck from Lake Schöhsee, Germany, inoculations from deep-frozen stock cultures; *Tetrahymena pyriformis* culture from CCAP 1630/1W). Coexistence of all three populations is possible because the predator *Tetrahymena* preys preferentially on the better competitor *Pedobacter*. Prey preference was tested using specific Cy3-labeled antibodies and unspecific staining with 4', 6-diamino-2-phenylindole DAPI (Porter & Feig 1983, Christofferson *et al.* 1997). Competition was determined in one stage chemostat experiments at different dilution rates (D = 0.2, 0.45, 0.5, 0.75, 0.9 d-1) as described below without the predator. *Brevundimonas* was always out-competed by *Pedobacter*. Feeding experiments using specific antibody staining showed that the rod-shaped *Pedobacter* was ingested four times more often than the coccus *Brevundimonas*.

Chemostat experiments were always started with the same inoculum (bacterial inoculum 100,000 cells/ml, ciliate inoculum 1,000 ind. /cells). Abundances of organisms in chemostats were determined daily. Living ciliates were counted under the phase-contrast microscope. Bacterial population sizes were determined after fixation (formaldehyde: final concentration 3%) and staining with DAPI. Evaluation of bacteria abundances was preformed under an epi-fluorescence microscope (Zeiss Axioskop with filter set 01). Experiments were performed at different dilution rates. Dilution rates were set to D = 0.5 d-1 and shifted to (a) D = 0.2 d-1, (b) D = 0.45 d-1, (c) D = 0.75 d-1 and (d) D = 0.9 d-1 respectively after 30 days. One experiment was conducted with a dilution rate of D = 0.75 d-1 for 30 days and a shift to D = 0.5 d-1 (e).

The largest Lyapunov exponents Λ were calculated from recorded abundances of *Tetrahymena* for a classification of the observed dynamics by using the algorithm by Rosenstein *et al.* (1993). Calculations were preformed with the TISEAN package, with a time delay of one day and an embedding dimension of 4 (Fig. 1a: $\Lambda = 0.11 \pm 0.14$; Fig. 1b: $\Lambda = 0.14 \pm 0.14$; Fig. 1c: $\Lambda =$

0.14 ± 0.11; Fig. 1d: Λ = 0.08 ± 0.13) (Chapter II). Due to the small data set, no calculations were done for the second part of the time series from the experiment after the shift as well as for the experiment with a dilution rate of D = 0.75 d-1 from the start of the experiment (Figs. 1e, 2e). Nevertheless the underlying dynamics could be determined from time series and by the constructed attractor in the time delay reconstruction. Time delay reconstruction graphs were drawn from time series abundances with a delay of one day. The duration of transition after the shift of the dilution rate was determined graphically by counting the days before the trajectories settle on a different type of attractor.

References:

Becks, L., Hilker, F.M., Malchow, H., Jürgens, K. & Arndt, H. Experimental demonstration of chaos in a microbial food web. *Nature* **435**, 1226-1229 (2005).

Bjørnstad, O.N. & Grenfell, B.T. Noisy clockwork: Time series analysis of population fluctuations in animals. *Science* **293**, 638-643 (2001).

Chen, X. & Cohen, J.E. Transient dynamics and food-web complexity in the Lotka-Volterra cascade model. *Proc. R. Soc. Lond. B* **268**, 869-877 (2001).

Christoffersen, K., Nybroe, O., Jürgens, K., & Hansen, M. Measurement of bacterivory by heterotrophic nanoflagellates using immunofluorescence labeling of ingested cells. *Aquat. Microb. Ecol.* **13**, 127-134 (1997).

Costantino, R.F., Cushing, J.M., Dennis, B. & Desharnais, R.A. Experimentally induced transition in the dynamic behaviour of insect populations. *Nature* **375**, 227-230 (1995).

Coulson, T. Rohani, P. & Pascual M. Skeletons, noise and population growth: the end of an old debate? *Trends Ecol. Evol.* **19**, 359-364 (2004).

De Angelis, D.L. & Waterhouse, J.C. Equilibrium and nonequilibrium concepts in ecological models. *Ecol. Monogr.* **57**, 1-21 (1987).

Dennis, B., Desharnais, R.A., Cushing, J.M. & Costantino, R.F. Transitions in population dynamics: equilibria to periodic cycles to aperiodic cycles. *J. Anim. Ecol.* **66**, 704-729 (1997).

Dennis, B., Desharnais, R.A., Cushing, J.M., Henson, S.M. & Costantino R.F. Estimating chaos and complex dynamics in an insect population. *Ecol. Monogr.* **71**, 277-303 (2001).

Earn, D.J.D., Rohani, P., Bolker, B.M. & Grenfell, B.T. A simple model for complex dynamical transition in epidemics. *Science* **287**, 667-670 (2000).

Ellner, S..P. & Turchin, P. Chaos in a noisy world: New methods and evidence from time-series analysis. *Am. Nat.* **145**, 343-375 (1995).

Fussmann, G.F., Ellner S.P., Shertzer K.W., Hairston, N.G. Jr. Crossing the Hopf bifurcation in a live predator-prey system. *Science* **290**, 1358-1360 (2000).

Gaspard, P., Briggs, M.E., Francis, M.K., Sengers, J.V., Gammon, R.W., Dorfman, J.R. & Clalabrese, R.V. Experimental evidence for microscopic chaos. *Nature* **394**, 865-868 (1999).

Hastings, A. & Higgins, K. Persistence of transients in spatially structured ecological models. *Science* **263**, 113-1136 (1994).

Hastings, A. Transient dynamics and persistence of ecological systems. *Ecol. Lett.* **4**, 215-220 (2001).

Hastings, A. Transients. The key to long-term ecological understanding? *Trends Ecol. Evol.* **19**, 39-45 (2004).

Hastings, A., Hom, C.L., Ellner S., Turchin, P. & Godfray, H.C.J. Chaos in ecology: Is Mother Nature a strange attractor? *Annu. Rev. Ecol. Syst.* **24**, 1-33 (1993).

Hegger, R., Kantz, H. & Schreiber, T. Practical implementation of nonlinear time series methods: The TISEAN package. *Chaos* **9**, 413-439 (1999).

Henson, S.M., King, A.A., Costantino, R.F., Cushing, J.M., Dennis, B. & Desharnais, R.A. Explaining and predicting patterns in stochastic population systems. *Proc. R. Soc. Lond. B* **270**, 1549-1553 (2003).

Higgins, K., Hastings A., Sarvela, J.N., Botsford, L.W. Stochastic dynamics and deterministic skeletons: Population behaviour of dungenesss crab. Science **276**, 1431-1435 (1997).

Huisman, J. & Weissing, F.J. Biodiversity of plankton by species oscillations and chaos. *Nature* **402,** 407-411 (1999).

Huisman, J. & Weissing, F.J. Fundamental unpredictability in multispecies competition. *Am. Nat.* **157**, 488-494 (2001).

Jessup, C. M., Kassen. R., Forde, S. E., Kerr, B., Buckling, A., Rainey, P.B. & Bohannan, B.J.M. Big questions, small worlds: microbial model systems in ecology. *Trends Ecol. Evol.* **19**, 189-197 (2004).

Kaitala, V., Ylikarjula, J., Ranta, E. & Lundberg, P. Population dynamics and the colour of environmental noise. *Proc. R. Soc. Lond. B* **264**, 943-948 (1997).

Martinerie, J. Adam, C., LeVanQuyen, M., Baulac, M., Clemencreau, S., Renault, B. & Varela F.J. Epileptic seizures can be anticipated by non-linear analysis. *Nature Medicine* **4**, 1173-1176 (1998).

May, R. M. Biological populations with nonoverlapping generations: stable points, stable cycles, and chaos. *Science* **186,** 645-647 (1974).

May, R. M. Simple mathematical models with very complicate dynamics. *Nature* **261**, 459-467 (1976).

Noonburg, E.G. & Abrams, P.A. Transient dynamics limit the effectiveness of keystone predation in bringing about coexistence. *Am. Nat.* **165**, 322-335 (2005).

Olsen, L.F. & Schaffer, W. M. Chaos versus noisy periodicity: alternative hypotheses for childhood epidemics. *Science* **249**, 499-504 (1990).

Porter, K.G. & Feig, Y.S. The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceangr.* **25**, 943-948 (1980).

Rosenstein, M.T., Collins, J.J. & De Luca, C.J. A practical method for calculating largest Lyapunov exponent from small data sets. *Physica D* **65**, 117-134 (1993).

Turchin, P. (2003). *Complex Population Dynamics: A Theoretical /Empirical Synthesis*. Princton Univ. Pres, Princton.

CHAPTER IV

PERSISTENCE OF COMPLEX POPULATION DYNAMICS IN COUPLED MICROBIAL POPULATIONS

The understanding and prediction of population dynamics of organisms either free-living or parasitic are an ongoing challenge for biology and medicine. Recently it became known that dynamics of real populations are not only determined by extrinsic but also by intrinsic mechanisms and the interactions between the two. Theoretically, local populations may differ regarding their dynamic behaviour and may comprise stable equilibrium, stable limit cycles as well as chaos. Due to the lack of suitable experimental systems, it is not known how local population dynamics may influence the dynamic behaviour of coupled populations when these populations are driven by intrinsic mechanisms. We studied the dynamics of a real world microbial community (2nd stage chemostat system) which received a continuous immigration of organisms from two local communities (1st stage chemostat systems) with different intrinsic dynamics. An experimental two-prey-one-predator system (bacteria, protozoa) served as a model community. Here we show that the dynamic behaviour of the coupled populations depends on intrinsic forces rather than on disturbances from intrinsic dynamics of the source populations. These results point to the stability of intrinsically forced dynamics. Determining the key factors of intrinsic dynamics should allow controlling population dynamics and diseases.

The role of complex deterministic population dynamics received continuous attention since the early work of Robert May in the 1970´s (May 1974). Different deterministic dynamic behaviours are exhibited in simple population models in dependence of one or more control parameters showing equilibria, limit cycles and chaos (e.g. May 1974). However, empirical evidence for such complex dynamics is very rare. To our knowledge, a bifurcation into all types of dynamic behaviour was shown only for a one-species system (the flour beetle, *Tribolium castaneum* (Costantino *et al.* 1997)) and recently for a three-species system (microbial two-prey-one-predator system (Becks *et al.* 2005). The occurrence and persistence of complex deterministic dynamics – stable or fluctuating - depends on the spatial structure of the system (Malchow 1993, Blasius *et al.*1999). Both, the

Tribolium (Costantino *et al.* 1997) and the microbial system (Becks *et al.* 2005) assume that the populations in separate experimental systems (milk bottle and chemostat, respectively) are uniform homogeneous populations. Most organisms and populations in nature have a patchy distribution and create small local subpopulations with possible inherently different local dynamics creating all together one metapopulation (Levins 1969, Hanski 1998, Holyoak *et al.* 2005). Metapopulation dynamics are driven by migration or dispersal between local populations. Theoretical studies have shown that the persistence and occurrence of dynamic behaviour of a population can change when heterogeneity is considered (Rohde & Rohde 2001, Dhamala *et al.* 2001, Maionchi *et al.* 2006). Spatial heterogeneity between local populations might be expressed by differences in demographic parameters, like the intrinsic growth rate (Rohde & Rohde 2001). It was shown that dispersal may either stabilise unstable dynamics in local populations caused by predator-prey interactions (e.g. parasite and host dynamics Hassel & May 1973, Ives 1992) or cause replacement of regular oscillations by chaos (rotifer and algae dynamics, Medvinsky *et al.* 2005).

There is a major gap between the theory of population interactions with specific intrinsically forced dynamics and empirical data from the real world. Here we show for the first time experimental studies of coupled populations with unidirectional coupling exhibiting different dynamic behaviour ranging from stable equilibrium to chaos. Using a system of combined chemostats, we were able to address the following questions: How stable is the dynamic behaviour of food web components when disturbed by chaotic, oscillating or constant immigrations? What type of dynamic behaviour is established in coupled communities when couples were originally characterised by different types of dynamic behaviour including chaos?

Laboratory microcosms were used to test the influence of local dynamic behaviour on coupled populations. The chemostat systems allows to exhibit different complex population dynamics like damped oscillations, stable limit cycles and chaos in a two-prey-one-predator system (c.f. Chapter II, compare to Becks *et al.* 2005). Different dynamic behaviours for all three

populations can be obtained for different dilution rates D: damped oscillations for D = 0.75 d^{-1}, stable limit cycles for D = 0.45 d^{-1} and chaos for D = 0.5 d^{-1}. For each type of dynamic behaviour we established two local populations in the first-stage of the chemostat system (Table 1a-h).

Table 1: Summary of chemostat set up and dynamic behaviour in the two-stage-chemostat systems of the bacterivorous ciliate *Tetrahymena pyriformis* and the two bacteria *Pedobacter* spec. and *Brevundimonas* spec. Lyapunov exponents were determined according to Rosenstein *et al.* 1993 (Hegger & Kantz 1999, c.f. Chapter II) for *Tetrahymena* time series. Due to a lack of a sufficient number of data, values could not be calculated for chemostat o.

1ST-STAGE CHEMOSTAT	DILUTION RATE D [D^{-1}] IN1ST STAGE	LYAPUNOV EXPONENT Λ	DYNAMIC BEHAVIOUR*	2ND STAGE CHEMOSTAT (TAKEN EQUALLY FROM FIRST STAGE CHEMOSTAT)	DILUTION RATE D [D^{-1}] IN 2ND STAGE	LYAPUNOV EXPONENT Λ	DYNAMIC BEHAVIOUR*
a	0.45	0.005	stable limit cycles	i (a +b)	0.45	-0.037	stable limit cycles
b	0.45	0.003	stable limit cycles	j (c +d)	0.5	0.04	chaos
c	0.5	0.14	chaos	k (e +f)	0.75	-0.034	damped oscillations
d	0.5	-0.089	chaos	l (a +c)	0.45	-0.037	stable limit cycles
e	0.75	-0.05	damped oscillations	m (b + d)	0.5	0.029	chaos
f	0.75	-0.065	Damped oscillations	n (f + g)	0.5	0.027	chaos
g	0.5	0.091	chaos	o (e + h)	0.75	--	damped oscillations
h	0.5	0.195	chaos				

*Determined by Lyapunov exponents

We used a defined microbial system of the ciliate *Tetrahymena pyriformis* (axenic culture from CCAP 1630/1W) as the predator and the two bacterial strains *Pedobacter* sp. (preferred and superior competitor) and *Brevundimonas* sp. (less preferred and inferior competitor) as prey bacteria (kindly provided by Klaus Jürgens) in the chemostats systems (185ml). The local populations were coupled in second stages of chemostats with different dilution rates receiving equal volumes of both of the coupled chemostats as the inflows (Table 1 i-o). The chemostats were handled using sterile techniques to prevent contaminations. If contaminations occurred,

experiments were terminated. Population abundances (fixation with Norris-Powel solution (Koch 1994)) were determined daily using the frame spotting method for the two bacteria (Maruyama *et al.* 2004). *Tetrahymena* abundances were enumerated by phase-contrast microscopy.

The dynamic behaviour of coupled populations in our experiments depended only on the dilution rate of the second chemostat and not on the inflow (immigration) from local populations (Figs. 1, 2). Intrinsically driven population dynamics in 2nd-stage-chemostats persisted in their dynamic behaviour when disturbed by a continuous immigration of organisms from two local communities (1st stage chemostat systems) with different intrinsic dynamics (abundances at equilibrium, stable limit cycles or chaos). A coupling of two first-stage chemostats with damped oscillations (D = 0.75 d^{-1}; Figs. 1, 2 e, f, k) resulted in damped oscillations when exposed to a dilution rate D = 0.75 d^{-1}, which created a damped oscillation. When damped oscillating populations of three species were combined with chaotically fluctuating populations, again damped oscillations of microbes were created when running at D = 0.75 d^{-1} independent of the dramatic fluctuations of the inflowing abundances of microbes. However, when the same combination of communities was running at D = 0.5 d^{-1}, the resulting dynamic was chaotic (Figs. 1 and 2 f, g, n). When two chaotic microbial communities were combined at chaos driving dilution rate (D = 0.5 d^{-1}) chaos occurred. And also, if a chaotic fluctuating community was coupled with a community running at stable limit cycles, a chaotic dynamic resulted when held a dilution rate of D = 0.5 d^{-1} (Figs. 1, 2 b, d, m). When the same combination of microbial communities was held at a dilution rate of D = 0.45 d^{-1} in the second stage, the resulting dynamics were damped oscillations according to estimates of Lyapunov exponents (Table 1), but were close to the establishment of stable limit cycles when analysing the time delay reconstructions.

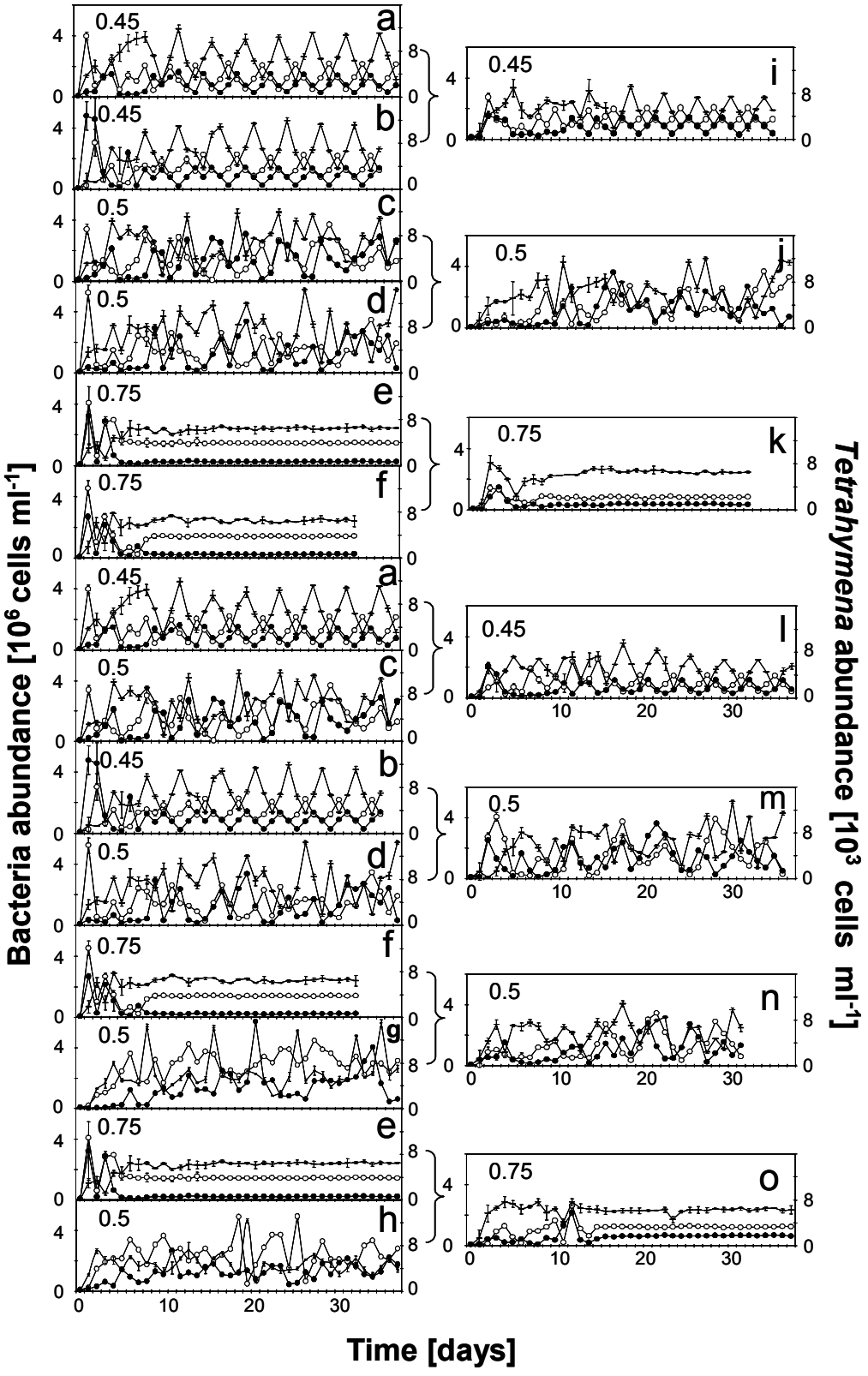

Bacteria abundance [10^6 cells ml^-1]
Tetrahymena abundance [10^3 cells ml^-1]
Time [days]
0.45
0.5
0.75
a
b
c
d
e
f
g
h
i
j
k
l
m
n
o

Fig. 1: Experimental results showing the population dynamics of bacteria-ciliate two-stage-chemostat systems. Left panels show the dynamics of the first-stage chemostats (a-h), right panels show dynamics in the coupled two-stage chemostats (i-o). Open circles, *Pedobacter* (preferred prey, superior competitor); filled circles, *Brevundimonas* (less preferred prey, inferior competitor); horizontal bar, *Tetrahymena* (predator). Vertical bars indicate the standard deviation of triplicate samples taken separately from one chemostat. Numbers represent the dilution rate D [d^{-1}] of the chemostats. Damped oscillations are shown for a dilution rate of D = 0.75 d^{-1} (e, f, k, and o), chaotic dynamics for a dilution rate of D = 0.5 d^{-1} (c, d, g, h, j, m and n), and stable limit cycles for a dilution rate of D = 0.45 d^{-1} (a, b,i , and l), respectively.

The same was true, when two limit cycling communities were coupled under similar conditions. It seems that both communities in Fig. 1 i and l were still in transition to stable limit cycles as it was obvious from the dynamics of the two faster reproducing bacteria species (Fig. 2 i and l).

All experiments indicated that the dynamic behaviours of the combined populations were only triggered by the demographic parameter – in this case the dilution rate - and react independently of the dynamic behaviour of the stage before. Unexpectedly, the great variations in the abundances in the 1st stage chemostats had only a very minor effect on dynamics in the 2nd stage chemostats. One explanation why empirical evidence for chaos and complex population dynamics is scarce might be due to the fact that intrinsically driven dynamics are not persistent when disturbed. Model analyses showed that the establishment and persistence of complex dynamics requires particular conditions (parameter settings) and small differences may lead to a shift in the dynamic behaviours (Scheffer 1991). The persistence of intrinsically triggered population dynamics of our three-species food web was surprisingly stable. Hence, the experimental driven complex dynamics were not fragile, as generally assumed and predicted by models. Chaotic dynamics show sensitivity to initial conditions. Thus, small perturbations of state variables are suggested to control chaos in terms of stabilising population densities (Shinbrot *et al.* 1993, Ott *et al.* 1990).

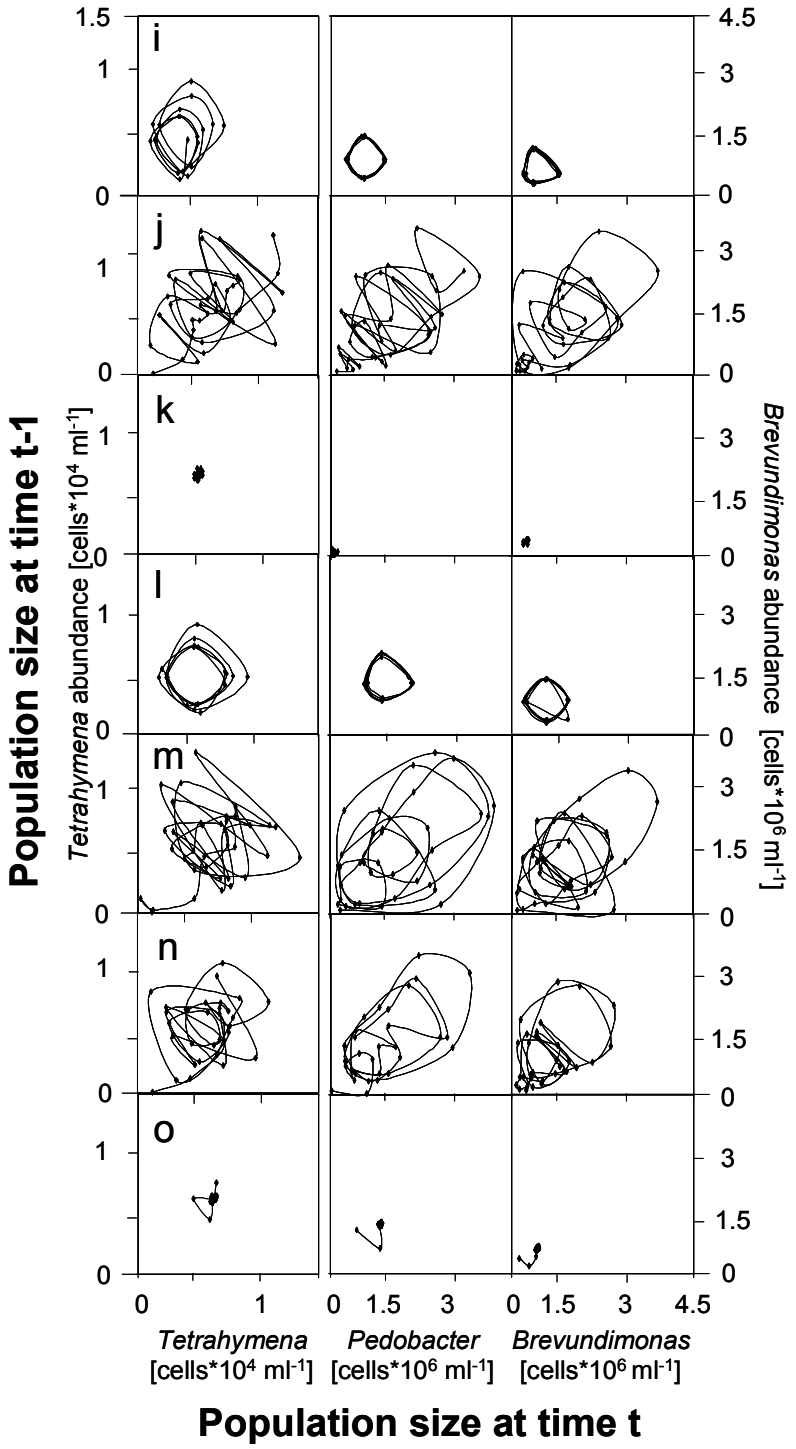


Fig. 2: Time delay reconstructions of experimental results (from day 15 on) showing the population abundances of Tetrahymena, *Pedobacter, and Brevundimonas* at time t plotted versus the population abundances at time t-1 (the day before) for the coupled chemostats (i-o in Table 1 and Fig. 1).

However, no stabilisation effects occurred in our experimental system, when chaotic systems were disturbed by fluctuating inflow concentrations (e.g. Figs.1, 2 l). In conclusion, intrinsically driven population dynamics may be more stable as commonly assumed. Stable coexistence at equilibrium, stable limit cycles, and chaotic dynamics may appear in habitats with a constant unidirectional flow of organisms and resources - such as aquatic organisms in streams, rivers and oceanic currents, and water drainage to groundwater.

References:

Becks, L., Hilker, F. M., Malchow, H., Jürgens, K. & Arndt, H. Experimental demonstration of chaos in a microbial food web. *Nature* **435**, 1226-1229 (2005).

Blasius, B., Huppert, A. & Stone, L. Complex dynamics and phase synchronization in spatially extended ecological systems. *Nature* **399**, 354-359 (1999).

Costantino, R.F., Desharnais, R.A., Cushing, J.M. & Dennis, B. Chaotic dynamics in an insect population. *Science* **275**, 389-391 (1997).

Dhamala, M., Lai, Y.C. & Holt, R.D. How often are chaotic transients in spatially extended ecological systems? *Phys. Lett. A.* **280**, 297-302 (2001).

Hanksi, I. Metapopualtion dynamics. *Nature* **396**, 41-49 (1998).

Hassel, M.P. & May, R. M. Stability in insect host-parasite models. *J. Animal. Ecol.* **42**, 693-736 (1973).

Hegger, R., Kantz, H. & Schreiber, T. Practical implementation of nonlinear time series methods: The TISEAN package. *Chaos* **9**, 413-439 (1999).

Holyoak, M., Leibold, M.A. & Holt, R.D. *Metacommunities* (The University of Chicago Press, 2005).

Ives, A.R. Continuous-time models of host-parasitoids interactions. *Am. Nat*. **140**, 1-29 (1992).

Koch, A. L.: *Growth measurement.* In: Gerhart, P., Murray, R.G.E., Wood, W.A., Krieg, N.R. (eds). *Methods for general and molecular biology.* (American Society for Microbiology, 1994) 248-277.

Levins, R. Some demographic and genetic consequences of environmental heterogeneity for biological control. *Bull. Entomol. Res.* **15**, 237-240 (1969).

Machioni, D.O., dos Reis, S.F. & de Aguiar, M.A.M. Chaos and pattern formation in a spatial tritrophic food chain. *Ecol. Modl.* **191**, 291-303 (2006).

Malchow, H. Spatiotemporal pattern formation in a nonlinear nonequilibrium plankton dynamics. *Proc. R. Soc. B.* **251**, 103-109 (1993).

Maruyama, F., Yamaguchi, N., Kenzaka, T., Tani, K., & Nasu, M. Simplified sample preparation using the frame spotting method for direct counting of total bacteria by fluorescence microscopy. *J. Microbiol. Methods* **59**, 427-431 (2004)**.**

May, R. M. Biological populations with nonoverlapping generations: stable points, stable cycles, and chaos. *Science* **186**, 645-647 (1974).

Medvinsky, A.B., Gonik, M.M., Berezovskaya, F.S., Li, B.L. & Malchow, H. Rotifer population dynamics in two coupled habitats: invasion of chaos. *Biol. Invas.* **7**, 877-883 (2005).

Ott, E., Grebogi, C. & Yorke, J.A. Controlling chaos. *Phys. Rev. Lett.* **64** (11), 1196 (1990).

Rohde, K. & Rohde, P.P. Fuzzy chaos: Reduced chaos in the combined dynamics of several independently chaotic populations. *Am. Nat.* **158**, 553-556 (2001).

Rosenstein, M.T., Collins, J.J. & De Luca, C.J. A practical method for calculating largest Lyapunov exponent from small data sets. *Physica D* **65**, 117-134 (1993).

Scheffer, M. Should we expect strange attractors behind plankton dynamics – and if so, should we bother? *J. Plankt. Research* **13**, 403-427 (1991).

Shinbrot, T. Grebogi, C., Ott, E. & Yorke, J.A. Using small perturbations to control chaos. *Nature* **363**, 411-417 (1993).

ABSTRACT

The study of spatial and temporal population dynamics has a long history in ecology, going back to the beginning of the 1900´s. Both intrinsic and extrinsic mechanisms are involved in determining the temporal and spatial occurrences of populations and species. Different dynamic patterns result from the strength and the interplay of the two mechanisms. The fact that intrinsic driven population dynamics are woven together with extrinsic, often stochastic dynamics makes analyses of intrinsic mechanisms difficult and led to a controversial discussion about the relevance in nature. However, there is a gap between results from mathematical modelling showing the occurrence and meaning of intrinsically driven dynamics, and empirical proves. Recently, laboratory experiments under clearly defined and controlled conditions were shown to be a suitable tool to study intrinsic, deterministic population dynamics. Deterministic chaos is one type of dynamic behaviour exhibited by a change in one or more intrinsic parameters beside extinction, damped oscillations, and stable limit cycle. Most discussed is the relevance of chaotic behaviour in population dynamics, due to the fact that empirical evidence is limited to a simple one-species system. Furthermore, chaotic fluctuations are thought to lead to extinction of a population, because chaotic dynamics can obtain very small population sizes, even more vulnerable when mixed together with stochastic events. The question, if chaos occurs in the real world and under which circumstances chaos may be found in nature, is still open.

Clearly defined laboratory experiments were established to analyse intrinsically driven dynamics in a multi-species system. Different dynamic behaviours were found in chemostat experiments with a two-prey-one-predator system of a bacterivorous ciliate as the predator and two bacteria strains as the prey organisms. The different population dynamics - extinction, damped oscillations, stable limit cycles and chaos - were triggered by a change in the dilution rate of the chemostat system and verified by calculations of the corresponding Lyapunov exponents. Therewith, chaos was shown in an experimental three-species system for the first time. The different dynamics in the microbial food web revealed a surprisingly short transition (4-7 days) to a different dynamic behaviour when the dilution rate as the control parameter was changed. All dynamics persisted in experiments when different local populations with different dynamics (chemostats with different dilution rates) were coupled. Experiments showed that the dynamic behaviours of the coupled

populations were only triggered by the demographic parameter – in this case the dilution rate - and reacted independent of the constant inflow of organisms from populations with different dynamics.

Here, we were able to shed more light on the question about the relevance of chaos in the real world. In conclusion spatio-temporal chaos might be more common in nature than generally assumed. Microbial communities with fast reproduction rates might be favoured candidates to show chaos and other complex dynamics in nature. Intrinsically driven dynamics might be persistent when perturbated by a constant fluctuating inflow of organisms and might lead to the establishment of chaos in habitats with constant flows (e.g. aquatic organisms in rivers and oceanic currents, and water drainage to groundwater). The fast transition to a different dynamic behaviour after a change in a control parameter shows how distinct intrinsic driven processes might be. A reason why chaotic dynamics in nature are not observed might be due too the large sampling intervals in most field studies.

KURZZUSAMMENFASSUNG

Die Erforschung von zeitlichen und räumlichen Populationsdynamiken hat eine lange Geschichte und geht zurück auf das frühe 20. Jahrhundert. Sowohl intrinsische als auch extrinsische Mechanismen sind am zeitlichen und räumlichen Auftreten von Populationen und Arten beteiligt. Dabei können sich verschiedene dynamische Muster in Abhängigkeit von der Stärke und dem Wechselspiel der beiden Mechanismen ergeben. Deterministischem Chaos, das Aussterben einer oder mehrere Populationen, gedämpfte Oszillationen und stabile Grenzzyklen sind intrinsisch gesteuerte dynamischen Muster, die durch die Änderung intrinsischer Parameter – Kontrollparameter –auftreten können. Die Tatsache, dass intrinsisch gesteuerte Populationsdynamiken mit extrinsischen, oft zufälligen Dynamiken, interagieren, macht Analysen von intrinsischen Dynamiken schwierig, was zu einer andauernden Diskussion über die Bedeutung intrisich gesteuerter Dynamiken in der Natur geführt hat. Insbesondere die große Diskrepanz zwischen empirischen Nachweisen und Ergebnissen mathematischer Modelle belebt die Diskussion immer wieder. Theoretische Arbeiten zeigen deutlich die Bedeutung intrinsisch gesteuert Dynamiken während eindeutige empirische Nachweise selten sind. Die Frage nach der Bedeutung von Chaos für Populationen hat die größte Kontroverse hervorgerufen. Neben der Sensitivität gegenüber kleinsten Störungen und evolutionären Argumenten, ist vor allem der fehlende empirische Nachweis ein Argument, dass gegen das Auftreten von Chaos in natürlichen Gemeinschaften spricht. Denn bis jetzt war der Nachweis auf ein Ein-Arten System beschränkt. Somit ist die Frage, ob Chaos in der 'realen' Welt vorkommt und unter welchen Umständen Chaos beobachtet werden kann, bis heute offen.

Um intrinsisch gesteuerte Dynamiken in einem Mehr-Arten System zu analysieren, wurden klar definierte Laborexperimente durchgeführt. Verschiedenen dynamischen Verhaltensweisen konnten in Chemostatexperimenten mit einem bakterivoren Cilliaten als Räuber und zwei Bakterienarten als Beuteorganismen aufgezeigt werden. Die verschiedenen Populationsdynamiken - Aussterben, gedämpfte Oszillationen, stabile Grenzzyklen und Chaos - konnten durch Änderungen der Verdünnungsrate (Kontrollparameter) eingestellt und mittels der

Berechnung des korrespondierenden Lyapunov Exponenten verifiziert werden. Dies ist der erste experimentelle Nachweis für Chaos in einem Drei-Arten System. In weiteren Versuchen konnte gezeigt werden, dass das mikrobielle Nahrungsgewebe erstaunlich schnell zwischen den verschiedenen dynamischen Verhaltensweisen wechselt (4 - 7 Tage), wenn die Verdünnungsrate (Kontrollparameter) geändert wird. Die Dynamiken in den Experimenten waren weiterhin beständig gegenüber einem konstanten oder fluktuierenden Zulauf von Organismen aus vorgeschalteten Chemostaten. Das dynamische Verhalten der Populationen war allein von der etablierten Verdünnungsrate im Chemostaten abhängig.

Die vorliegende Arbeit konnte mehr Aufschluss über die Bedeutung von Chaos und intrinsisch gesteuerten Dynamiken geben. Räumlich und zeitlich begrenztes Chaos ist wahrscheinlicher, als allgemein angenommen. Dafür spricht vor allem der hier erbrachte Nachweis von chaotischen Dynamiken in einem Mehrarten-System. Des Weiteren sind intrinsisch gesteuerte Dynamiken persisierend, auch wenn sie durch einen konstanten oder fluktuierenden Zulauf von Organismen gestört werden, woraus man schließen kann, das sich komplexe Dynamiken wie Chaos in konstanten Lebensräumen ausbilde kann (z. B. in Fliessgewässern, ozeanischen Strömungen oder Grundwasserabflüssen). Der schnelle Wechsel zwischen verschiedenen Dynamiken nach einem Wechsel des Kontrollparameters zeigt, wie stark intrinsische Kräfte auf ein System einwirken können. Mikrobielle Gemeinschaften mit ihren hohen Vermehrungsraten sind mögliche Kandidaten, die Chaos und andere intrinsisch gesteuerte, komplexe Dynamiken in der Natur zeigen können. Denn in mikrobiellen Gemeinschaften können sich komplexe Dynamiken – darunter eben auch chaotische Dynamiken – schneller etablieren, als dass sie von außen gestört werden. Zusätzlich können zu kleine Probenameintervalle dazu führen, dass Chaos, aber auch andere Dynamiken in der Natur übersehen werden.

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- Becks, L. & Arndt, H. Persistence of complex population dynamics in coupled microbial populations.

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