Influence of temperature on the complex dynamic behavior of a microbial food web

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You are not safer in first class. Banksy. Wall and Piece. Ed. Century 2005

Because what happens will never happen,
and because what has happened
endlessly happens again,

we are as we were, everything
has changed in us, if we speak
of the world
it is only to leave the world

unsaid. Early winter: the yellow apples still
unfallen
in a naked tree, the tracks
of invisible deer

in the first snow, and then the snow
that does not stop. We repent
of nothing. As if we could stand
in this light. As if we could stand in the silence of this single
moment

of light.

Narrative. Paul Auster. Desapariciones Poemas. Ed. Pre-textos poesía.

Danksagung.

Content

Introduction

The study of population dynamics began at the early years of the last century (e.g. Volterra (1926)) with mathematical models of predator-prey systems that predicted stable limit cycles. The experimental investigation of the model followed some years later (Gause, 1935). Intrinsic processes in populations derive from density dependent population parameters like growth rate or feeding rate (see Turchin (2003)) and the interactions between the organisms in a food web. Regarding the interactions between species, interspecific competition and predation have been widely studied from the theoretical point of view. Mathematical models have been developed to investigate the relation between interspecific competition and resources availability (e.g. Hairston et al., 1960). Other models demonstrated that predation allowed the coexistence of two competing prey species when the best competitor is the preferred prey of the predator (Takeuchi and Adachi, 1983). Competition and predation are considered driving factors of the population dynamics (Chase et al., 2002).

Extrinsic factors affect organisms and their interactions but are not related either with the organisms or with their interactions. Some of those factors depend on climate parameters and are known to show nonlinear dynamics (Lorenz, 1963). One of those extrinsic factors that most affect organisms is the temperature (Clarke, 2006). Several population parameters are temperature dependent like growth rate (Savage et al., 2004) or developmental time (Gillooly et al., 2002).

The interaction between species and the temporal dynamics derived of these interactions, can be subjected to direct and indirect temperature effects. Direct effects are constituted by temperature

dependent population parameters like growth rate (Savage et al., 2004), feeding rate or other metabolic processes. Such direct effects can be described through the Q_{10} factor which assumes a linear relation between a determined parameter and temperature, though this linear relation has been criticised (Montagnes et al., 2003). Indirect effects are food web mediated (see Begon et al.(2006)). Natural ecosystems are characterised by a complex network of organisms which makes it difficult to assess those indirect temperature effects (Davis et al., 1998), though their effects may be as large as the ones derived directly from temperature (Harmon et al., 2009, Stenseth and Mysterud, 2002).

Ecosystems are set to climatic variations (Stenseth et al., 2002) and the population dynamics are strongly influenced by climate parameters (Post and Forchhammer, 2002) Climate change is already affecting ecosystems (Walther et al., 2002, Root et al., 2003) and their activity (Bond-Lamberty and Thomson, 2010), thus deeper knowledge of the mechanisms driving effects on populations and their interactions is urgently needed (Winder and Schindler, 2004, Jiang and Morin, 2004, Stenseth, 2010). Several catastrophic events have been related to the actual climate change (e.g. increase of malaria risk (Paaijmans et al., 2009), amphibian mass extinction (Pounds et al., 2006)).

The main hypothesis investigated in this work was that extrinsic temperature changes can promote shifts in the dynamic behaviour of the system. Each species in the food web had a specific temperature response and thus the interactions strength should change with the temperature.

In order to assess the effects of extrinsic temperature changes on the intrinsic population dynamics is necessarily to exclude external influences from the experiments. The experiments presented in this

work were carried out with chemostats in a temperate bath. Such an experimental setup permitted an accurate control on external conditions, so the dynamic behaviour showed by the food web was considered to be intrinsic. A two-prey-one-predator food web was studied, this simple composition of the food web and the highly controllable experimental conditions allow a better understanding of the relation between the species reaction to temperature and its effects at the food web level.

Investigations presented here, used microbes as model organisms in order to analyze the effects of temperature on the dynamic behaviour of a two-prey-one-predator food web. Microorganisms are especially appropriate for laboratory experiments thanks to the short generation times and the small sizes (Jessup et al., 2004) as well as to their relevance for aquatic food webs (Caron et al., 1982).

According to the model analysis made by Takeuchi and Adachi (1983), a two-prey-one-predator food web may present several dynamic behaviours including stable equilibrium, stable limit cycles and chaos. The first model predictions of intrinsic chaotic dynamics were done in the 70's (May, 1974), though experimental demonstrations of the aforementioned dynamics are scarce (in a three species microbial food web (Becks et al., 2005), in a natural planktonic food web (Beninca et al., 2008) and in a flour beetle population (Costantino et al., 1997)).

I investigated the temperature reaction norm for each experimental species in the food web. These results were used to develop a mathematical model with temperature dependent growth rates. Numerical analyses of the model were used to investigate the food web reaction to extrinsic temperature changes, which could not be derived directly from the single species temperature norm.

The experimental food web that I used for my investigations was similar to that presented by Becks et al. (2005), and consisted on *Tetrahymena pyriformis* as predator ciliate, *Pedobacter sp.*, and *Acinetobacter johnsonii* as bacterial preys. *Acinetobacter* presented the ability of forming grazing resistant morphologies. This is a common feature in natural systems (Juergens and Matz, 2002), and take several phenotypic expressions like production of toxins or morphological shifts in form of colonies and filaments (reviews by Juergens and Matz, 2002; and by Pernthaler, 2005).

In this study, I developed a mathematical model in cooperation with David Heckmann in order to analyze the effect of grazing resistance on the dynamic behavior of the system. Two modeling approaches were investigated and compared with a model without grazing-resistant prey: (1) a constitutive grazing resistance (the prey population presents grazing-resistant morphologies independently of the presence or absence of the predator) and (2) inducible grazing resistance (triggered by the predator abundance).

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Chapter I

Temperature effects on a microbial food web. Single species temperature response in model analyses of the food web.

Introduction.

Species interactions and their temporal dynamics can be affected by temperature in two different ways: directly through temperature dependent population parameters like growth rate (Savage et al., 2004), feeding rate or other metabolic processes; and indirectly through food web mediated effects (Davis et al., 1998, Durant et al., 2007). Those indirect mechanisms are not easy to asses in real ecosystems due to the complexity of natural food webs (Winder and Schindler, 2004), though their effects may be as large as the ones derived directly from temperature (Harmon et al., 2009, Stenseth and Mysterud, 2002).

Due to the short generation times and the small sizes, microorganisms are especially appropriate for laboratory experiments (Jessup et al., 2004). Microorganisms are at the basis of aquatic food webs (Caron et al., 1982) and are adapted to the temperature regime of their environment (bacteria (Hahn and Pockl, 2005) and also protozoa (Gaechter and Weisse, 2006)). The experiments presented in this work run under very accurate conditions, and may help to disentangle the interaction between extrinsic and intrinsic processes affecting food webs. This knowledge is of great interest because of the ecological effects of the actual climate change (Walther et al., 2002)

The objective of my investigations was to analyze the effects of temperature on the population dynamics of a three species food web. This consisted of two prey bacteria: *Acinetobacter johnsonii* and *Pedobacter sp.*; and a predator ciliate: *Tetrahymena pyriformis*. Thus I assessed the effects of temperature on the growth rates of all three species with the objective of developing a model based on experimental data. Numerical analyses of the model were run in order test following hypothesis at a theoretical level: firstly temperature

changes can affect the dynamic behaviour of a system; secondly, these changes cannot be extrapolated from the single species temperature response.

Materials and Methods

1. Organisms and axenic cultivation.

Tetrahymena pyriformis CCAP 1630/1W (Ciliophora, Hymenostomatia, average size 41x22 μm; provided by the Culture Collection of Algae and Protists, Windermere, U.K) was cultivated at 10°±1°C without lighting in a proteose pepetone yeast medium (PPY: 20g/l proteose pepetone, 2,5g/l yeast extract). Every 14 days the ciliates were inoculated in fresh medium.

The two bacteria were *Pedobacter sp.* KB11 (heparinolytic bacteria, Sphingobacteriaceae) and *Acinetobacter johnsonii* (α-proteobacteria, Caulobacteriaceae). *Pedobacter sp.* is a rod-shaped bacterium, 2x1 μm in size and was kindly provided by Klaus Juergens (Warnemuende, Germany). *Acinetobacter johnsonii* measures 2.5x2.5 μm in its single cell form and was kindly provided by Michael Steinert (University Kiel, Germany). Bacterial strains were kept at -80°C and cultured overnight with LB medium (10g trypton, 10g NaCl, 5g yeast extract) for 24 hours at ambient temperature before the experiments began.

2. Batch experiments

Experiments were run in sterile glass vessels filled with 150 ml of PPY100 medium (2g/l proteose peptone, 0,25g/l yeast extract). Gentle aeration served for homogeneous mixing and sufficient oxygen content. Bacteria were inoculated from an overnight culture with an initial abundance of 1×10^5 cells ml^{-1} and grown for 60 hours. Samples were taken with sterile syringes at 0, 12, 24, 28, 32, 36, 40, 44, 48,

52, 56 and 60 hours, and fixed with 2% formol in PBS buffer (8.0 gl^{-1} NaCl; 0.2 gl^{-1} KCL, Merck, Darmstadt, Germany; 1.42 gl^{-1} Na$_2$HPO$_4$ x H$_2$O, Merck, Darmstadt, Germany; 0.2 gl^{-1} KH$_2$PO$_4$, Applichem, Darmstadt, Germany; pH 7.4) with SDS. Ciliates were inoculated after 60 hours because prior experiments showed that bacteria had reached the maximal possible abundance at that time. The initial abundance of ciliates was 5x10^3 cells ml^{-1}. Samples were taken every 12 hours during 60 hours. The total last of the experiments was composed of the 60 hours of the bacterial growth and the 60 hours of the ciliate growth (120 hours total duration).

Samples were dyed following the frame spotting method (Maruyama et al., 2004) with Propidium iodide (50 µg ml^{-1}). Bacteria were enumerated with an epifluorescence microscope (Zeiss Axioskop, HXP 120, filter set 43), and the ciliate under light microscope (Zeiss Axiophot 2).

Temperature was kept constant with a thermostat (Julabo FC 600) for the experiments at 5, 15, 17.5, 25, 30, 35 °C. Experiments at 10 and 20°C were kept at constant room temperature in climate rooms.

Growth rate was calculated for the exponential growth phase. Natural logarithms of the abundances during this period were calculated and were fitted to a linear model. The slope of this model represented the growth rate.

3. Temperature dependent functions

Several temperature dependent functions were fitted to the data with R version 2.10.1. Besides the fit of the temperature dependent models for the growth rate to the data (given by R^2), the criteria followed to decide which function should be chosen were: the number of parameters should be kept as low as possible and as far as

possible, those parameters should measure biological processes; that means, that they should have a biological interpretation. Also the performance of the function at the minimum and maximum temperatures where positive growth was possible was taken in account. Following functions were analyzed:

$$r = r_{\max} \left(\frac{(T_{\max} - T)}{(T_{\max} - T_{opt})} \right) e^{\frac{(x(T - T_{opt}))}{(T_{\max} - T_{opt})}} \qquad (1)$$

where

$$x = \frac{W^2}{400} \left(1 + \sqrt{1 + \frac{40}{W}} \right)^2 \qquad (2)$$

and

$$W = Q_{10} - (T_{\max} - T_{opt}) \qquad (3)$$

$r_{\max}$ represents the maximal growth rate, $T_{\max}$ the maximal temperature where positive growth is possible, T_{opt} is the temperature where the growth rate takes the maximal value, T is the temperature in °C, the Q_{10} factor measures how much the growth rate changes when the temperature increases 10°C.

$$r = r_{\max} e^{(aT - bT^{cT})} \qquad (4)$$

$$r = r_{\max} e^{(aT)(1 - be^{cT})} \qquad (5)$$

for both functions $r_{\max}$ is the maximal growth rate. The parameters a, b and c have no biological interpretation. T is the temperature in °C.

$$r = r_{\max} \left(1 + b \left(\left(R_1^{T - T_{opt}} - 1 \right) - \frac{\ln R_1}{\ln R_2} \left(R_2^{T - T_{opt}} - 1 \right) \right) \right) \qquad (6)$$

where r_{max} is the maximal growth rate, T is the temperature in °C, T_{opt} is the temperature where maximal growth rate takes place. Parameters R_1 and R_2 have no biological interpretation.

$$r = \frac{r_{max}}{1 + e^{\left(\left(\frac{T_{al}}{T_k}\right) - \left(\frac{T_{al}}{T_l}\right)\right)} + e^{\left(\left(\frac{T_{ah}}{T_h}\right) - \left(\frac{T_{ah}}{T_k}\right)\right)}} \qquad (7)$$

r_{max} represents the maximal growth rate, T_k is the temperature in °K, T_h and T_l are respectively the maximal and minimal temperatures where positive growth is possible. T_{al} and T_{ah} are respectively the low and high Arrhenius temperatures and are related with the concept of activation energy of the Arrhenius function.

4. Mathematical model

The mathematical model was implemented with R version 2.10.1. The same program was used for the statistical analyses.

Results

1. Temperature dependent growth rates

Pedobacter sp.: the best fit ($R^2 = 0.96$) was given by equation 7 with the following parameter values: r_{max} is 3.56 d^{-1}, T_{al} and T_{ah} are 40422.29, 75273.69 respectively, T_l is 277. 63°K, and T_h is 305.91°K.
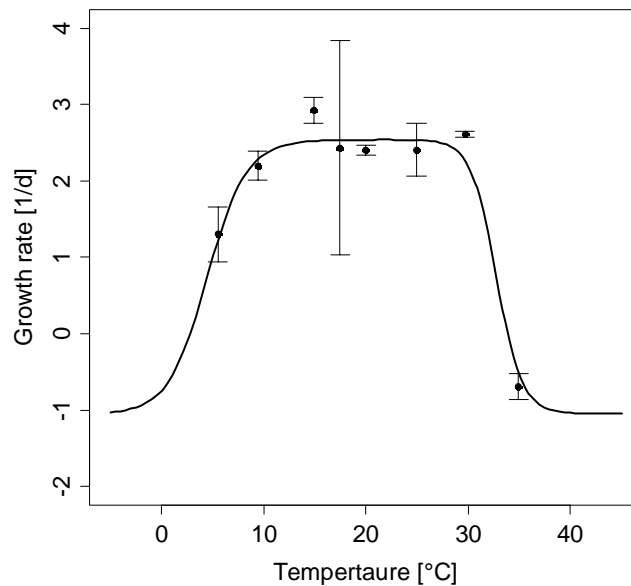
Fig.1: Mean growth rate (d^{-1}) of triplicate experiments vs. temperature (°C) for *Pedobacter sp.*. Line represents the function (7) fitted to experimental data.

This function presented a broad range where the growth rate took maximum values, between 10 and 30°C. The maximal growth rate obtained experimentally was 2.92±0.17 d^{-1} at 15°C. The minimal temperature where there was still a positive growth rate was below 5.5°C. The maximal temperature at which positive growth rate was possible must lie between 30°C and 35°C. All those characteristics obtained experimentally were reflected by the model.

Acinetobacter: the function that best fitted (R^2=0.79) the data was equation (6) where r_{max} is 2.71 d-1, T_{opt} is 24.55°C, and b is 13.33, R_1 is 1.07 and R_2 1.09.
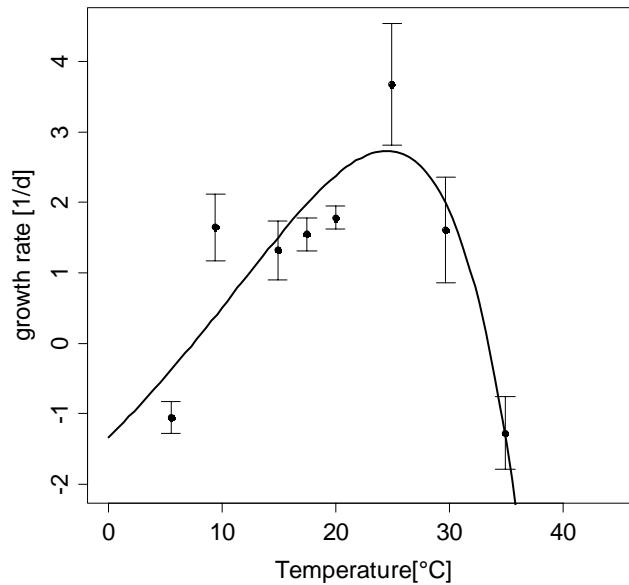
Fig.2: Mean growth rate (d^{-1}) of triplicate experiments vs. temperature (°C) for *Acinetobacter sp.*. Line represents the function (6) fitted to experimental data.

The maximal growth rate obtained experimentally was 3.67±0.87 d^{-1} at 25°C. The minimum temperature with a positive growth rate was between 5°C and 10°C, the maximum temperature allowing a growth should lie between 30°C and 35°C. The temperature dependent function fulfilled all those characteristics of the experimental data, although the maximal growth rate (with an optimized parameter value of 2.73 d^{-1}) was underestimated, and the optimal temperature given by the model (24.55°C) was slightly lower than the experimental value.

Tetrahymena pyriformis: both bacteria strains were offered as a food source separately so a direct comparison of *Tetrahymena* growth rates feeding on each bacterium was possible, allowing the consideration of a possible food preference of *Tetrahymena* (Fig.4).

The function that best fitted the data in both cases ($R^2=0.77$ for *Tetrahymena* feeding on *Pedobacter*, and $R^2=0.83$ when feeding on *Acinetobacter*) was equation (7) where the parameter values are (values in brackets are from the experiment where *Tetrahymena* fed on *Acinetobacter*) 1.98 d^{-1} (2.28 d^{-1}) for r_{max}, T_{al} was 42081.57

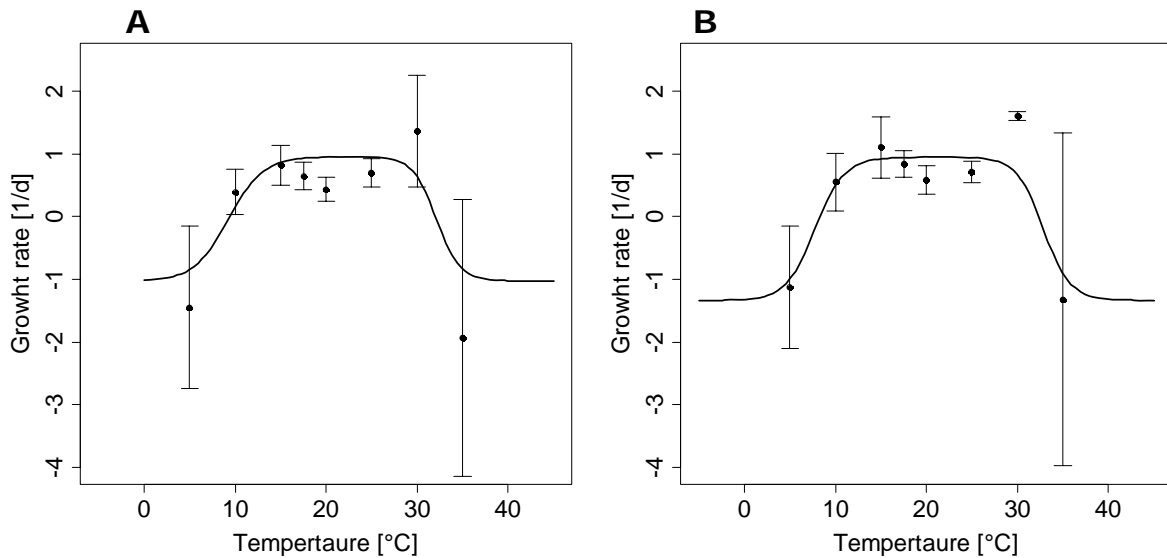(49382.93), T_{ah} was 72444.94 (63887.17), T_l was 282.42 °K (280.90°K), and T_h was 305.27°K (305.99°K).

Fig.3: Mean growth rate (d^{-1}) of triplicate experiments vs. temperature (°C). Line represents the function (7) fitted to experimental data. A: *Tetrahymena* feeding on *Pedobacter*, and B: *Tetrahymena* feeding on *Acinetobacter*.

In order to asses if the growth rate differed with each bacterium as a food source, the data were divided in three groups (from 5 to 15°C, from 15 to 30 °C, and from 30 to 35°C) and fitted to linear models. The slopes of the linear regressions were compared with a two-way ANOVA. The analysis showed no significant differences of the growth rate depending on the food source (P=0.93, F=0.01 for the group from 5 to 15°C; P=0.45, F=0.88 for the group from 15 to 30°C; P=0.82, F=0.80 for the group from 30 to 35°C.).

Mathematical model

A mathematical model of the Lotka-Volterra type was developed to test the effects of temperature on the population dynamics. The model was based on experimental results of the growth rates of the organisms that formed the food web. Experiments showed that the growth rate for *Pedobacter* and *Tetrahymena* did not change

significantly for temperatures between 15 and 25°C (ANOVA: F=0.4256, p>0.05 for *Tetrahymena*; F=2.193, p>0.05), and the temperature dependent function showed consequently maximal growth rates for a broad range of temperatures. In order to keep the model as simple as possible, only the function with temperature dependent growth rate for *Acinetobacter* was introduced.

The model consisted of the following differential equations:

$$\frac{\partial C}{\partial t} = (C_0 - C)D - \varepsilon_1 \mu_1(C)N_1 - \varepsilon_2 \mu_2(C)N_2 \quad , \quad (8)$$

$$\frac{\partial N_1}{\partial t} = N_1 \mu_1(C) - P\varphi_1(N_1) - DN_1 \quad , \quad\quad\quad (9)$$

$$\frac{\partial N_2}{\partial t} = N_2 \mu_2(C) - P\varphi_2(N_2) - DN_2 \quad , \quad\quad\quad (10)$$

$$\frac{\partial P}{\partial t} = P\beta_1\varphi_1(N_1) + P\beta_2\varphi_2(N_2) - DP \quad , \quad\quad\quad (11)$$

where C is the nutrient concentration, N_1 the abundance of *Acinetobacter*, N_2 the abundance of *Pedobacter* and P the abundance of *Tetrahymena*. The growth rates of the bacteria, μ_1 and μ_2 follow the Monod function, the same as *Tetrahymena* feeding response, φ_1 and φ_2, which was assumed to be of the Holling II type:

$$\mu_i = \frac{\mu_{\max_i} C}{K_{s_i} + C} \quad (12)$$

$$\varphi_i(N_i) = \frac{\varphi_{\max_i} N_i}{K_{N_i} + N_i} \quad (13)$$

The parameter ε_i represents the bacterial yield and took the same value for ε_1 and ε_2 ($2\times10^6 \mu gC/ind_{prey}$); β_i is the predator yield and took the value $1/4000 \ ind_{predator}/ind_{prey}$ for both β_1 and β_2; C_0 represents the inflow nutrient concentration and was 3 µgC/ml. The

bacterial growth rate was controlled by two parameters, for *Pedobacter* those parameters were $\mu_{max1}=0.15$ h^{-1} and $K_{s1}=0.0274$ µgC/ml; for *Acinetobacter* μ_{max2} was substituted through the temperature dependent function (equation (2)), $K_{s2}=0.002$ µg/ml. The feeding response of *Tetrahymena* followed the expression (9) which parameters are $\varphi_{max1}=150$ ind$_{prey}$/h ind$_{predator}$, and $K_{N1}=422000$ind$_{prey}$/ml for Pedobacter; and $\varphi_{max2}=450$ ind$_{prey}$/ind$_{predator}$ and $K_{N2}=400000$ind$_{prey}$/ml. The units for all the state variables were µC/ml, so a transformation from individuals in these units was done with the following factors: 1.241×10^{-7} µgC/ind for *Acinetobacter*, 5.72×10^{-8} µgC/ind for *Pedobacter*, and 6.5507×10^{-3} µgC/ind for *Tetrahymena*.

The flow rate was used as bifurcation parameter for numerical analysis at 20 and 25°C. According to the experimental results, in this temperature range species react differentially, the growth rate of *Acinetobacter* increased significantly (1-way ANOVA, $P=0.0012$, $F=14.83$) while *Tetrahymena* and *Pedobacter* did not show any changes. I analyzed how the different temperature reaction norms of the species affected the reaction to temperature of the whole food web. Sensitivity analysis of the model at 20°C showed that coexistence for all three species was possible for flow rates between 0.2d^{-1} and 1.52d^{-1}, at a flow rate of 1.53 *Pedobacter* was predicted to go extinct. The model predicts a stable equilibrium for all analyzed flow rates. At 25°C the coexistence interval was smaller than at 20°C and ranged from 0.2d^{-1} to 1.19 d^{-1} higher flow rates led to the extinction of *Pedobacter*. The dynamical behaviour predicted in this case was also stable equilibrium for all flow rates analyzed.

In a second investigation of the model, the temperature was changed during the run. At low flow rates, the stabilization period was very long, and the temperature effect almost unseeable (Fig. 4, A).

With increasing flow rates, the abundances relation changed after the temperature had reached 25°C. At a flow rate of 0.8 d^{-1} (Fig 4, B) the abundance of *Acinetobacter* was higher than the abundance of *Pedobacter* after the temperature had changed. Pedobacter went extinct at a flow rate of 1.2 d^{-1}. In this scenario the coexistence range was smaller than when the temperature was constant during the complete run (at 20 and 25°C).The phase space diagrams show that the system reaches different attractors at 25°C (Fig. 4).
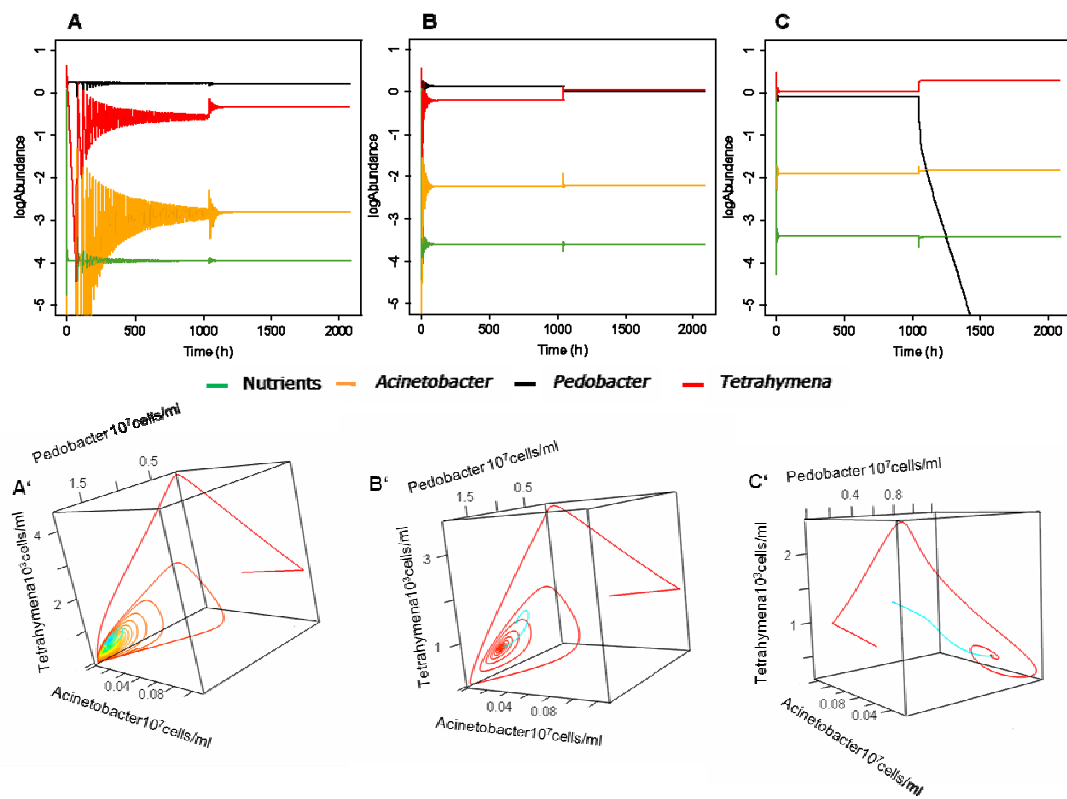

Fig.4: Time series and phase space diagrams of model results. A run at 0.4 d-1, B at 0.8 d-1 and C at 1.2 d-1. A', B', and C' represent the corresponding phase space diagrams; the temporal development follows the rainbow colors beginning in the red region and finishing in blue.

Discussion

The results of the experiments presented here lighten the following main assertion: the different temperature response of the species in a food web can affect the species interactions.

Temperature dependence of the growth rate is an autecological question that is linked to the concept of ecological niche (Begon et al., 2006). This has turned to be a major question in ecology derived from the necessity of predicting the consequences of climate change. These predictions are based on mathematical models, but oversimplification can lead to false conclusions (Soetaert and Herman, 2009). Temperature reaction norms are species specific (Gaechter and Weisse, 2006, Clarke, 2006, Hahn and Pockl, 2005), and this ecological complexity has to be taken in account in the model predictions.

The growth rate data for *Tetrahymena* presented here differ slightly from other data found in the literature, although these data are also discrepant within the different authors. Schmid (1967) measured the optimal growth rate of *Tetrahymena* at 28°C , while Slater (1954) did it at 25°C and Elliott (1973) at 32.5°C . Our results show a broad range of temperatures (from 15 to 25°C) where the growth rate of the ciliate did not change significantly (ANOVA: F=0.4256, p>0.05) and the temperature dependence function that best fitted the data also had this characteristic. The publications cited above concentrated on determining such parameters as optimal or maximal temperature and Schmid (1967) also differentiated between growth rate (somatic growth) and multiplication rate (population growth), this hindered a direct comparison of the results because our interest was to find a continuous function which would express the growth rate as a temperature dependent equation. Regarding the

maximal growth rate, the model fitted for each bacterium as a food source gave similar results in both cases (32.12°C feeding on *Pedobacter* and 32.74°C feeding on *Acinetobacter*) and both values were lower than those found by Slater (1954) and by Elliott (1973) (35 and 36.6 °C respectively). This difference can be due to the food resource: none of the bacteria was able to grow at 35°C so no food resources were available at this temperature for *Tetrahymena*. It is possible that our strain of *Tetrahymena* is able to grow at temperatures higher than 32°C but our interest lay not only in the temperature dependence but also on the food web interactions, so no other food source was analyzed. Regarding the minimal temperature where positive growth is possible, experimental data indicated that it must be between 5 and 10°C for *Tetrahymena* feeding on both bacterial strains. Both models supported this supposition with optimized parameter values at 9.27°C for the experiments with *Pedobacter* as food source, and 7.75°C for those experiments where *Tetrahymena* fed on *Acinetobacter.*

The bacteria used for our experiments: *Pedobacter sp*. and *Acinetobacter johnsonii* were isolated from lake Schoehsee (Germany) by Kristin Beck (Beck, 2000) and very few is known about their ecological performance except some experiments done previously in our working group for *Pedobacter*.(Becks, 2003). The temperature range where both bacteria were able to grow represented the range of a temperate lake in central Europe indicating an adaptation of both bacteria at the temperature regime from the region where they were isolated (Hall et al., 2009). Nevertheless they showed significant differences. On one hand the growth rate of *Pedobacter* did not change significantly between 15 and 25°C (ANOVA: F=2.193, p>0.05), instead of that, *Acinetobacter* presented a clear optimal temperature at 25°C. On the other hand *Pedobacter* showed a better performance at low temperatures than

Acinetobacter being able to grow at 5°C. However, the temperature dependent function fit performed quite well and predicted a negative growth rate at 4.48°C. Both bacteria showed negative growth rates at 35°C, the model fitted to *Pedobacter* data had the maximal temperature where positive growth is possible as a parameter and the optimized value was 32.76°C which is a possible value according to the experimental results; the model fitted to *Acinetobacter* did not have this extreme temperature as a parameter but performed well graphically (see Fig. 5).

Sensitivity analysis of the model revealed a temperature effect on the coexistence range of the system. At 25°C, *Pedobacter* went extinct at a lower flow rate than at 20°C. This could be due to a food web effect because *Acinetobacter* has a higher growth rate at 25°C than at 20°C and therefore the competition pressure on *Pedobacter* is higher at 25°C.

Our results show that the population dynamics change when the temperature increases from 20 to 25°C. Observation of the time series (see Fig.4) indicated that the only species that benefits of the temperature increase is *Acinetobacter*. The maximal growth rate for *Acinetobacter* was predicted to be at 24.55 °C. The complexity of the food web response can be seen in the phase-space diagrams (see Fig. 5). The first thing visible on those diagrams is that the system changes the attractor when the temperature increases. This new attractor is set in the three dimensional space at higher abundances of *Acinetobacter* and *Tetrahymena*, reflecting also a benefit for the predator that was not observable on the time series. This indicates a food web effect, and means that although *Tetrahymena* does not grow better at 20 than at 25°C, it profits from the higher prey abundance. This food web effects regarding temperature response have been already observed experimentally in several investigations (competition between *Colpidium* and *Paramecium* (Jiang and Morin,

2004) field experiments with pea Aphids (Harmon et al., 2009) changing food web structure (Petchey et al., 1999)). Until now no data are available regarding dynamic behaviour.

The model presented here is based on experimental data and shows a complex response of a two-prey-one-predator food web to temperature that cannot directly be derived from the species specific temperature norm. The complexity of food web interactions and the interplay between those interactions and the single species autecology needs to be better understood in order to improve our management capacity concerning the actual climate change (Stenseth, 2010). Experimental microbial food webs can permit deeper insights of this interplay.

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Chapter II

Extrinsic temperature impact on intrinsic dynamic behaviour of an experimental food web

Introduction

Intrinsic population dynamics have been of interest for biologists since the early 20th century from a theoretical point of view (Volterra, 1926) and also experimentally (Gause, 1935). Intrinsic population dynamics derive from the organisms densodependent growth and their interactions. Within those interactions, interspecific competition and predation have been widely studied (HilleRisLambers and Dieckmann, 2003, Jost et al., 1973) and are considered driving factors of the population dynamics (Chase et al., 2002). At the early 70's the existence of intrinsic chaotic dynamics in biological systems was first theoretically shown (May, 1974), this opened a discussion about the importance of this type of dynamics in natural systems (Cushing et al. 2003). Since then, only few experiments showed the existence of chaotic dynamics: in a three species microbial food web (Becks et al., 2005), in a natural planktonic food web (Beninca et al., 2008) and in a flour beetle population (Costantino et al., 1997)

Extrinsic factors affect organisms and their interactions but do not derive from the organisms themselves. Some of those factors depend on climate and weather and are known to show chaotic behaviour (Lorenz, 1963). Temperature is one of those extrinsic factors that most affect organisms (Clarke, 2006), but each population has a different reaction to temperature (Hahn and Pockl, 2005, Gaechter and Weisse, 2006). The interaction between direct and indirect (food web mediated) temperature effects on populations need to be better understood in frame of the actual global warming. As a consequence of climate change, the global warming is already affecting ecosystems (Walther et al., 2002, Stenseth et al., 2002), thus deeper knowledge of the mechanisms underlying the relation between intrinsic and extrinsic processes in food webs is urgently

needed. in order to avoid catastrophic consequences of global warming (Wake and Vredenburg, 2008).

In this work I studied the effects of a temperature increase from 20 to 25°C on a two-prey-one-predator microbial food web. Chemostat experiments allow long term investigations under very constant conditions besides allowing the experimenter to determine some external parameters. In this case the flow rate and temperature were manipulated. This simple food web structure allows a deep analysis of the populations but it still offers the possibility of complex nonlinear dynamics (Takeuchi and Adachi, 1983, Becks et al., 2005). Experiment results show that temperature increase induces a change in the population dynamics of the system that cannot be explained only through the single species temperature reaction norm. Model analyses support qualitatively these results.

Materials and Methods

1. Organisms and axenic cultivation.

The prey organisms were *Pedobacter sp.* (heparinolytic bacteria, Sphingobacteriaceae fam. nov.) kindly provided by Klaus Juergens (Warnemuende, Germany) and *Acinetobacter johnsonii* (α-proteobacteria, Caulobacteriaceae), kindly provided by Michael Steinert (Braunschweig, Germany). *Pedobacter sp*. is a rod-shaped bacterium, 2x1 µm in size. *Acinetobacter johnsonii* measures 2.5x2.5 µm in its single cell form. Bacterial strains were kept at -80°C and the experimental inoculum was obtained from overnight cultures with LB medium (10g trypton, 10g NaCl, 5g yeast extract) set 24 hours at ambient temperature before the experiments began. The predator *Tetrahymena pyriformis CCAP 1630/1W* (Ciliophora, Hymenostomatia, average size 85x22 µm; provided by the Culture Collection of Algae and Protists, Windermere, U.K) was cultivated at

20°C under dark conditions in a proteose pepetone yeast medium (PPY: 20g/l proteose pepetone, 2.5g/l yeast extract). Every 14 days the medium was renewed.

2. Experimental setup

One stage chemostats were filled after sterilization with 190 ml medium (proteose peptone yeast medium in a 1:100 dillution: 2g/l proteose peptone, 2.5 g/l yeast extract). Continuous fresh medium inflow was provided by syringe pumps (Cavro XLP6000 Tecan ®, Crailsheim, Germany) at three flow rates: $0.45\pm0.004 d^{-1}$, 0.5 ± 0.004 d^{-1}, $0.75\pm0,004$ d^{-1}. Temperature was kept constant with a thermostat (Julabo FC 600).at the desired temperature ±0.3 °C. Gentle aeration ensured oxygenic conditions and homogeneous mixing.

Triplicate samples were taken daily through a computer controlled system consisting of a robot (RSP9000 Cavro Tecan ® Crailsheim, Germany), a syringe pump (Cavro XLP6000 Tecan ®, Cairlsheim, Germany) and a valve system (Smart valve, Tecan®, Carlsheim, Germany) 0.5 ml sample were fixed with 2% formol in PBS buffer (Phosphate buffered saline: 8.0 gl^{-1} NaCl; 0.2 gl^{-1} KCL, Merck, Darmstadt, Germany; 1.42 gl^{-1} Na_2HPO_4 x H_2O, Merck, Darmstadt, Germany; 0.2 gl^{-1} KH_2PO_4, Applichem, Darmstadt, Germany; pH 7.4). Samples were stained following the frame spotting method (Maruyama et al., 2004) with CYBR green I (Invitrogen, Karlsruhe, Germany, 1:5000 dilution from original stock with distilled sterile water). Enumeration took place under epifluorescence microscope (Zeiss Axiophot, filter set 43, 1250 times magnification for the bacteria, 125 times for the ciliate). Lyapunov exponents were calculated with the TISEAN package (Hegger et al., 1999) implemented with R following the algorithm described by Rosenstein (Rosenstein et al., 1993)

3. Mathematical model

Numerical analyses of the mathematical model were implemented with R 2.10.1 (see supplementary material).

Results

Chemostat experiments could be run under constant conditions and free of contamination through other organisms for 95 days (see Fig. 1). In order to avoid a temperature shock, the heating process took place progressively from day 59 to day 63 (1°C per day) for the onsets A, B, D, F and G; for onsets C and E temperature was gradually enhanced from day 55 to day 61.

Population dynamics were assessed through observation of the time series and supported by the calculation of the Lyapunov exponents. Only when both analyses coincided the resulting population dynamic was considered to be true.

At 20°C almost all onsets showed irregular dynamics, only in onset B, observation of the time series indicates that *Pedobacter* reached a stable equilibrium after a long period of stabilization of 14 days; calculation of the Lyapunov exponent between days 14 and 47 confirm this first impression and take negative values (-0.18±0.017). For onset A, the Lapunov exponent for *Acinetobacter* at 20°C takes values near to 0 (0.025±0.028) which is indicative of stable limit cycles, this is not observable on the time series and therefore, according to the criteria aforementioned, not taken in account for the conclusions. The values of the Lyapunov exponents for all species in all onsets are positive (see Fig 2), this indicates the existence of

chaotic behaviour and coincides with the observation of the time series.
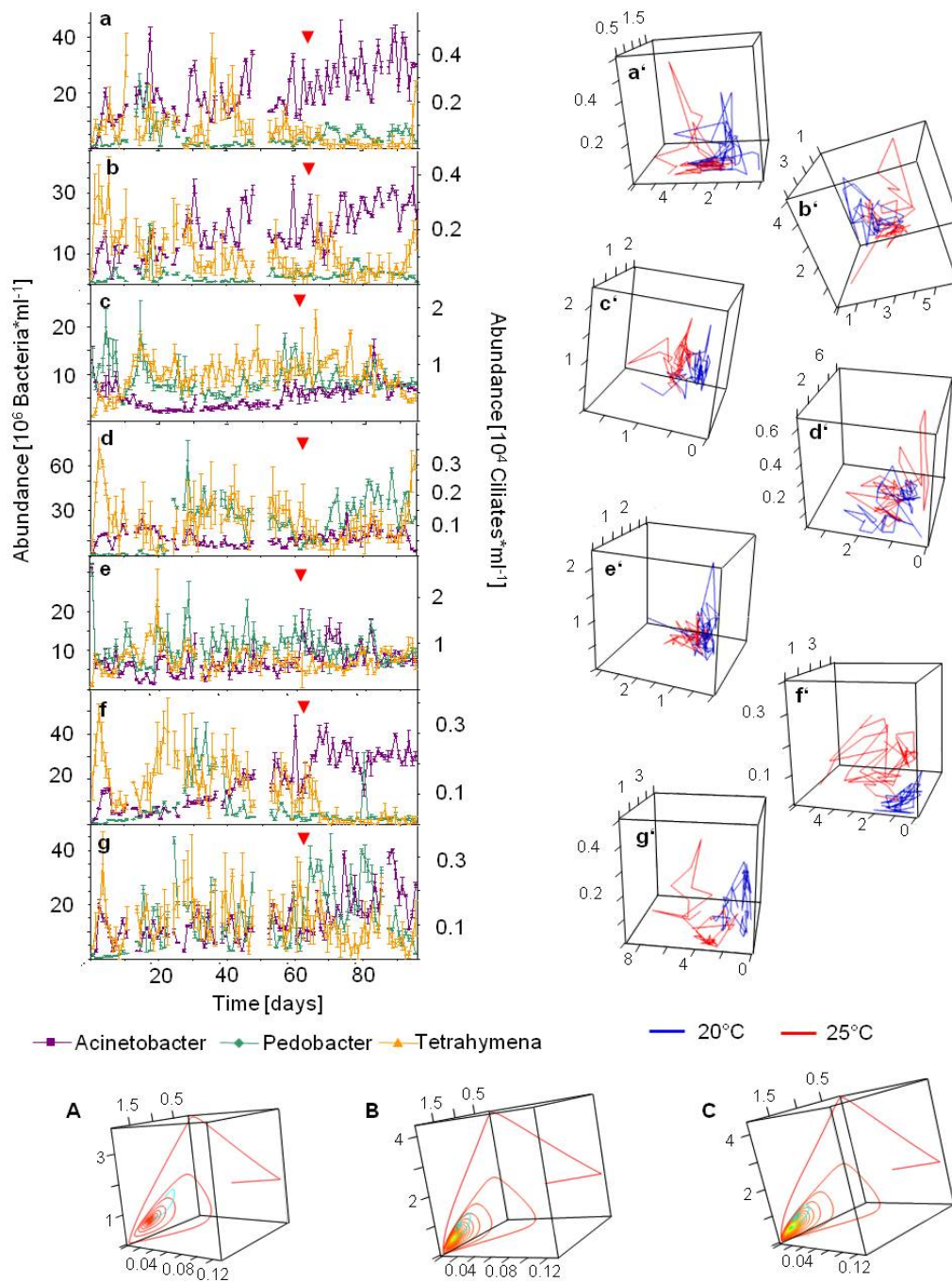
Fig 1: Time series of chemostat experiments and the corresponding phase space diagrams. Onsets a, b, and c run at a flow rate of 0.75 d^{-1}; d and e at 0.5 d^{-1}; and f and g at 0.45 d^{-1}. Red arrows indicate the point where temperature increase began. On the left side are the corresponding phase space diagrams. A, B and C are the phase space diagrams of the model analyses at 0.75 d^{-1}, 0.5 d^{-1}, and 0.45d^{-1} respectively.

At 25°C and for onsets A and B, *Pedobacter* and *Tetrahymena* showed stable limit cycles that were clearly observable especially in onset A (see Fig. 1). This dynamic behaviour cannot be seen for *Acinetobacter*. The Lyapunov exponents are in this case near to zero for all three species and both onsets supporting the impression given by the time series. In onset G, *Pedobacter* and *Tetrahymena* showed in the time series a stable equilibrium, but the Lyapunov exponents took positive values, thus again, this statement was not considered conclusive.
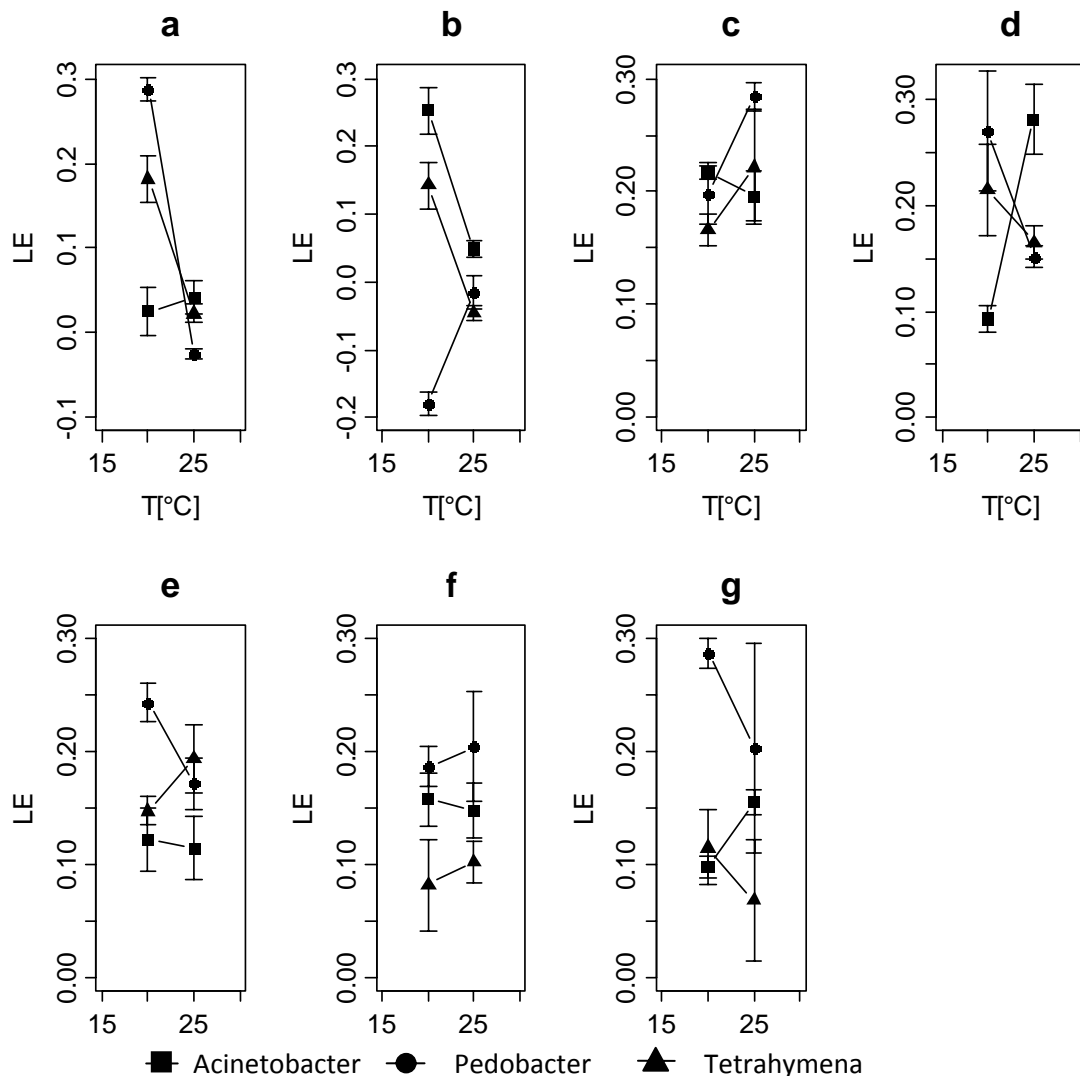

Fig 2: Lyapunov exponents at 20 and 25°C. a, b, and c run at a flow rate of 0.75 d^{-1}, d and e at 0.5 d^{-1}, f and g at 0.45 d^{-1}.

Phase space diagrams for all experimental onsets were done in order to observe changes in the dynamic behaviour that could not be assessed with the Lyapunov exponents or through the observation of the time series. In those diagrams it can be seen that the system shifted to another attractor after the temperature was changed (see Fig. 1). The general tendency of this new attractor was directed towards higher abundances of *Acinetobacter* for all the experimental onsets. However, phase space diagrams lighten a different reaction depending on the flow rate, then the attractor shifted more abruptly at a flow rate of 0.45 d^{-1} (onsets F and G) than for the other flow rates analyzed, and this shift was bidimensional because *Tetrahymena* and *Acinetobacter* reached higher abundances. For the onsets that run at 0.5 d^{-1} and 0.75 d^{-1} the attractor only changed slightly, however it still can be seen that the system shifted towards higher abundances of *Acinetobacter* reaching so a new attractor.

The same scenario was analyzed with a mathematical model that consisted on four differential equations:

$$\frac{\partial C}{\partial t} = (C_0 - C)D - \varepsilon_1 \mu_1(C)N_1 - \varepsilon_2 \mu_2(C)N_2 \qquad (1)$$

$$\frac{\partial N_1}{\partial t} = N_1 \mu_1(C) - P\varphi_1(N_1) - DN_1 \qquad (2)$$

$$\frac{\partial N_2}{\partial t} = N_2 \mu_2(C) - P\varphi_2(N_2) - DN_2 \qquad (3)$$

$$\frac{\partial P}{\partial t} = P\beta_1 \varphi_1(N_1) + P\beta_2 \varphi_2(N_2) - DP \qquad (4)$$

where C represents the nutrient concentration, N_1 and N_2 the abundances of *Pedobacter* and *Acinetobacter* respectively, and P the abundance of *Tetrahymena*. The growth rates of the prey organisms, μ_1 and μ_2 follow the Monod function, the same as the predator feeding response for each bacterial prey, φ_1 and φ_2, which was assumed to be of the Holling II type:

$$\mu_i = \frac{\mu_{\max_i} C}{K_{s_i} + C} \quad (5) \qquad \varphi_i(N_i) = \frac{\varphi_{\max_i} N_i}{K_{N_i} + N_i} \quad (6)$$

The parameter ε_i is the bacterial yield and took the value $2 \times 10^6 \mu g/\text{ind}_{prey}$ for ε_1 and ε_2; β_i is the predator yield and took the value $1/4000 \text{ ind}_{predator}/\text{ind}_{prey}$ for both β_1 and β_2; C_0 represents the nutrient concentration of the inflow and was 3 µg/ml. The feeding response of *Tetrahymena* followed the expression (9) which parameters are $\varphi_{max1}=150$ ind_{prey}/h $\text{ind}_{predator}$, and $K_{N1}=422000\text{ind}_{prey}/ml$ for Pedobacter; and $\varphi_{max2}=450$ $\text{ind}_{prey}/\text{ind}_{predator}$ and $K_{N2}=400000\text{ind}_{prey}/ml$. The bacterial growth rate was controlled by two parameters μ_i and K_{si}, for *Pedobacter* those parameters were $\mu_{max1}=0.15$ h^{-1} and $K_{s1}=0.0274$ µg/ml; for *Acinetobacter* K_{s2} was 0.002 µg/ml, and μ_{max2} was variable and took its values from the temperature dependent function:

$$r = r_{\max}\left(1 + b\left(\left(R_1^{T-T_{opt}} - 1\right) - \frac{\ln R_1}{\ln R_2}\left(R_2^{T-T_{opt}} - 1\right)\right)\right) \qquad (7)$$

where r_{max} is the growth rate at the optimal temperature (2.71 d-1), T_{opt} is the temperature where maximal growth rate occurs (24.55°C), and b (13.33), R_1 (1.07) and R_2 (1.09) are parameters without biological interpretation.

The model was run at the same flow rates as the experiments; temperature was increased at the equator of the modeling time span.

Two characteristics of the experimental system were good reflected by the model. An increase of the abundances of *Acinetobacter* after the temperature changes and phase space diagrams show a shift in the attractor of the system. This new attractor shifted to higher abundances of *Acinetobacter* and of *Tetrahymena*. This shift was more pronounced for higher flow rates (see Fig. 1 point attractor in blue) in the model predictions, in the experiments this was observed for lower flow rates.

Discussion

The results presented here show that, species interactions change and promote qualitative shifts in the dynamic behaviour as an effect of temperature increase although other extrinsic parameters remained constant. The shift is observed both in experimental results as in numerical analysis of the model. Although the model did not capture the whole complexity of the experiments, reflects qualitatively some experimental observations. The higher abundances of *Acinetobacter* predicted by the model are also observed experimentally, and the complex food web response reflected by the shift in the attractor coincides in the model and the experiments. Although the coincidences between model and experiment are sufficient to consider the model as a good approach, some discrepancies were found: the change in the attractor was more

pronounced for higher flow rates in the model predictions, the contrary was observed in the experiments where at lower flow rates the attractor change was more abrupt.

Although external conditions were kept constant, our experiments showed irregular dynamics through all dilution rates analyzed. Two main processes could generate this variability: 1- *Acinetobacter* shows the capacity to form filaments and colonies; this mechanism of protection against predation is widely distributed in natural systems (Corno and Jurgens, 2006, Juergens and Matz, 2002, Salcher et al., 2005). However, several theoretical studies propose inducible defences against predation as a stabilizing mechanism of the predator-prey dynamics (Leibold, 1989, Bohannan and Lenski, 1999). On the other hand, density dependent mechanisms are characteristic for non-linear dynamics, and therefore open the possibility for chaotic behaviour (see Turchin, 2003). 2- The chemostat experiments run for up to 90 days, due to the short generation times of the microorganisms forming the food web, we cannot exclude the possibility that evolution occurs (Jones and Ellner, 2007, Bennett et al., 1990). Several theoretical studies analyzed the effect of food web length and omnivory on the population dynamics (Tanabe and Namba, 2005, Gross et al., 2005), those evolutionary processes could affect the food web composition and interactions within the organism, and therefore population dynamics.

In the frame of global warming it is of vital importance to understand how intrinsic processes respond to extrinsic drivers (Stenseth et al., 2002, Dillon et al., 2010). On one hand, the dynamic behaviour of a system has been related to essential ecological

questions like persistence of species (Ruokolainen et al., 2007, McLaughlin et al., 2002); on the other hand, the different temperature reaction norms of the species forming a food web can have large effects, for example in the phenology of species (Durant et al., 2007). Other effects derived from differential temperature reaction may be of catastrophic nature like mass extinction (Pounds et al., 2006) or changes in the distribution of human diseases (Paaijmans et al., 2009).

Here I showed for the first time experimentally that temperature changes induced qualitative shifts in the population dynamics, this conclusion is based on the observation of experimental time series and supported by the calculation of the Lyapunov exponents

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Chapter III

Predator induced colony formation of bacteria. Effects on the dynamics of a three species microbial food web model.

1. Introduction

The "competitive exclusion principle" (Hardin, 1960) allows coexistence of two competing species only for exactly balanced parameters, which is an extremely improbable scenario in nature (Smith and Waltman, 1994). The presence of a predator preying preferentially on the superior competing prey organism was predicted by Takeuchi and Adachi (1983) to allow coexistence of all three species for certain parameter values. This theoretical finding was experimentally proofed for a microbial food web in chemostat experiments (Becks et al., 2005), where the flow rate was used as a control parameter. In that work it was also shown that a two-prey-one-predator food web presented stable equilibrium, stable limit cycles and chaos. Recent experimental results indicated that, when one of the prey bacteria formed grazing resistant morphotypes (Schieffer et al. unpubl.), coexistence of species in such a food web is enhanced. The formation of grazing resistant forms is a common feature in natural systems (Tollrian and Harvell, 1999).

Bacteria present several strategies to avoid predation (Pernthaler, 2005), one of them is the formation of colonies and filaments. Few is known about the triggers that promote such grazing-resistant morphotypes, but selective grazing, chemical cues produced by the predator (kairomones) as well as availability of nutrients and thus growth rate effects are discussed as possible driving factors (reviewed by Juergens and Matz, 2002; Pernthaler 2005). Recent findings showed that protists can indeed induce grazing resistance via chemical cues (Corno and Jurgens, 2006)

The effect of grazing resistant morphotypes has been mathematically analyzed for a two-prey-one-predator food web with Daphnia feeding on algae (Kretzschmar et al., 1993). Kretzschmar et al. (1993) showed a stabilization of the system due to the grazing

resistance of the prey algae. Bohannan and Lenski (1999) demonstrated a shift between top-down and bottom-up control due to enrichment in a predator-prey system with resistant and susceptible *Escherichia coli* and the phage T4. Those investigations included chemostat experiments and model analysis (Levin et al., 1977).

In this study, I investigated the following hypotheses (1) varying morphologies result in a wider range of dilution rates permitting coexistence for all species, and (2) grazing resistant forms lead to a destabilization of the system and non-periodic behavior. With this aim, a model reflecting the chemostat experiments of Becks et al. (2005) was developed first. Two mechanisms driving the grazing resistance were analyzed through modifications of that first model. (1) selective grazing of determined morphotypes (Juergens and Matz, 2002) and (2) grazer induced (through kairomones) defense mechanisms (Corno and Jurgens, 2006) . The latter includes a possible switch-over between grazing resistant and non-resistant morphotypes. Model results are compared with the data of Becks et al. (2005) and recent studies by Willen et al. (subm.).

2. Description of the system

2.1. Two-prey-one-predator food web without grazing resistance (model 1)

This first model included four differential equations as follows:

$$\frac{dC}{dt} = (C_0 - C)D - \varepsilon_1 \mu_1(C)N_1 - \varepsilon_2 \mu_2(C)N_2 \quad,$$

$$\frac{dN_1}{dt} = N_1 \mu_1(C) - P\varphi_1(N_1) - DN_1 \quad,$$

$$\frac{dN_2}{dt} = N_2 \mu_2(C) - P\varphi_2(N_2) - DN_2 \quad,$$

$$\frac{dP}{dt} = P\beta_1\varphi_1(N_1) + P\beta_2\varphi_2(N_2) - DP \quad,$$

where C is the concentration of nutrients in the chemostat, N_1 the abundance of *Acinetobacter*, N_2 the abundance of *Pedobacter* and P the abundance of *Tetrahymena*. The parameter C_0 represents the concentration of nutrients in the reservoir, D the dilution rate, ε_1 and ε_2 the reciprocal yield of each prey organism and β_1 and β_2 the yield of *Tetrahymena* feeding on the bacteria.

The specific growth rate $\mu_i(C)$ of the bacteria follows the Monod function

$$\mu_i(C) = \frac{\mu_{\max_i} C}{k_{s_i}} \quad, \quad i = 1,2 \quad,$$

where μ_{maxi} is the maximum growth rate and K_{si} the half-saturation constan. Similarly, the ciliate feeding response of the Holling II type is given by Monod's model:

$$\varphi_i(N_i) = \frac{\varphi_{\max_i} N_i}{k_{N_i} + N_i} \quad, \quad i = 1,2 \quad,$$

where φ_{maxi} is the maximum feeding rate and K_{Ni} the half-saturation constant for the predator feeding on bacterium N_i.

Parameter values were chosen for a food web consisting on *Tetrahymena pyriformis* as predator ciliate, *Pedobacter* and *Acinetobacter* as prey bacteria. This food web has been experimentally investigated in our working group.

The model was run with following parameter values: C_0 = 3 [µg ml^{-1}]; D [h^{-1}] was taken as a control parameter, dynamics were investigated in the range from 0 h^{-1} to 1.4 h^{-1}; ε_1 = ε_2 = 2·10^{-6} [µg Ind.$_i^{-1}$] (Lenski ,1988); β_1 = β_2 = 1/4000 [Ind.$_{Tetra.}$ Ind.$_i^{-1}$]. The maximum growth rates given by this value were consistent with those found in the literature ($\approx$ 0.138 h^{-1}) for the ciliate when abundances of both bacteria are high- (Taylor, 1978); μ_{max1} = 0.150 [h^{-1}] and μ_{max2} = 0.172 [h^{-1}] were obtained in previous experiments; K_{s1} = 0.0274 [µg/ml]; K_{s2} = 0.0020 [µg/ml], these values are in a reasonable order of magnitude (Vayenas and Pavlou, 1999) and together with the maximum growth rates determine the better fitness of *Pedobacter.* The edibility of *Pedobacter* is given by φ_{max2} = 450 [Ind.$_{Pedo.}$ h^{-1} Ind.$_{Tetra.}^{-1}$] and of *Acinetobacter* is φ_{max1} = 150 [Ind.$_{Acin.}$ h^{-1} Ind.$_{Tetra.}^{-1}$]. The half-saturation constants K_{N1} = 422,000 [Ind.$_{Acin.}$ ml^{-1}] and K_{N2} = 400,000 [Ind.$_{Pedo.}$ ml^{-1}] were orientated on experiments performed by Becks (2003) with *Tetrahymena* grazing on *Pedobacter* and *Brevundimonas*. Initial conditions of the bacteria were 10^5 [Ind. ml^{-1}] and of *Tetrahymena* 500 [Ind. ml^{-1}]. The initial nutrient concentration is 0 [µg ml^{-1}]. Note that individual-based units were converted into µg carbon (*Acinetobacter*: 1.241·10^{-7} µg carbon Ind.$^{-1}$; *Pedobacter*: 6.65·10^{-8} µg carbon Ind.$^{-1}$; *Tetrahymena*: 6.5507·10^{-3} µg carbon Ind.$^{-1}$; nutrients: 0.4 µg carbon/µg gluc) before the model was run.

2.2. Two-prey-one-predator food web with grazing resistance (colony formation) (model 2)

In order to model the morphological heterogeneity observed for *Acinetobacter,* one part of the population was assumed to grow as single cells while the other would grow aggregated in colonies. The population fraction grown in colonies has a lower fitness due to overlap of nutrient depletion areas (Young, 2006) and the energy

costs of developing grazing resistance strategies. This trade-off was considered essential by Bohannan and Lenski (1999) and Levin (1977) as well. A complete grazing protection against predation for cells in colonies was assumed. In the model, this fraction was represented by an additional differential equation inside the system:

$$\frac{dC}{dt} = (C_0 - C)D - \varepsilon_1 \mu_1(C)N_1 - \varepsilon_2 \mu_2(C)N_2 - \varepsilon_R \mu_R(C)R$$

$$\frac{dN_1}{dt} = N_1 \mu_1(C) - P\varphi_1(N_1) - DN_1 \,,$$

$$\frac{dR}{dt} = R\mu_R(C) - DR \,,$$

$$\frac{dN_2}{dt} = N_2 \mu_2(C) - P\varphi_2(N_2) - DN_2 \,,$$

$$\frac{dP}{dt} = P\beta_1 \varphi_1(N_1) + P\beta_2 \varphi_2(N_2) - DP \,,$$

where R is the abundance of inedible *Acinetobacter*, ε_R the reciprocal yield coefficient of R, and μ_R its specific growth rate. μ_R is represented by a Monod function with following parameters μ_{maxR} (0.08 [1/h]) and K_{SR} (0.04 [µg/ml]) being the maximum specific growth rate and the half-saturation constant, respectively. Furthermore, the reciprocal yield coefficient ε_R took the same value as ε_1 and ε_2. Initial concentrations of R and N_1 were set to 50,000 [Ind./ml]. The remaining parameters and initial conditions were equal to the ones in model 1.

2.3. Two-prey-one-predator food web with predator induced colony formation (model 3)

In this model, the possibility of an exchange between the grazing resistant and the grazing vulnerable subpopulations is investigated. This exchange is modeled to be triggered by the abundances of *Tetrahymena*. With this aim, the flow terms $\psi_1(N_1,P)$ and $\psi_2(R,P)$ were introduced into model 2:

$$\frac{dC}{dt} = (C_0 - C)D - \varepsilon_1 \mu_1(C)N_1 - \varepsilon_2 \mu_2(C)N_2 - \varepsilon_R \mu_R(C)R \ ,$$

$$\frac{dN_1}{dt} = N_1 \mu_1(C) - P\varphi_1(N_1) - \Psi_1(N_1,P) + \Psi_2(R,P) - DN_1 \ ,$$

$$\frac{dR}{dt} = R\mu_R(C) + \Psi_1(N_1,P) - \Psi_2(R,P) - DR \ ,$$

$$\frac{dN_2}{dt} = N_2 \mu_2(C) - P\varphi_2(N_2) - DN_2 \ ,$$

$$\frac{dP}{dt} = P\beta_1\varphi_1(N_1) + P\beta_2\varphi_2(N_2) - DP \ ,$$

With ψ_1 given by:

$$\Psi_1(N_1,P) = \frac{P}{P_{crit}} \alpha N_1 \ ,$$

and ψ_2 given by:

$$\Psi_2(R,P) = \frac{P_{crit}}{P} \gamma R \ .$$

Here, α and γ represent the velocity at which cells attach or leave colonies, respectively. P_{crit} [Ind.$_{Tetra.}$/ml] is the critical abundance of *Tetrahymena* above which more *Acinetobacter* cells

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aggregate in colonies than leave them. A higher value for α (0.01 [h^{-1}]) was used than for γ (0.002 [h^{-1}]). Although these values are difficult to determine experimentally, detachment from colonies should require degradation of exopolymeric substances, which can be assumed to take more time than the attachment process. The value for P_{crit} was estimated from the resulting ciliate abundances of model 2: P_{crit} = 183 [Ind.$_{Tetra}$./ml]. The same initial conditions and parameter values as in model 2 were used.
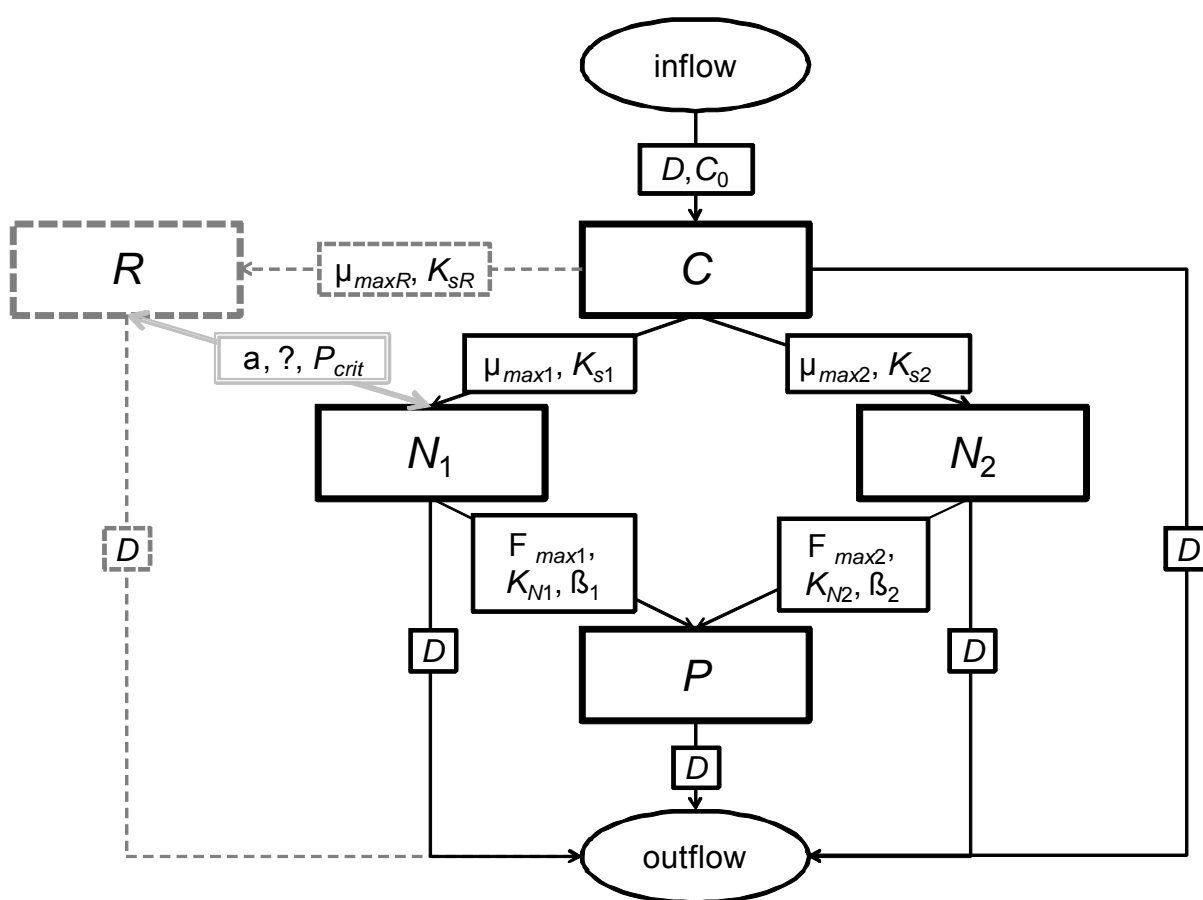
Fig. 1. Graphical illustration of the models. State variables are depicted in bold squares (C: Nutrients, N1: *Acinetobacter*, N2: *Pedobacter,* P: *Tetrahymena*). Arrows indicate flows between the compartments with the corresponding parameters (for explanation of parameter names, see text). Model 1 is shown in black solid lines, the modifications in model 2 in dark grey dashed lines and modifications in model 3 in light grey doubled lines. Circles depict flow of matter into and out of the system

2.4. Numerical model analyses

All model simulations were run with R 2.10.1. The models were solved numerically using the automatic step size algorithm "Livermore Solver for Ordinary Differential Equations" of the R-package "odesolve" (Hindmarsch, 1983, Petzold, 1983). The dynamical behavior of the system was analyzed with bifurcation diagrams. Additionally, sensitivity analyses were run.

3. Results

3.1. Two-prey-one-predator food web without grazing resistance (model 1)

The coexistence range was predicted between the dilution rates 0.64 d^{-1} and 1.06 d^{-1} (see Fig. 2, top panel). Acinetobacter reached higher abundances than Pedobcater always. At dilution rates lower than 0.64 d^{-1} *Pedobacter* dies off. The opposite is predicted for dilution rates higher than 1.06 d^{-1}.

The bifurcation diagram for *Acinetobacter* (top panel of Figure 3) shows that between dilution rates of 0.64 d^{-1} and 0.74 d^{-1}, the system reaches a stable equilibrium. Stable limit cycles are observed at flow rates between 0.74 d^{-1} and 0.93 d^{-1}, up to this flow rate, the system undergoes a period doubling process that leads to chaotic behavior at a flow rate of 1.0 d^{-1} until. 1.06 d^{-1}, where *Acinetobacter* goes extinct.

3.2. Two-prey-one-predator food web with grazing resistance (colony formation) (model 2)

Considering the population of Acinetobacter to be formed by a grazing-resistant and a grazing-vulnerable subpopulation increases the coexistence range of the three species, which is predicted between 0.04 d^{-1} and 1.29 d^{-1} (Fig. 2, center). The model does not

permit the coexistence of grazing-resistant and grazing-vulnerable forms. Between a dilution rate of 0.044 d^{-1} and 0.56 d^{-1}, no single *Acinetobacter* cells are predicted. Contrarily, between dilution rates of 0.67 d^{-1} and 0.97 d^{-1}, only grazing-vulnerable morphotypes are present. Above dilution rates of 0.97 d^{-1}, *Acinetobacter* shows only the grazing-resistant form until a dilution rate of 1.29 d^{-1}, where *Acinetobacter*   dies off
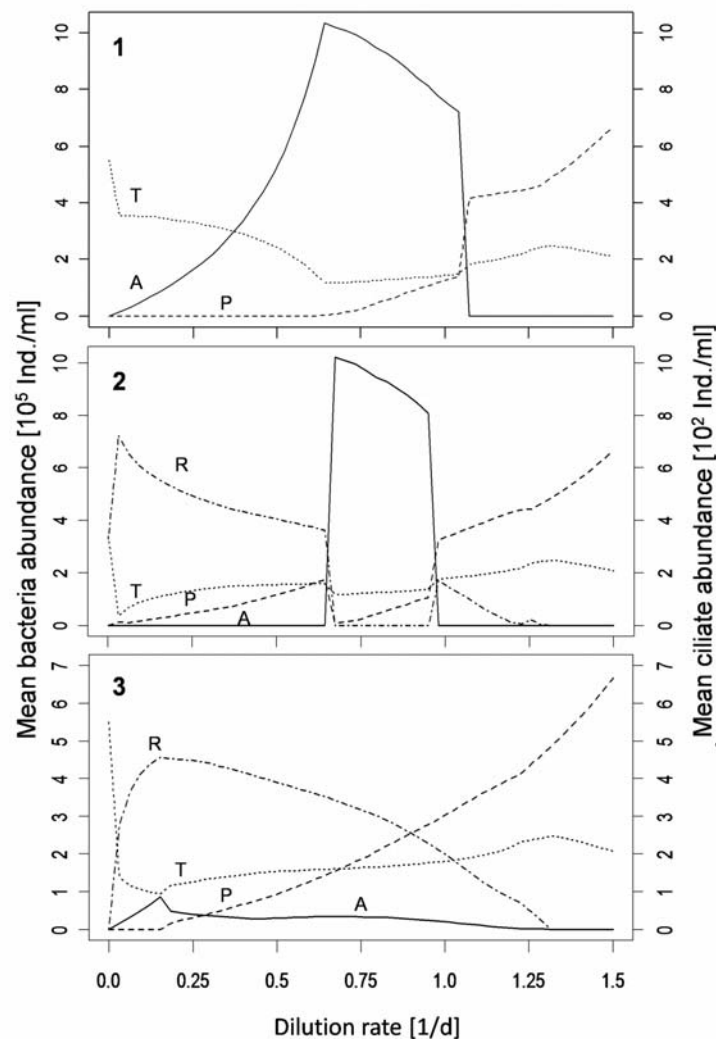

Fig. 2. Mean abundances of bacteria and ciliates at different dilution rates predicted by model 1, 2 and 3 for a time series of 625 days. The stabilization phase was omitted in the calculation of the mean abundances. A (solid line): *Acinetobacter*, R (dashed-dotted line): Grazing resistant *Acinetobacter*, P (dashed line): *Pedobacter*, T (dotted line): *Tetrahymena*.

The model predicts equilibrium up to a flow rate of 0.33 d^{-1} (Fig. 3, middle panel). For higher flow rates, stable limit cycles are predicted. When single cells become dominant at dilution rates higher than 0.67 d^{-1}, the system first stabilizes and then starts oscillating again. The amplitude of the oscillations is lower when resistant cells are dominant. At a dilution rate of 0.93 d^{-1} the system oscillates with period four, but no chaotic behavior can be observed.
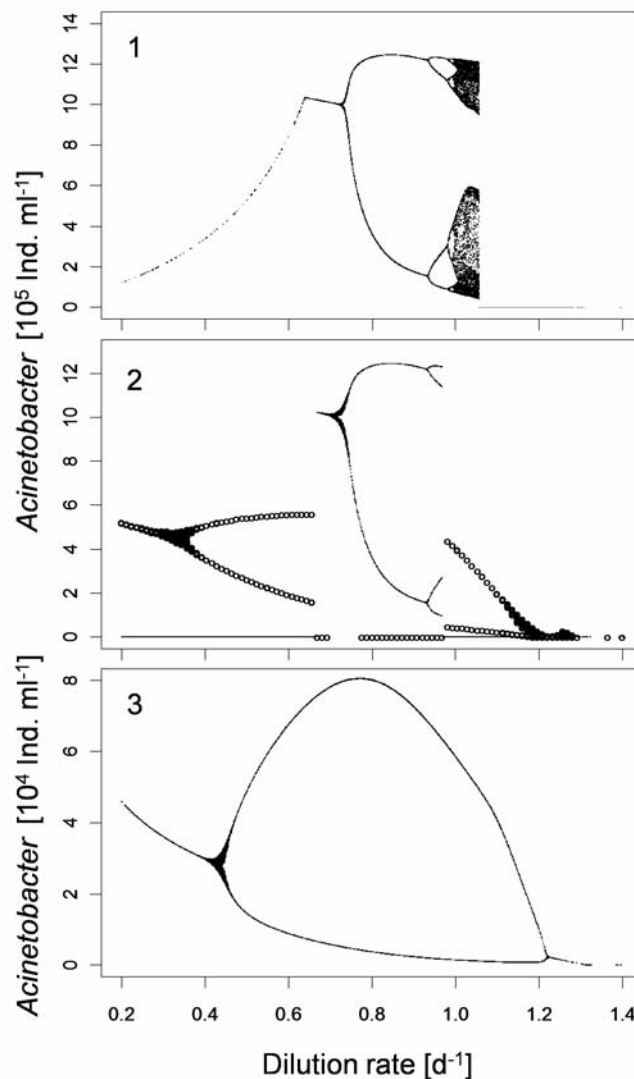

Fig. 3. Bifurcation diagrams for model 1, 2 and 3. The local maxima and minima of the abundance of *Acinetobacter* in time series of 417 days are plotted against the dilution rate. Stabilization phases are omitted. In model 2, grazing resistant forms of *Acinetobacter* are shown as open circles.

3.3. Two-prey-one-predator food web predator-induced colony formation (model 3)

In this case the coexistence range is similar as in model 2 and spans from 0.14 d^{-1} to 1.31 d^{-1} (Fig. 2, bottom panel), but the population of *Acinetobacter* is predicted to be heterogeneously constituted of grazing-resistant and grazing-vulnerable cells, though only 10% of the population occurs as single cells.

The model predicts a stable equilibrium for dilution rates between 0.14 d^{-1} and 0.45 d^{-1} (Fig. 3, bottom panel). Up to this point stable limit cycles are predicted until a dilution rate of 1.21 d^{-1}. The amplitude of these oscillations is considerably lower when compared to the system without defenses. At higher dilution rates a stable equilibrium of all three species is predicted until *Acinetobacter* dies off at dilution rates above 1.31 d^{-1}.

4. Discussion

The models analyzed here show that, formation of grazing resistant morphotypes, constitutive (model 2) or predator induced (model 3), enlarge the range of flow rates where coexistence is possible compared with a model where such protection against predation is not possible (model 1). Furthermore, the chaotic behavior observed in model 1, is not present in none of the models protections against predation is possible.

In model 1, where no protection against predation is considered, numerical analysis reflect the results obtained by Becks et al.(2005). The dynamic behavior observed experimentally is also predicted by the model, including stable equilibrium, stable limit cycles and chaos. Though, chaotic behaviour is predicted to occur at higher flow rates (above 1.0 d^{-1}) than experimentally observed (0.5 d^{-1} in Becks et al. (2005)). The model predicts the inferior competitor

to die off at high dilution rates. This is also the case in the experiments by Becks et al. (2005). In the experiments by Becks et al. (2005), increasing the flow rate drove different dynamic behaviours: at low flow rates (0.45 d^{-1}) stable limit cycles, followed by chaotic behavior (0.5 d^{-1}), and at high flow rates a stable equilibrium (0.75 d^{-1}). The model differed in this succession and predicted a stable equilibrium for low flow rates, followed of stable limit cycles, and at high flow rates predicted chaotic behavior until extinction.

Abundances predicted for the three species are in the same order of magnitude observed experimentally e.g. Becks et al. (2005) or Jost et al. (1973). The model was parameterised for Acinetobacter (which presents grazing-resistant morphologies) in order to be compared with the two other models presented in this work. Thus the differences between the experimental results by Becks et al. (2005) and the model 1 could be due to the different composition of the food web.

Analyze of model 2 with constitutive grazing protection for *Acinetobacter* showed that coexistence was possible for a broader range of flow rates. This can be due to the capacity of grazing-resistant cells to survive at higher flow rates compared to grazing-vulnerable cells, while intermediate flow rates favor unprotected morphotypes. Experiments done in our working group with a grazing-vulnerable bacterium (*Brevundimonas*, experiments by Becks unpubl.) showed coexistence from 0.1d-1 to 0.9 d-1, while substituting this prey bacterium by the grazing-resistant *Acinetobacter* allowed coexistence between 0.1d-1 and 1.2d-1 (Schieffer et al. unpubl.). This increase of the area of coexistence is predicted by model 2 and can thus be explained by the occurrence of grazing resistant morphologies.

Regarding the dynamic behavior of the system, no chaotic behavior is observed and the oscillations have reduced amplitude, indicating a stabilization of the system. Grazing-resistant cells are independent of the predators' oscillations and are thus under bottom-up control, depending only on the availability of nutrients. Stabilization of population dynamics has been considered theoretically in several papers (e.g. Kretzschmar et al. 1993; Vos et al. 2004), though the concept of stabilization has been defined as reduced oscillations' amplitude or increase of the coexistence range. I fist showed here that the dynamic behavior of the system lost chaotic oscillations due to grazing protection mechanisms in one prey bacterium.

Phenotypic shifts in prey activated by biological agents like predators or pathogens, are widespread in nature and have been shown for vertebrates, invertebrates, and plants in terrestrial, marine, and freshwater habitats (for a review see Tollrian and Harvell, 1999). Chemical cues produced by predators can promote shifts in the morphology of prey populations towards grazing resistant forms.(Juergens and Matz, 2002, Pernthaler, 2005, Blom et al., 2010). This phenomenon was analyzed in model 3. The range of flow rates that allow coexistence is similar to model 2 (Fig. 2, bottom panel). The main difference to model 2 is that both morphotypes of *Acinetobacter,* the grazing resistant and grazing vulnerable subpopulations, are predicted to coexist with the other bacteria species. This occurs due to the possible flow between the compartments representing the two morphotypes, which is controlled by the predator abundance. In recent chemostat experiments, the composition of the *Acinetobacter* population showed that grazing resistant forms coexisted with single cells (Willen et al., subm.), this is consistent with the predictions of model 3.

Model 3, like model 2, did neither show chaotic behavior (Fig. 3, center), and low amplitude oscillations were observed for a wide range of flow rates. This is indicative of a stabilization of the dynamic behavior in comparison with model 1. Vos et al. (2004) and van der Stap et al (2006) analyzed the influence of inducible defenses on a one-predator-one-prey rotifer-algae system and also found that their system's dynamics were stabilized by the presence of inducible defenses in the prey population. Nevertheless they considered the stabilization from the point of view of extinction risk (which was reduced when grazing resistance was possible), and of the amplitude of oscillations. None of these studies considered the qualitative change of dynamic behavior of the system.

While the hypothesis that grazing resistant morphotypes result in a destabilization of the system could not be supported by the model predictions, the hypothesis that grazing resistant phenotypes may cause the observed increase of parameter ranges allowing coexistence is supported by both modeling approaches. This has major implications for our view on the role of phenotypic plasticity in bacteria. While laboratory systems like chemostats allow us to observe and investigate coexisting populations for strictly regulated parameters, plankton organisms in natural habitats are permanently faced with fluctuating conditions. Phenotypic plasticity may be one way for bacteria to escape extinction in dynamically changing environments. For a better understanding of these important mechanisms, experimental studies are needed to understand the evolution of triggers regulating phenotypic plasticity in bacteria.

The models presented in this work indicate that predator-induced grazing resistance may explain the coexistence of grazing and non-grazing resistant morphotypes of prey organisms (Yoshida et al., 2007, Becks et al., 2010).

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Discussion

The results presented here intended to analyze the interplay of intrinsic population dynamics and extrinsic temperature effects on a microbial food web. The hypothesis studied was: the differential temperature reaction of the species in a food web can promote qualitative shifts in the dynamical behaviour when extrinsic temperature changes. The hypothesis was analyzed experimentally for a microbial two-prey-one-predator food web, and theoretically with model analyses.

With this aim I assessed the temperature reaction norms of the three species experimentally. The data obtained for *Tetrahymena* differed slightly from the values found in the literature (Schmid, 1967, Elliott, 1973, Slater, 1954). This discrepancy could be due to the experiments objective: that was to analyze a determined food web composition, so the food sources of *Tetrahymena* were the prey bacteria of the analyzed food web, *Pedobacter sp*. and *Acinetobacter johnsonii.* Regarding the bacteria, no literature data were available but the temperature range where positive growth was possible could represent the ambient temperature of central Europe, given that the bacteria were isolated from lake Schoesee in Germany (Beck, 2000) by Kristin Beck, they would be adapted to the temperature regime of the natural habitat where they were isolated (Hall et al., 2009).

Chemostat experiments were done to analyze the food web response to extrinsic temperature changes. I was able to show that a temperature increase from 20 to 25°C promoted a shift in the dynamic behaviour of the system, for a flow rate of 0.75 d^{-1}, the system showed chaotic behaviour at 20°C and stable limit cycles at 25°C. Flow rates of 0.5 d^{-1} and 0.45 d^{-1} were also analyzed. Experiments run under those flow rates showed a shift in the attractor towards higher abundances of *Acinetobacter*.

A mathematical model based on a Lotka Volterra predator-prey model (see Turchin, 2003) was developed. The model included temperature dependent growth rates. Numerical analysis of the model for different temperature scenarios revealed that the food web reacted to temperature in a complex manner and that the reaction could not be extrapolated from the single species temperature response. The model showed stable equilibrium for all flow rates permitting coexistence of all three species. Despite this discrepancy, the temperature reaction shown by the model was similar to that observed experimentally. The system shifted to another attractor when the temperature increased, and this shift depended on the flow rate analyzed.

The results obtained by Becks et al.(2005) showed several dynamic behaviours, like stable equilibrium, stable limit cycles and chaos. The food web analyzed by Becks et al.(2005) consisted on *Pedobacter sp.*, *Brevundimonas sp.* and *Tetrahymena pyriformis* and was very similar to the food web analyzed in my experiments. The different food web composition introduced additional complexity because *Acinetobacter* was able to develop grazing-resistant morphologies while *Brevundimonas* was always present in a single cell form.

The discrepancy regarding the dynamic behaviour between model predictions and experimental results was interpreted as a consequence of oversimplification (see Soetaert et al., 2009), although the temperature response was qualitatively well reflected. I hypothesised that the chaotic behaviour was a consequence of the grazing resistance showed by *Acinetobacter*. Grazing resistance strategies are widely observed in natural systems (Juergens and Matz, 2002) and take several phenotypic forms (Pernthaler, 2005). In this case, *Acinetobacter* formed large filaments. In order to test if

colony formation can drive chaotic dynamics in a two-prey-one-predator food web, I developed a mathematical model to analyze theoretically this question.

Two types of grazing resistance mechanisms were analyzed in different models: a constitutive and a predator induced grazing resistance. Both models were compared with a model without protection against predation. Numerical analyses showed that both grazing resistance strategies stabilized the food web in two manners: on one hand the range of parameters permitting coexistence is enlarged, on the other hand, the chaotic dynamic behaviour predicted by the model without grazing resistance, disappears when a grazing-resistant subpopulation is present.

The results of the model are consistent with experimental results obtained by Becks et al. (2005) and Jost et al. (1973) regarding the abundances predicted, both for the bacteria and for the ciliate. Furthermore a comparison between the results showed in Becks et al. (2005), where no grazing resistant bacterium was present, and results obtained by Schieffer et al (unpubl.) with the same food web analyzed here, coincide with the prediction of enlargement of the flow rates range permitting coexistence.

The model with constitutive grazing-resistant morphologies did not allow the coexistence of grazing-resistant and grazing-vulnerable forms. This was possible when inducible defences against predations were modelled. The latter represented a more realistic scenario for the food web analyzed and has been observed in several experiments (Becks et al 2010, Willen et al 2010 (subm.)).

The results presented in this work show that, experimental microbial food webs can help to disentangle the interaction between intrinsic and extrinsic processes affecting food webs. The actual

global warming makes necessary the understanding of such interactions (e.g. Stenseth et al., 2002). Furthermore, the dynamical behaviour of a system is of ecological relevance because it is related with the persistence of species (Ruokolainen et al., 2007, McLaughlin et al., 2002). Also the different temperature reaction of the species forming a food web can have large effects, for example on the phenology of species (Durant et al., 2007). Other effects derived from differential temperature reaction may be of catastrophic nature like mass extinction. This is the case of the amphibians; some authors indicate that the best performance of the parasitic fungus *Batrachochytrium dendrobatidis* at higher temperatures, combined with other factors like habitat destruction and changes in the local climate are responsible for the extinctions observed in many amphibian species (Pounds et al., 2006, Wake and Vredenburg, 2008). Climate change has also been related to changes in the distribution of malaria (Paaijmans et al., 2009). All those consequences are related with the interaction of intrinsic population dynamics and extrinsic temperature drivers.

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Commented R script for the mathematical model with temperature dependent growth rates (Chapter II)

```r
require(odesolve)
levin<-function(t,x,p){ ## definition of the parameters
        C0<-p["C0"]             #µg/ml
        D<-p["D"]               #1/h
        ep<-p["ep"]             #reciprocal yield prey 1
        ep2<-p["ep2"]           #reciprocal yield prey 2
        mumax<-p["mumax"]       # maximal growth rate of prey 1
        Ks<-p["Ks"]             # half saturation constant of prey 1
        be<-p["be"]               # Bacteria needed by the predator for
reproduction
        C<-x[1]                  # [µg/ml]
        N<-x[2]                  # [Ind/ml]
        N2<-x[3]                 # [Ind/ml]
        P<-x[4]                  # [Ind/ml]

        mumaxP<-p["mumaxP"]     # [IndN/(IndP*h)]
        KsP<-p["KsP"]           #half saturation constant of the predator

        #parameters oft he temperature dependent function
        kmax<-p["kmax"]         #maximal growth rate #fit parameter
        b<-p["b"]               #fit parameter
        R1<-p["R1"]             #fit parameter
        Topt<-p["Topt"]         # optimal temperature
        R2<-p["R2"]             #fit parameter

        Ks2<-p["Ks2"]           # half saturation constant of prey 2
        mumaxP2<-p["mumaxP2"]   # [IndN/(IndP*h)]
        KsP2<-p["KsP2"]         #half saturation constant of the predator
        be2<-p["be2"]           # Bacteria needed by the predator for
reproduction


###Temperature dependent function

mumax2<-(kmax*(1 + b*(((R1^(Temp[t+1]-Topt))-1)-
(log(R1)/log(R2))*((R2^(Temp[t+1]-Topt))-1)))))

dC<- (C0-C)*D - (ep*N*mumax*C/(Ks+C)) - (ep2*N2*mumax2*C/(Ks2+C))    #
Nutrients

dN<- (N*mumax*C/(Ks+C)) - P*(mumaxP*N/(KsP+N))- D*N                  #
prey1

dN2<- (N2*mumax2*C/(Ks2+C)) - P*(mumaxP2*N2/(KsP2+N2))- D*N2         # prey
2

dP<-  be*P*(mumaxP*N/(KsP+N)) + be2*P*(mumaxP2*N2/(KsP2+N2)) - D*P   #
Predator

list(c(dC,dN,dN2,dP))
        }

### Transformation µgC into individuals
### Acinetobacter: 1.241*10^-7    µgC
### Pedobacter: 5.72* 10^-8       µgC
### Tetrahymena: 6.5507*10^-3     µgC
```

Supplementary material

```r
dt<-1
times<-seq(0,50000,dt)

# Temperature vector: length(Temp) = length(times)
Temp1<-rep(20,times=25000)
Temp2<-rep(30,times=25001)
Temp<-c(Temp1,Temp2)

##Parameter values
parms<-c( C0=3  *0.4,
        D=0.4            /24,
        ep=2*10^-7      *(0.4/(1.241*10^-7)),
        ep2=2*10^-6    *(0.4/(5.72* 10^-8)),
        be=1/4000      *((6.5507*10^-3)/(1.241*10^-7)),
        be2=1/4000     *((6.5507*10^-3)/(5.72* 10^-8 )),
        mumax=0.18,
        Ks=0.00274      *0.4,
        kmax = 0.93,
        Topt =29.0,
        b = 7.3,
        R1 = 1.12,
        R2 = 1.15,

        Ks2=0.002      *0.4,
        mumaxP=150     *(1.241*10^-7)/(6.5507*10^-3),
        KsP=422000     *1.241*10^-5,
        mumaxP2=500     *((5.72* 10^-8 )/(6.5507*10^-3)) ,
        KsP2=400000    *5.72* 10^-8
        )

##Initial values  ##Graphics
xstart<-c(C=3      *0.4,
        N=1000000  *1.241*10^-7,
        N2=1000000 *5.72* 10^-8 ,
        P=200    *6.5507*10^-3
        )

##Graphics
n=10

par(mfrow=c(n/2,2))

Dseq<-data.frame(D=seq(0.2 /24, 0.9 /24,length=n))
for (i in 1:n){
        parms["D"]<-Dseq$D[i]
        out<-as.data.frame(lsoda(xstart,times,levin,parms,hmax=0.3))
        plot(times/24,log(out$N,10),type="l",ylim=c(-
5,2),main=Dseq$D[i]*24)
        lines(times/24,log(out$N2,10),col="orange")
        lines(times/24,log(out$P,10),col="red")
        lines(times/24,log(out$C,10),col="#4AA02C")
        }

outlevin<-as.data.frame(lsoda(xstart,times,levin,parms))

require(rgl)
plot3d(outlevin$N,outlevin$N2,outlevin$P,type="l",xlab="N",ylab="R",zlab="P
",col=rainbow(length(times)))
```

Commented R-script for the creation of the bifurcation diagram in Chapter III

```r
require(odesolve)

levin<-function(t,x,p){

        # parameters:
        C0<-p["C0"]            # reservoir concentration of nutrients
        D<-p["D"]              # dilution rate
        ep<-p["ep"]            # reciprocal yield for Acinetobacter
        ep2<-p["ep2"]          # reciprocal yield for Pedobacter
        mumax<-p["mumax"]      # maximum growth rate for Ac.
        Ks<-p["Ks"]            # half saturation constant for Ac.
        be<-p["be"]            # yield for Tetra. on Ac.
        mumaxP<-p["mumaxP"]    # maximum feeding rate for Tetra. on Ac.
        KsP<-p["KsP"]          # half saturation constant for Tetra feeding on
Ac.
        mumax2<-p["mumax2"]    # maximum feeding rate for Tetra. on Ac.
        Ks2<-p["Ks2"]          # half saturation for Pedo.
        mumaxP2<-p["mumaxP2"]  # maximum feeding rate for Tetra. on Pedo.
        KsP2<-p["KsP2"]        # half saturation constant for Tetra feeding on
Pedo.
        be2<-p["be2"]          # yield for Tetra. on Pedo.

        # state variables:

        C<-x[1]                # nutrients
        N<-x[2]                # Ac.
        N2<-x[3]               # Pedo.
        P<-x[4]                # Tetra.

        # differential equations

        dC<- (C0-C)*D - (ep*N*mumax*C/(Ks+C)) - (ep2*N2*mumax2*C/(Ks2+C))
        dN<- (N*mumax*C/(Ks+C)) - P*(mumaxP*N/(KsP+N))- D*N
        dN2<- (N2*mumax2*C/(Ks2+C)) - P*(mumaxP2*N2/(KsP2+N2))- D*N2
        dP<-  be*P*(mumaxP*N/(KsP+N)) + be2*P*(mumaxP2*N2/(KsP2+N2)) - D*P
        list(c(dC,dN,dN2,dP))
        }

### Transformation µgC into individuals
### Acinetobacter: 1.241*10^-7     µgC
### Pedobacter: 6.65* 10^-8        µgC
### Tetrahymena: 6.5507*10^-3      µgC

dt<-1
times<-seq(0,10000,dt)
parms<-c( C0=3  *0.4,              # parameter-values with conversion factors
        D=0.5           /24,
        ep=2*10^-6      *(0.4/(1.241*10^-7)),
        ep2=2*10^-6     *(0.4/(6.65* 10^-8)),
        be=1/4000       *((6.5507*10^-3)/(1.241*10^-7)),
        be2=1/4000      *((6.5507*10^-3)/(6.65* 10^-8)),
        mumax=0.15,
        Ks=0.0274       *0.4,
        mumax2=0.172,
        Ks2=0.002       *0.4,
        mumaxP=150      *(1.241*10^-7)/(6.5507*10^-3),
        KsP=422000      *1.241*10^-7,
        mumaxP2=400      *((6.65* 10^-8)/(6.5507*10^-3)) ,
        KsP2=400000     *6.65* 10^-8
        )
```

```
xstart<-c(C=0        *0.4,              # Initial conditions with conversion
factors
          N=100000  *1.241*10^-7,
          N2=100000 *6.65* 10^-8,
          P=500     *6.5507*10^-3
          )

peaks <- function(x) {                  # selection of local maxima and minima
          l <- length(x)
          xm1 <- c(x[-1], x[l])
          xp1 <- c(x[1], x[-l])
          x[x > xm1 & x > xp1 | x < xm1 & x < xp1]
          }

Dmin<-0.6/24                    # range of analysed dilution rates
Dmax<-1.2/24

# create an empty plot:

plot(0,0, xlim=c(Dmin*24,Dmax*24),
      ylim=c(0,0.16), type="n", xlab="D [1/d]", ylab="N")

# repeated model solutions for dilution rates between Dmin and Dmax
# and add local maxima and minima to the plot:

for (D in seq(Dmin,Dmax,0.0001)){
        parms["D"] <- D
        out <- as.data.frame(lsoda(xstart,times,levin,parms))
        l <- length(out$N) %/% 4
        out <- out[(1*l):(4*l),]
        p <- peaks(out$N)
        l <- length(out$N)
        xstart <- c(C=out$C[l], N=out$N[l], N2=out$N2[l],P=out$P[l])
        points(rep(D*24, length(p)), p, pch=".")
        }
```

Abstract

Ecosystems are set to extrinsic drivers like climate parameters. These are known to show non-linear dynamics and potential chaotic behaviour. One of the most important drivers is temperature; it influences a large variety of ecological processes (e.g. growth rate and other metabolic rates). On the other hand, populations show density dependent, intrinsic, non-linear dynamics including complex, irregular patterns. The interactions between extrinsic and intrinsic dynamic behaviour are difficult to determine in natural ecosystems and have been discussed in literature.

The assessment of the consequences derived from climate change represents a great challenge for ecologist. Deeper knowledge on the mechanisms driving temperature effects on natural food webs is needed. In this work I investigated a well defined simplified microbial food web consisting of two prey bacteria (*Pedobacter sp.* and *Acinetobacter johnsonii*) and one predator ciliate (*Tetrahymena pyriformis*). This simple food web permits the study of intrinsic dynamics as well as the influence of extrinsic disturbances.

The experimental setup developed by my colleagues and me, consisted of chemostats where parameters like the flow rate were computer controlled, so external noise was reduced to the minimum. Experimental parameters could be determined with great precision, and therefore the dynamic behaviour showed by the experiments is considered to be purely intrinsic.

A mathematical model based on experimental data was developed with the aim of analyzing the temperature scenario investigated experimentally. The model included temperature dependent growth rate functions that were fitted to experimental data. Although the model did not capture the whole complexity of the

food web, reflected some qualitative temperature effects observed experimentally.

The bacterium *Acinetobacter johnsonii* showed grazing-resistant morphologies. I hypothesised that this morphological plasticity was responsible for part of the irregular fluctuations of the abundances observed in the chemostat experiments. In cooperation with David Heckman I developed a mathematical model with the aim of testing this hypothesis at a theoretical level. Numerical analysis showed a stabilization of the food web represented by two characteristics: the possibility of coexistence for a wider range of external parameters, and the absence of chaotic fluctuations.

I was able to show for the first time experimentally, that changes on extrinsic temperatures may shift population dynamics to different attractors depending on the specific temperature response of populations. I analyzed the impact of a temperature increase from 20°C to 25°C. The results presented here suggest that the ecological responses to temperature can affect the dynamic behaviour in food webs.

Zusammenfassung

OEkosysteme werden durch extrinsische Faktoren wie klimatische Parameter beeinflusst. Solche Faktoren zeigen nicht-lineare Dynamiken und ein potentiell chaotisches Verhalten. Einer der wichtigsten Klimafaktoren stellt die Temperatur dar, welche viele verschiedene oekologische Prozesse (z.B. Wachstumsrate und andere metabolische Prozesse beeinflusst. Andererseits zeigen Populationen dichteabhaengig- intrinsische, nicht-lineare Dynamiken die manchmal komplexe, irregulaere Muster annehmen koennen. Die Interaktionen zwischen extrinsisch- und intrinsischen dynamischem Verhalten sind schwer zu bestimmen und sind fuer natuerliche OEkosysteme in der wissenschaftlichen Literatur oft diskutiert worden.

Das Abschaetzen der Konsequenzen des Klimawandels ist eine Herausforderung fuer die OEkologen. Tiefere Kenntnisse ueber die Mechanismen, die die Temperatureffekte in natuerlichen Nahrungsnetzen steuern sind noch nicht vorhanden. In dieser Arbeit praesentiere ich ein von mit untersuchtes, vereinfachtes und streng definiertes Nahrungsnetz. Dieses Nahrungsnetz besteht aus zwei Beutebakterien (*Pedobacter sp.* und *Acinetobacter johnsonii*) und einem raeuberischen Cilliat (*Tetrahymena pyriformis*). Solch ein vereinfachtes Nahrungsnetzt erlaubt die Untersuchung der intrinsischen Dynamiken und den Einfluss extrinsischer Stoerungen auf das System.

Der experimentelle Aufbau den meine Kollegen und ich entwickelt haben, besteht aus einem Chemostatsystem, welches durch komplett automatisierte Parametersteuerung erlaubt, dass externe Rauschen zu einem Minimum zu reduziert. Die experimentellen Parameter konnten mit hoher Praezision bestimmt werden, sodass das dynamische Verhalten, welches in den

Experimente gefunden wurde als rein intrinsisch betrachtet werden kann.

Ein experimentbasiertes Model wurde entwickelt, um das Temperaturszenario, welches der experimentell untersucht wurde zu analysieren. Das Model beinhaltet temperaturabhaengige Wachstumsratefunktionen, die auf experimentellen Daten basieren und an die verwendeten mathematischen Gleichungen angepasst wurden. Das Model konnte nicht die ganze Komplexitaet des System erfassen, nichts desto trotz konnte es jedoch einige qualitative Effekte der Temperatur zeigen beziehungsweise nachvollziehbar machen.

Das Bakterium *Acinetobacter johnsonii* zeigte fraßresistente Morphologien. Ich habe die Hypothese erstellt dass, die morphologische Plastizitaet verantwortlich war fuer die irregulaeren Schwankungen, welche ich in den Chemostatversuchen beobachten koennte. In Zusammenarbeit mit David Heckman habe ich ein mathematisches Populationsmodel erstellt, mit dem Ziel dieser Hypothese theoretisch nachzugehen. Numerische Analysen des Models zeigten eine Stabilisierung des Nahrungsnetzes hinsichtlich zweier Sachverhalte: Eine Koexistenz war fuer eine breitere Spanne verschiendener Parameterwerte moeglich und chaotische Schwankungen wurden gedaempft beziehungsweise nicht mehr zu beobachten.

Folglich konnte ich erstmals experimentell zeigen, dass die untersuchten Temperaturszenarien (als extrinsischer Faktor) die Populationsdynamiken zu einem anderen Attraktorgebiet im Phasenraum verschieben koennen und dass diese Verschiebung der einzelnen Arten von deren (intrinsischen) artspezifischen Temperaturreaktion abhaengig ist. In den experimentellen Systemen habe ich den Effekt einer kontinuierlichen Temperaturerhoehung von

20 bis 25°C untersucht. Die hier praesentierten Ergebnisse deuten an, dass die oekologische Reaktion bezueglich der Temperatur das dynamische Verhalten eines Nahrungsnetzes veraendern kann.

Koeln, den 09. Dezember 2010

Erklaerung

Ich versichere, dass ich die von mir vorgelegte Dissertation selbstaendig angefertigt, die benutzten Quellen und Hilfsmittel vollstaendig angegeben und die Stellen der Arbeit - einschließlich Abbildungen und Tabellen - die anderen Werken im Wortlaut oder dem Sinn nach entnommen habe, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultaet oder Universitaet zur Pruefung vorgelegen hat; dass sie noch nicht veroeffentlicht worden ist sowie, dass ich solche Veroeffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde.

Die Bestimmungen dieser Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Prof. Dr. Hartmut Arndt betreut worden.

_____

Mar Monsonís Nomdedeu

Curriculum vitae

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