The interplay of intrinsic and extrinsic parameters on the dynamic behaviour of a microbial food web

Inaugural-Dissertation

zur

Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultät

der Universität zu Köln

vorgelegt von

Christine Willen

aus Wildeshausen

Köln 2010

Berichterstatter: Prof. Dr. Hartmut Arndt

Prof. Dr. Eric von Elert

Tag der mündlichen Prüfung: 18. November 2010

Danksagung

Bei Herrn Professor Doktor Hartmut Arndt bedanke ich mich herzlich für die Bereitstellung des Themas und für die Möglichkeit zur Promotion.

Innerhalb der Graduate School of Biological Sciences danke ich dem Thesis Committee bestehend aus: Professor Doktor Hartmut Arndt, Professor Doktor Eric von Elert und Professor Doktor Horst Malchow, für die kritischen Auseinandersetzungen zum Thema und für zusätzliche Anregungen hinsichtlich der experimentellen Untersuchungen.

Ich bedanke mich bei der gesamten AG Arndt für ihre Hilfe und Unterstützung.

Meinen direkten Arbeitskollegen Mar Monsonís Nomdedeu und Andre Schieffer danke ich für die fachliche Unterstützung und für die schöne Zeit zusammen in der Arbeitsgruppe.

Ich danke Nicole Nopper für Rechtschreibkorrekturen in der vorliegenden Arbeit.

Ein ganz besonderer Dank geht an meine Eltern und an meine Geschwister, da sie mir Rückhalt bieten.

Content

Introduction

The interplay of intrinsic and extrinsic parameters on the dynamic behaviour of a microbial food web

Background

Ecologists study the dynamic behaviour of organisms in order to understand their underlying driving forces (Turchin 2003). The interpretation of the observed time series data for their biological implications is a fundamental issue in ecology. Population dynamics are triggered by intrinsic and extrinsic parameters. Extrinsic parameter can be distinguished in biotic factors and abiotic factors. Abiotic factors include amongst others temperature, light and habitat structure. The most important biotic factors are for example competition for the present resources (nutrients and space) and predator-prey dynamics (Tollrian 1999; Persson *et al.* 2001). Population ecologists collect time series data in the field of single species or of key stone species within food webs in order to understand the underlying mechanisms of coexistence in the trophic cascades (Turchin 1995; Giovannoni & Stingl 2005; Corno *et al.* 2008; van der Stap *et al.* 2008).

However, for ecologists it is rather difficult to distinguish density depended and therefore purely intrinsic population dynamics from the influences of environmental factors (Kaitala *et al.* 1997; Upadhyay & Rai 1997; Bjornstad & Grenfell 2001). Experiments in the field are faced with randomness which can alter population dynamics like unfavourable weather phenomena or unforeseen differences in the nutrient availability. Furthermore, they have to take in account short-term and long-term effects (for example daily temperature changes and seasonal temperature changes) on time series data (Hastings 2004; Rohani *et al.* 2004; Vasseur & Yodzis 2004). Therefore, it may be very difficult to understand the underlying mechanisms of population dynamics from field-data. One possibility to solve this problem are laboratory experiments. Quasi-natural food web compositions serve as model-systems in which a selection of extrinsic parameters like temperature and predation can be studied separately (Cadotte *et al.* 2005).

Several model organisms have already been studied in the lab for example invertebrates, protists and bacteria (Costantino *et al.* 1997; Miramontes & Rohani 1998; Hahn & Höfle 1999; Laakso *et al.* 2003; van der Stap *et al.* 2009). Laboratory food webs consisting of bacteria and protists seem to be a useful tool to study in detail population dynamics because of short generation times and simple cultivation techniques (Jessup *et al.* 2004). Before one can apply any extrinsic parameter, one must study the intrinsic, density dependent population dynamics. Such laboratory experiments revealed different intrinsic population dynamics over time like stable equilibrium, predator-prey cycles or chaos (Fussmann *et al.* 2000; Beninca *et al.* 2008).

In natural habitats microbial food webs consisting of bacteria, algae and protozoans play an important role in the trophic cascade (e.g. within the microbial loop) of aquatic ecosystems like rivers and lakes (Azam *et al.* 1983; Weisse *et al.* 1990; Weitere & Arndt 2003; Weitere *et al.* 2005). Protozoans for example heterotrophic flagellates and ciliates have a broad impact on the bacterial community structure (Beaver & Crisman 1989a; Boenigk & Arndt 2002; Wey *et al.* 2008). Ciliates can constitute an important component of plankton in freshwater ecosystems depending on the lake trophic status (Simek *et al.* 1994). In mesothrophic and eutrophic lakes the ciliate abundance can range from 70 cells ml^{-1} up to 150 cells ml^{-1} (Beaver & Crisman 1989b). Bacterivory by pelagic ciliates is considered to be important in freshwater when bacterial abundances reach more than 5 x 10^6 ml^{-1} (Simek *et al.* 1994). Concerning microbial aquatic food webs protists are seen as a major source of mortality for both heterotrophic and autotrophic bacteria as they are ubiquitous and abundant in all types of habitats (Sherr & Sherr 2002). The protist *Tetrahymena pyriformis* is ubiquitous and abundant in several freshwater habitats and served as a model organism, grazing on two different bacterial strains in the present laboratory system.

The experimental setup

A simplified predator-prey system was designed not to mimic natural conditions but to examine in detail the impact of intrinsic and extrinsic parameters on the population dynamics over time. Therefore, my colleagues and me established a highly controllable and to the greatest possible extent automated experimental

setup for chemostat experiments (Fig. 1). External inaccuracies like fluctuations in the dilution rate (peristaltic pumps were replaced by more accurate syringe pumps (Supplementary Material Fig.1)), unintended temperature fluctuations and the risk of contaminations with other bacteria or fungi could be minimized due to an automated sampling robot. The first aim of the present work was to study purely intrinsic driven population dynamics at two trophic levels separately. The microbial model system consisted of three species, two different bacterial strains and a protist predator.

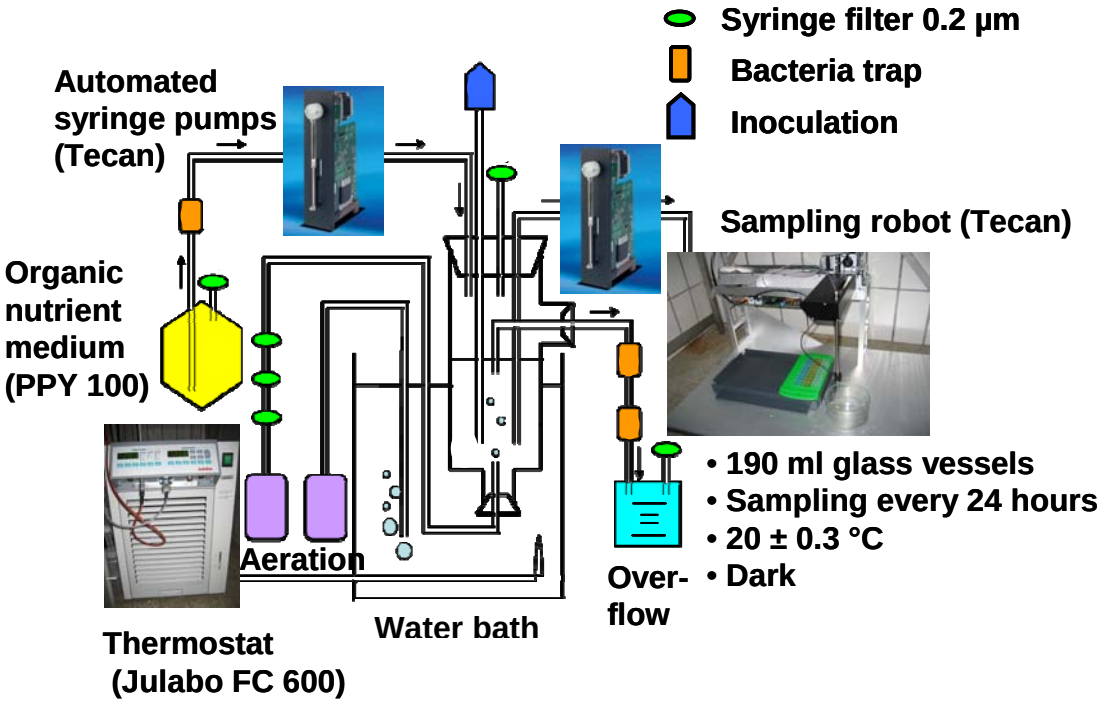

Fig. 1. Schematic setup for chemostat experiments.

In previous works (Becks *et al.* 2005) the two bacterial preys consisted of *Brevundimonas* sp. (alpha-proteobacteria, 1.5 x 1.5 µm, morphological stable) and *Pedobacter sp.* (cytophaga-flexibacter group, 1 x 2 µm, morphological stable, Fig. 2 B and B`). Grazing by *Tetrahymena* led to different predator-prey population dynamics like stable limit cycles, stable equilibrium and chaotic dynamics using the dilution rate as bifurcation parameter (the established dilution rates were 0.45, 0.5 and 0.75 per day). Coexistence of all three species (in a certain range of dilution rate per day between 0.1 and 0.9) was possible because *Brevundimonas* was the inferior competitor and the less preferred

prey. The observed dynamics at the different flow rates had been previously predicted by a simplified mathematical model (Takeuchi & Adachi 1983).

Chemostat experiments and model analyses

In the present work the less preferred bacterial prey, *Brevundimonas sp.* was replaced by *Acinetobacter johnsonii* (gamma-proteobacteria, Fig. 2 A and A`) to investigate a more realistic system including morphological changes of prey organisms in response to predation as it is common in the field (Hahn & Höfle 2001; Pernthaler 2005). We included *Acinetobacter* which is able to form different cell sizes with volumes ranging from 3 – 30 µm^3 (single cells as well as chains of different lengths) without any external trigger in continuous culture as well as in over night grown batch cultures.

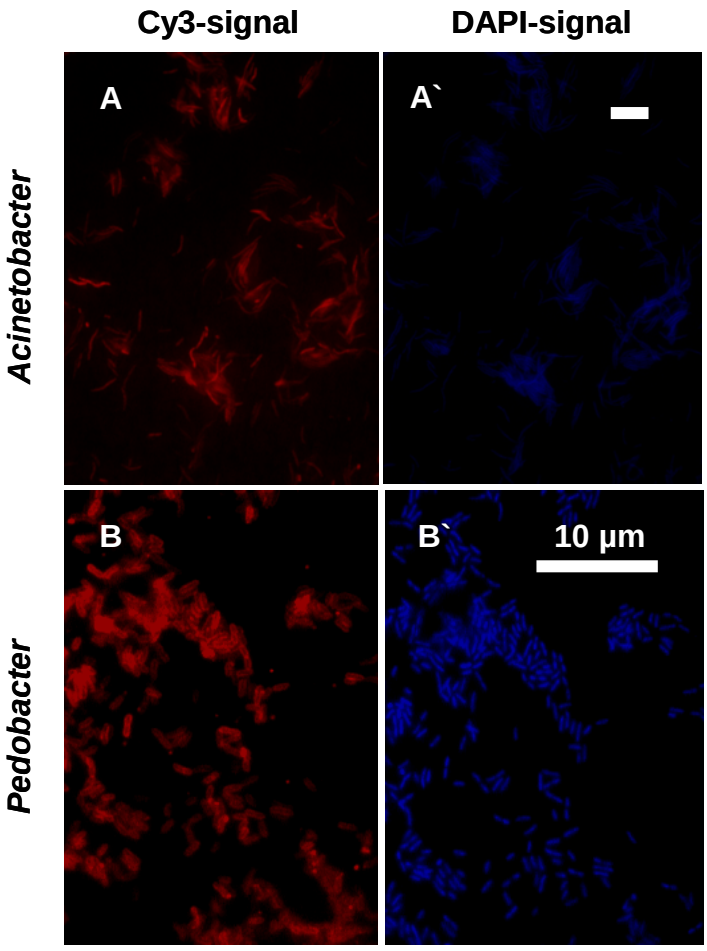

Fig. 2. Specifically labelled cells of *Acinetobacter* (A: Cy3-CARD-FISH-signal and A` unspecific DAPI counterstain) and *Pedobacter* (B: Cy3-antibody-signal and B` unspecific DAPI counterstain) from over night grown stock cultures. Scale bars indicate 10 µm.

The second hypothesis of the present work was, if the morphology of *Acinetobacter* changes towards ciliate grazing. Single species and two-species predator-prey long-term chemostat experiments as well as short-term batch experiments were performed in order to study if there was a detectable shift towards larger, inedible growth forms. David Heckmann, a bachelor candidate of our working group, performed in 2009 corresponding model-analyses based on (Bohannan & Lenski 1999). He examined the influences of inducible defence mechanisms of one bacterial strain in a three species predator-prey model. His findings showed that predator triggered grazing resistance results in an increase of the range of dilution rates which allow coexistence of all three species (dilution rates of 0.1-1.3, Supplementary Material Fig. 2A) in comparison to a three species model without phenotypic plasticity with coexistence for a narrow range of dilution rates of 0.6-1.0, (Supplement Material Fig. 2B). Based on the model predictions of David Heckmann, chemostat experiments were performed in the present study in order to test the hypothesis, if the introduction of a morphological heterogeneous strain like *Acinetobacter* results in a higher range of coexistence in a three species microbial food web, concerning dilution rates in comparison to previous experiments performed by (Becks *et al.* 2005).

Temperature effects

Beside changes in the nutrient availability (dilution rate), predator-prey interactions are also triggered by temperature (Weisse *et al.* 2002; Gächter & Weisse 2006; Viergutz *et al.* 2007). Temperature is one of the most important extrinsic factors influencing the overall intrinsic biological processes like metabolic growth, respiration, ingestion, reproduction (Laybourn & Finlay 1976; Gillooly *et al.* 2001; Savage *et al.* 2004; Weitere *et al.* 2009). The influence of temperature gained more and more attention ever since the awareness of the global climate change (IPCC 2007). The response to global warming in ecosystems is a complex interplay of individuals and their temperature dependent reaction-norms. The study of temperature mediated changes in the trophic interaction modes revealed for example unexpected or harmful changes (Winder & Schindler 2004; Jiang & Morin 2007). Almost every kind of interaction ranging from the feeding behaviours, competitive relationships and predator

attacks react sensitively towards changing temperatures (McLaughlin *et al.* 2002; Norf *et al.* 2007; Viergutz *et al.* 2007). In the field, it is almost impossible to separate the impact of temperature from other internal or external triggers. In the background of climate change, it is of rising interest to which degree this extrinsic factor alters intrinsic population dynamics. Temperature fluctuations are very common in natural systems from daily temperature fluctuations on small scale changes to large scale changes due to e.g. seasonal shifts. This applies in particular to shallow aquatic environments like lakes, where stratification is normally absent and temperature refuges are minimal (McKee *et al.* 2002). Temperature may fluctuate in the littoral zone up to 10°C within one day (Montagnes & Weisse 2000), differences of up to 15°C may occur within one month. In the present study daily changing temperatures were fluctuating chaotically around 16.7 and 29.7°C in a course of 30 days. Chemostat experiments as well as corresponding model analyses should disentangle the impact of temperature fluctuations of the present three species microbial food web.

Summing up

In the present study the microbial food web consisted of three species (Fig. 3): a bacterivorous ciliate *Tetrahymena pyriformis* and two bacterial organisms *Acinetobacter johnsonii* and *Pedobacter sp*. In the first Chapter, highly controllable chemostat experiments were performed in order to study purely intrinsic driven population dynamics of the microbial food web at constant external parameters. The dynamical behaviour in each trophic level from single species to three species predator-prey interactions were analysed in detail. Beside the quantitative analyses, qualitative studies of the morphological heterogeneous strain *Acinetobacter* were carried out to test the hypothesis if grazing by *Tetrahymena* results in a shift of morphotypes towards larger growth forms (Chapter II). With this background and according to model predictions, the experimental microbial food web was analysed at two different external parameters in Chapter three. At first, the dilution rate was used as bifurcation parameter to test the hypothesis if the coexistence of all three species is enlarged when one bacterial strain exhibited phenotypic plasticity. Second,

chaotic temperature fluctuations were applied as an extrinsic trigger to study whether intrinsic population dynamics could be changed.

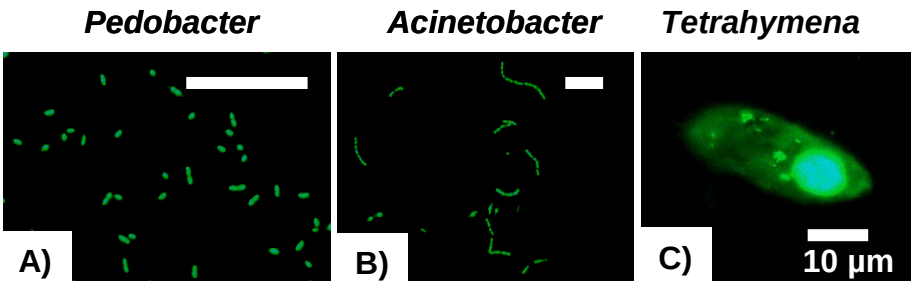

Fig. 3. Photographs of CYBR-Green stained cells of the three species microbial food web. Scale bars indicate 10 µm in all pictures.

References

Azam F., Fenchel T., Field J.G., Gray J.S., Meyerreil L.A. & Thingstad F. (1983). THE ECOLOGICAL ROLE OF WATER-COLUMN MICROBES IN THE SEA. *Marine Ecology-Progress Series*, 10, 257-263.

Beaver J.R. & Crisman T.L. (1989a). ANALYSIS OF THE COMMUNITY STRUCTURE OF PLANKTONIC CILIATED PROTOZOA RELATIVE TO TROPHIC STATE IN FLORIDA LAKES. *Hydrobiologia*, 174, 177-184.

Beaver J.R. & Crisman T.L. (1989b). THE ROLE OF CILIATED PROTOZOA IN PELAGIC FRESH-WATER ECOSYSTEMS. *Microbial Ecology*, 17, 111-136.

Becks L., Hilker F.M., Malchow H., Jurgens K. & Arndt H. (2005). EXPERIMENTAL DEMONSTRATION OF CHAOS IN A MICROBIAL FOOD WEB. *Nature*, 435, 1226-1229.

Beninca E., Huisman J., Heerkloss R., Johnk K.D., Branco P., Van Nes E.H., Scheffer M. & Ellner S.P. (2008). CHAOS IN A LONG-TERM EXPERIMENT WITH A PLANKTON COMMUNITY. *Nature*, 451, 822-U7.

Bjornstad O.N. & Grenfell B.T. (2001). NOISY CLOCKWORK: TIME SERIES ANALYSIS OF POPULATION FLUCTUATIONS IN ANIMALS. *Science*, 293, 638-643.

Boenigk J. & Arndt H. (2002). BACTERIVORY BY HETEROTROPHIC FLAGELLATES: COMMUNITY STRUCTURE AND FEEDING STRATEGIES. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology*, 81, 465-480.

Bohannan B.J.M. & Lenski R.E. (1999). EFFECT OF PREY HETEROGENEITY ON THE RESPONSE OF A MODEL FOOD CHAIN TO RESOURCE ENRICHMENT. *American Naturalist*, 153, 73-82.

Cadotte M.W., Drake J.A. & Fukami T. (2005). CONSTRUCTING NATURE: LABORATORY MODELS AS NECESSARY TOOLS FOR INVESTIGATING COMPLEX ECOLOGICAL COMMUNITIES. *Advances in Ecological Research, Vol. 37: Population Dynamics and Laboratory Ecology*, 37, 333-353.

Corno G., Caravati E., Callieri C. & Bertoni R. (2008). EFFECTS OF PREDATION PRESSURE ON BACTERIAL ABUNDANCE, DIVERSITY,

AND SIZE-STRUCTURE DISTRIBUTION IN AN OLIGOTROPHIC SYSTEM. *Journal of Limnology*, 67, 107-119.

Costantino R.F., Desharnais R.A., Cushing J.M. & Dennis B. (1997). CHAOTIC DYNAMICS IN AN INSECT POPULATION. *Science*, 275, 389-391.

Ellner S.P. & Turchin P. (2005). WHEN CAN NOISE INDUCE CHAOS AND WHY DOES IT MATTER: A CRITIQUE. *Oikos*, 111, 620-631.

Fussmann G.F., Ellner S.P., Shertzer K.W. & Hairston N.G. (2000). CROSSING THE HOPF BIFURCATION IN A LIVE PREDATOR-PREY SYSTEM. *Science*, 290, 1358-1360.

Gächter E. & Weisse T. (2006). LOCAL ADAPTATION AMONG GEOGRAPHICALLY DISTANT CLONES OF THE COSMOPOLITAN FRESHWATER CILIATE MESERES CORLISSI. I. TEMPERATURE RESPONSE. *Aquatic Microbial Ecology*, 45, 291-300.

Gillooly J.F., Brown J.H., West G.B., Savage V.M. & Charnov E.L. (2001). EFFECTS OF SIZE AND TEMPERATURE ON METABOLIC RATE. *Science*, 293, 2248-2251.

Giovannoni S.J. & Stingl U. (2005). MOLECULAR DIVERSITY AND ECOLOGY OF MICROBIAL PLANKTON. *Nature*, 437, 343-348.

Hahn M.W. & Höfle M.G. (1999). FLAGELLATE PREDATION ON A BACTERIAL MODEL COMMUNITY: INTERPLAY OF SIZE-SELECTIVE GRAZING, SPECIFIC BACTERIAL CELL SIZE, AND BACTERIAL COMMUNITY COMPOSITION. *Applied and Environmental Microbiology*, 65, 4863-4872.

Hahn M.W. & Höfle M.G. (2001). GRAZING OF PROTOZOA AND ITS EFFECT ON POPULATIONS OF AQUATIC BACTERIA. *Fems Microbiology Ecology*, 35, 113-121.

Hastings A. (2004). TRANSIENTS: THE KEY TO LONG-TERM ECOLOGICAL UNDERSTANDING? *Trends in Ecology & Evolution*, 19, 39-45.

IPCC (2007). FOURTH ASSESSMENT REPORT OF THE INTERGOVERNMENTAL PANEL ON CLIMATE CHANGE. IPCC (WG I & II). Cambrige University Press, Cambridge.

Jessup C.M., Kassen R., Forde S.E., Kerr B., Buckling A., Rainey P.B. & Bohannan B.J.M. (2004). BIG QUESTIONS, SMALL WORLDS:

MICROBIAL MODEL SYSTEMS IN ECOLOGY. *Trends in Ecology & Evolution*, 19, 189-197.

Jiang L. & Morin P.J. (2007). TEMPERATURE FLUCTUATION FACILITATES COEXISTENCE OF COMPETING SPECIES IN EXPERIMENTAL MICROBIAL COMMUNITIES. *Journal of Animal Ecology*, 76, 660-668.

Kaitala V., Ylikarjula J., Ranta E. & Lundberg P. (1997). POPULATION DYNAMICS AND THE COLOUR OF ENVIRONMENTAL NOISE. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 264, 943-948.

Laakso J., Loytynoja K. & Kaitala V. (2003). ENVIRONMENTAL NOISE AND POPULATION DYNAMICS OF THE CILIATED PROTOZOA TETRAHYMENA THERMOPHILA IN AQUATIC MICROCOSMS. *Oikos*, 102, 663-671.

Laybourn J. & Finlay B.J. (1976). RESPIRATORY ENERGY-LOSSES RELATED TO CELL WEIGHT AND TEMPERATURE IN CILIATED PROTOZOA. *Oecologia*, 24, 349-355.

McKee D., Atkinson D., Collings S., Eaton J., Harvey I., Heyes T., Hatton K., Wilson D. & Moss B. (2002). MACRO-ZOOPLANKTER RESPONSES TO SIMULATED CLIMATE WARMING IN EXPERIMENTAL FRESHWATER MICROCOSMS. *Freshw. Biol.*, 47, 1557-1570.

McLaughlin J.F., Hellmann J.J., Boggs C.L. & Ehrlich P.R. (2002). CLIMATE CHANGE HASTENS POPULATION EXTINCTIONS. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 6070-6074.

Miramontes O. & Rohani P. (1998). INTRINSICALLY GENERATED COLOURED NOISE IN LABORATORY INSECT POPULATIONS. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 265, 785-792.

Montagnes D.J.S. & Weisse T. (2000). FLUCTUATING TEMPERATURES AFFECT GROWTH AND PRODUCTION RATES OF PLANKTONIC CILIATES. *Aquatic Microbial Ecology*, 21, 97-102.

Norf H., Arndt H. & Weitere M. (2007). IMPACT OF LOCAL TEMPERATURE INCREASE ON THE EARLY DEVELOPMENT OF BIOFILM-ASSOCIATED CILIATE COMMUNITIES. *Oecologia*, 151, 341-350.

Pernthaler J. (2005). PREDATION ON PROKARYOTES IN THE WATER COLUMN AND ITS ECOLOGICAL IMPLICATIONS. *Nature Reviews Microbiology*, 3, 537-546.

Rohani P., Miramontes O. & Keeling M.J. (2004). THE COLOUR OF NOISE IN SHORT ECOLOGICAL TIME SERIES DATA. *Mathematical Medicine and Biology-a Journal of the Ima*, 21, 63-72.

Savage V.M., Gillooly J.F., Brown J.H., West G.B. & Charnov E.L. (2004). EFFECTS OF BODY SIZE AND TEMPERATURE ON POPULATION GROWTH. *American Naturalist*, 163, 429-441.

Sherr E.B. & Sherr B.F. (2002). SIGNIFICANCE OF PREDATION BY PROTISTS IN AQUATIC MICROBIAL FOOD WEBS. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology*, 81, 293-308.

Simek K., Vrba J. & Hartman P. (1994). SIZE-SELECTIVE FEEDING BY CYCLIDIUM SP ON BACTERIOPLANKTON AND VARIOUS SIZES OF CULTURED BACTERIA. *Fems Microbiology Ecology*, 14, 157-167.

Takeuchi, Y., & N. Aadachi. 1983. EXISTENCE AND BIFURCATION OF STABLE EQUILIBRIUM IN TWO-PREY-ONE-PREDATOR COMMUNITIES. *Bulletin of Mathematical Biology*, 45, 877-900.

Tollrian R. (1999). THE ECOLOGY AND EVOLUTION OF INDUCIBLE DEFENSES. *Princeton University Press, Princeton.*

Turchin P. (2003). COMPLEX POPULATION DYNAMICS: A THEORTHICAL/EMPIRICAL SYNTHESIS. *Princeton University Press, Princeton.*

Turchin P. (1995). CHAOS IN MICROTINE POPULATIONS. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 262, 357-361.

Upadhyay R.K. & Rai V.K. (1997). WHY CHAOS IS RARELY OBSERVED IN NATURAL POPULATIONS. *Chaos Solitons & Fractals*, 8, 1933-1939.

van der Stap I., Vos M., Kooi B.W., Mulling B.T.M., van Donk E. & Mooij W.M. (2009). ALGAL DEFENSES, POPULATION STABILITY, AND THE RISK OF HERBIVORE EXTINCTIONS: A CHEMOSTAT MODEL AND EXPERIMENT. *Ecological Research*, 24, 1145-1153.

van der Stap I., Vos M., Tollrian R. & Mooij W.M. (2008). INDUCIBLE DEFENSES, COMPETITION AND SHARED PREDATION IN PLANKTONIC FOOD CHAINS. *Oecologia*, 157, 697-705.

Vasseur D.A. & Yodzis P. (2004). THE COLOR OF ENVIRONMENTAL NOISE. *Ecology*, 85, 1146-1152.

Viergutz C., Kathol M., Norf H., Arndt H. & Weitere M. (2007). CONTROL OF MICROBIAL COMMUNITIES BY THE MACROFAUNA: A SENSITIVE INTERACTION IN THE CONTEXT OF EXTREME SUMMER TEMPERATURES? *Oecologia*, 151, 115-124.

Weisse T., Muller H., Pintocoelho R.M., Schweizer A., Springmann D. & Baldringer G. (1990). RESPONSE OF THE MICROBIAL LOOP TO THE PHYTOPLANKTON SPRING BLOOM IN A LARGE PREALPINE LAKE. *Limnology and Oceanography*, 35, 781-794.

Weisse T., Stadler P., Lindstrom E.S., Kimmance S.A. & Montagnes D.J.S. (2002). INTERACTIVE EFFECT OF TEMPERATURE AND FOOD CONCENTRATION ON GROWTH RATE: A TEST CASE USING THE SMALL FRESHWATER CILIATE *UROTRICHA FARCTA*. *Limnology and Oceanography*, 47, 1447-1455.

Weitere M. & Arndt H. (2003). STRUCTURE OF THE HETEROTROPHIC FLAGELLATE COMMUNITY IN THE WATER COLUMN OF THE RIVER RHINE (GERMANY). *European Journal of Protistology*, 39, 287-300.

Weitere M., Scherwass A., Sieben K.T. & Arndt H. (2005). PLANKTONIC FOOD WEB STRUCTURE AND POTENTIAL CARBON FLOW IN THE LOWER RIVER RHINE WITH A FOCUS ON THE ROLE OF PROTOZOANS. *River Research and Applications*, 21, 535-549.

Weitere M., Vohmann A., Schulz N., Linn C., Dietrich D. & Arndt H. (2009). LINKING ENVIRONMENTAL WARMING TO THE FITNESS OF THE INVASIVE CLAM CORBICULA FLUMINEA. *Global Change Biology*, 15, 2838-2851.

Wey J.K., Scherwass A., Norf H., Arndt H. & Weitere M. (2008). EFFECTS OF PROTOZOAN GRAZING WITHIN RIVER BIOFILMS UNDER SEMI-NATURAL CONDITIONS. *Aquatic Microbial Ecology*, 52, 283-296.

Winder M. & Schindler D.E. (2004). CLIMATE CHANGE UNCOUPLES TROPHIC INTERACTIONS IN AN AQUATIC SYSTEM (vol 85, pg 2100, 2004). *Ecology*, 85, 3178-3178.

Chapter I

Unbalanced variability at different trophic levels in an experimental microbial food web

1. 1 Introduction

Chaotic and therefore unpredictable dynamical behaviours have initially been detected in mathematical models of physical systems like weather forecasts (Lorenz 1963). (May 1974) stated that the simplest nonlinear difference equation for the growth of a single population (with non-overlapping generations) can exhibit stable points, stable cycles as well as chaotic population dynamics over time depending on the intrinsic growth rate.

These findings led to an ongoing and controversial discussion about the importance of chaos in natural ecosystems between theoreticians and biologists (Hastings *et al.* 1993; Tel *et al.* 2004). Mathematical models predict chaotic population dynamics in simple food chains as well as complex food webs (May & Oster 1976; Huisman & Weissing 2001; Fussmann & Heber 2002; Gross *et al.* 2005). For biological experimentalists it is rather difficult to discern deterministic chaos from environmental noise (Kaitala *et al.* 1997; Bjornstad & Grenfell 2001; Vasseur & Yodzis 2004; Ellner & Turchin 2005). Unlike physical systems, experiments in the field are faced with randomness which can alter population dynamics like unfavourable weather phenomena or differences in the nutrient availability. Furthermore, it is impossible to neglect short-term and long-term effects (for example daily temperature changes and seasonal temperature changes) from the time series data (Hastings 2004; Rohani *et al.* 2004). Therefore it seems to be very difficult to detect chaotic population dynamics in field data (Turchin 1995; Upadhyay & Rai 1997).

Fortunately, there is a possibility to combine both controllable external conditions and a quasi-natural food web composition in laboratory systems (Jessup *et al.* 2004; Cadotte *et al.* 2005). Invertebrates, protists and bacteria have already been studied at controllable experimental conditions. Their population densities revealed a broad range of possible dynamics over time from stable equilibrium to stable limit cycles (Tsuchiya *et al.* 1972; Swift *et al.*

1982; McCauley *et al.* 1999; Fussmann *et al.* 2000). Chaos has been detected in an insect population (Costantino *et al.* 1997) in a three species microbial food web (Becks *et al.* 2005), in a nitrifying bacterial community (Graham *et al.* 2007) and in a multispecies and complex plankton community (Beninca *et al.* 2008). In the present study the dynamic behaviour of a microbial food web in one- and two-trophic level systems was studied in a defined chemostat environment. Intra- and interspecific dynamics were analyzed for two bacterial strains; the dynamic behaviour of predator-prey interactions was studied in one-prey-one-predator chemostat systems as well as in two-prey-one-predator systems. Two morphological different bacterial strains, namely *Acinetobacter johnsonii* and *Pedobacter sp.,* and a ciliate predator, *Tetrahymena pyriformis* were used as model organisms.

1. 2 Methods

Experimental setup. The microbial food web experiments were carried out with two different bacterial strains. *Acinetobacter johnsonii* (gamma-proteobacteria, single cells as well as chains of different lengths, ~1 µm x 1.5-30.0 µm in continuous culture without predation, γ-proteobacteria; kindly provided as an isolate by Michael Steinert, Univerity of Braunschweig, Germany) exhibits a heterogeneous morphology. The second bacterium, *Pedobacter sp*. (cytophaga flexibacter group, exclusively single cells, ~1 µm x 2 µm, isolated by Kristin Beck, kindly provided by Klaus Jürgens, IOW, Warnemünde, Germany) has a constant morphology. Both bacterial strains could be grazed by the ciliate *Tetrahymena pyriformis* (strain CCAP 1630/1W; Culture Collection of Algae and Protists, Windermere, U.K., average length and width 41 µm x 22 µm) which was the predator in predator-prey systems. All species were cultivated under monoxenic conditions.

Chemostat experiments were carried out in sterile one-stage glass reactors with an average fluid content of 190 ml. All chemostat experiments were performed with the same experimental setup at the same external conditions, namely temperature (20°C ± 0.3), nutrient supply (proteose peptone, yeast extract, diluted 100-times, PPY 100) and dilution rate (nutrient inflow of 0.75 per day ± 0.004). A complex (organic) nutrient source was chosen in order

to establish a non-selective environment. We wanted to study intrinsic population dynamics which were not influenced by growth limitation or selection pressures like starvation due to the lack of certain minerals or vitamins (Graham *et al.* 2007).

Constant dilution rates were established by automated syringe pumps (Cavro XLP 6000, Modular Syringe Pump, TECAN, Crailsheim, Germany). The sampling was computer controlled and took place every 24 hours by a sampling robot (RSP 9000 Cavro, TECAN, Crailsheim, Germany, triplicate samples, 0.5 ml each). All samples were fixed 1:1 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) and about 0.01 gl^{-1} SDS (sodium dodecyl sulphate, Merck, Darmstadt, Germany) containing 4 % formaldehyde (Merck, Darmstadt, Germany). Samples were stained with the fluorescent dye CYBR Green I (Invitrogen, Karlsruhe, Germany, 1:5000 dilution of original stock with sterile distilled water). Staining and sample preparation was carried out according to the frame spotting method protocol of (Maruyama *et al.* 2004). Both bacterial strains (each at least 900 cells in 15 µl total sample volume) and *Tetrahymena* (all cells in 15 µl sample volume) abundances were enumerated by epifluorescence microscopy (Zeiss Axiophot, Zeiss filter set 43; BP 550/25, FT 570, BP 605/70, 1250-times magnification in case of both bacteria, 125-times magnification for *Tetrahymena* enumeration).

In order to discover any contamination by other bacteria, fungi or viruses, chemostat samples were checked every day, during enumeration. On occasion, plate pouring on agar plates took place during the experiments (LB-agar: 0.1 gl^{-1} tryptone, DIFCO, Michigan, USA; 0.05 gl^{-1} yeast extract; 0.1 gl^{-1} NaCl, Merck, Darmstadt, Germany, 5 gl^{-1} agar Merck, Darmstadt, Germany), Furthermore, stock cultures and chemostat probes were specifically labelled by Catalysed Reporter Deposition Fluorescence in Situ Hybridisation (CARD-FISH) (Schönhuber *et al.* 1997) towards *Acinetobacter* cells and antibody-techniques were applied to identify *Pedobacter* cells.

Calculation of the Lyapunov exponents. The dynamic behaviour of the time series data of the chemostat experiments were characterised by the

corresponding Lyapunov exponent. The Lyapunov exponent (λ) quantifies the exponential separations of initially close trajectories (Eckmann & Ruelle 1985). It was calculated with the TISEAN package (3.0.0 Nonlinear Time Series Analysis 2000; (Hegger *et al.* 1999) using the algorithm of (Rosenstein *et al.* 1993) (embedding dimension: m = 1,…, 6; number of iterations in time: 15). For evaluation of the delay, the autocorrelation function (ACF) and mutual information revealed different reconstruction delays between 1 - 3. Transient data points at the beginning of the experiments were omitted for the analyses. The Lyapunov exponent was estimated from the slope of a strait line fitted to the linear part of the ln-transformed divergence. Here, the values for the Lyapunov exponents are given for the embedding dimension m = 4. The corresponding Lyapunov exponents were calculated from time series data of *Acinetobacter*, *Pedobacter* and *Tetrahymena*. Positive estimates of the Lyapunov exponent confirm a chaotic behaviour according to theoretical expectations, whereas negative values indicate coexistence at equilibrium. Stable limited cycles are characterised by values close to zero.

1. 3 Results

Single-species population dynamics. Even though each chemostat experiment was running at the same external conditions, the intrinsic population dynamics differed strongly within each setup (Fig. 1). *Acinetobacter* reached different steady state abundances (dynamics from day 10 onwards). In one case (Fig. 1A) it was ranging between 4 and 8 x 10^7 cells ml^{-1}, whereas in the other case (Fig. 1B) it was ranging between 1 and 4 x 10^7 cells ml^{-1}. Steady state dynamics are dominated by irregular fluctuating population dynamics in both experiments. Differences in the steady state abundances are also detectable for the monoxenic chemostat experiments with *Pedobacter* (Fig. 1C and D). In order to characterise these nontrivial population dynamics, the corresponding Lyapunov exponents (λ) were calculated of these time series data (see values of λ in table 1). Every Lyapunov exponent was positive and ranged between 0.22 and 0.25 (± 0.05). The values indicated that the system exhibited chaotic population dynamics. If there was chaos, it should be sensitive towards the initial conditions (Hastings *et al.* 1993). Both *Acinetobacter*

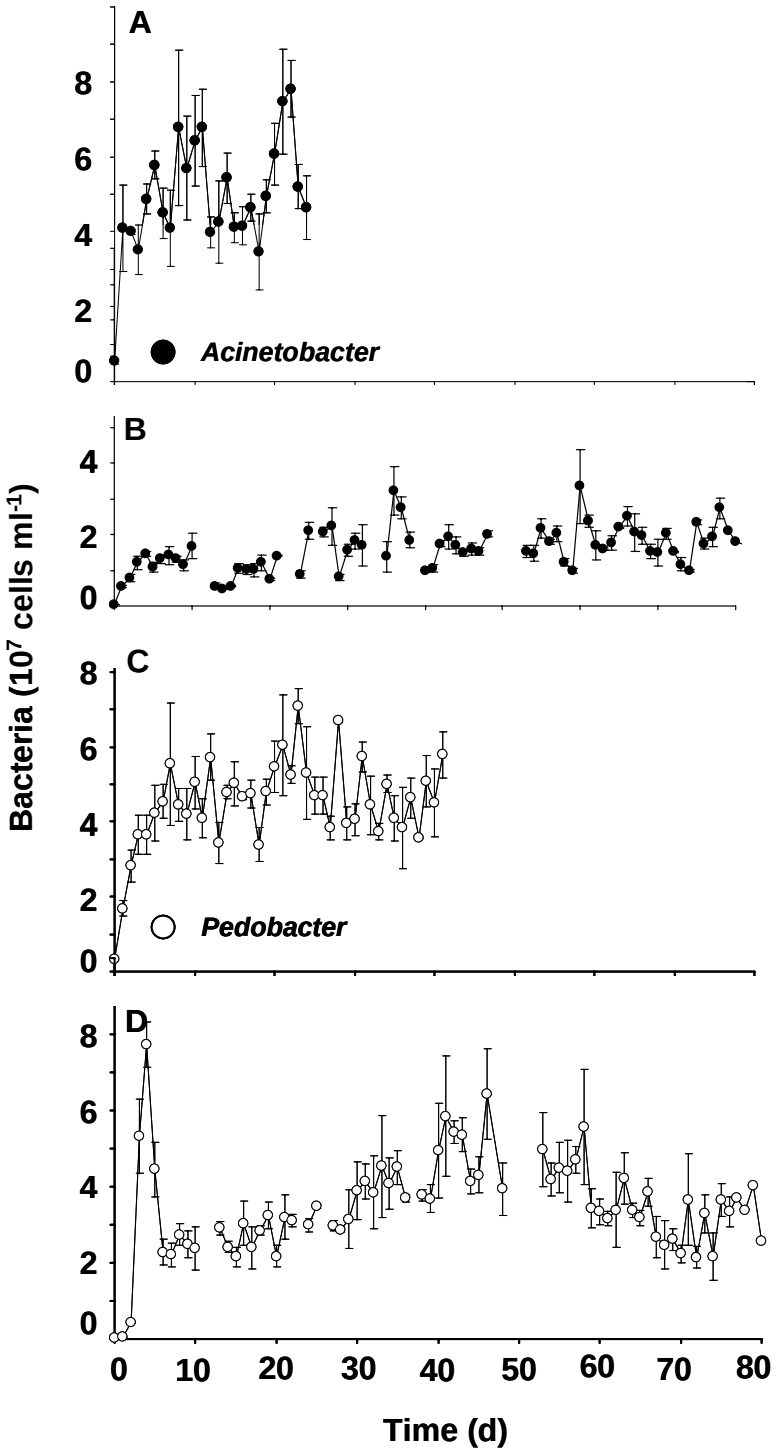

Fig. 1. Time series data of monoxenic bacterial chemostat experiments at a dilution rate of 0.75 per day and at constant temperature (20°C). A and B represent the time series data of *Acinetobacter johnsonii.* C and D represent the time series data of *Pedobacter*. Vertical bars represent the standard deviation of triplicate samples.

experiments differed only in their inoculation densities, on one occasion the inoculation density was 5.4 x 10^6 cells ml^{-1} at starting point zero (Fig. 1A). In another experiment (Fig. 1B) the inoculation density of *Acinetobacter johnsonii*

was 2.3 x 10^5 cells ml^{-1}. In the following course, both experiments showed huge differences in their dynamical behaviour and in their steady state abundances. This phenomenon is also true for the experiments with *Pedobacter*, here the inoculation densities varied between 3.0 x 10^6 cells ml^{-1} (Fig. 1C) and 1.7 x 10^5 cells ml^{-1} (Fig. 1D).

Two species competition experiments. For the two species competition experiments a mixture of *Acinetobacter* and *Pedobacter* cells was inoculated in a chemostat. Both bacterial strains coexist during the whole course of the experiments at different population densities (Fig. 2). *Acinetobacter* shows higher abundances at any time in one experiment in comparison to *Pedobacter*. In another experiment the dominance of both bacterial strains was vice versa since day seven. *Acinetobacter* and *Pedobacter* were able to coexist at irregular fluctuating abundances. The calculated Lyapunov exponent (see Table 1) was positive for both bacterial strains indicating a chaotic dynamic pattern over time.

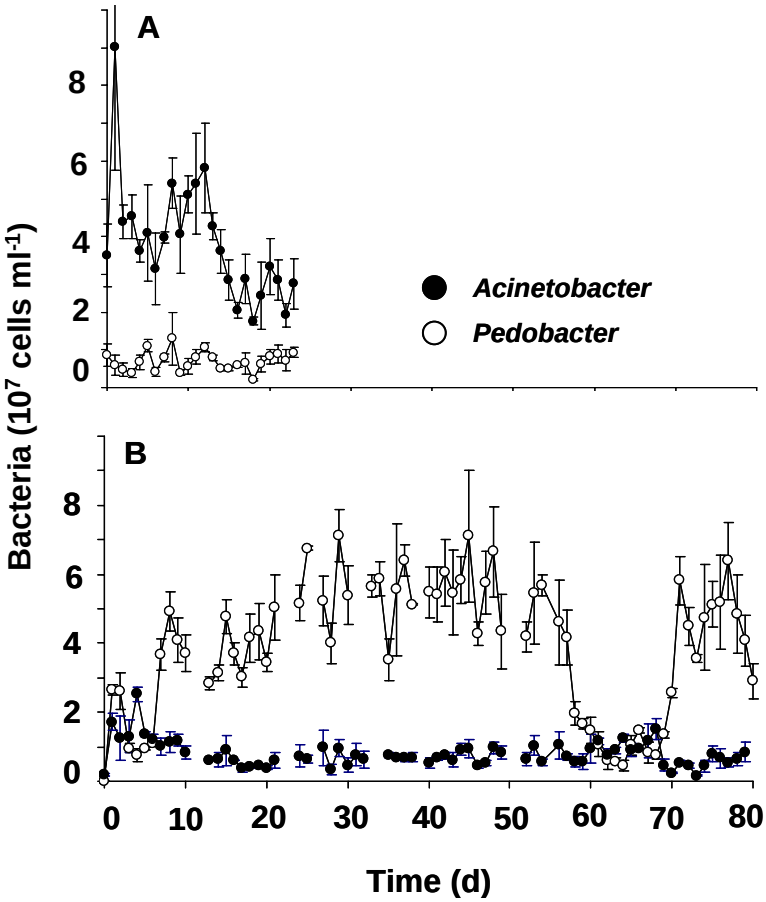

Fig. 2. Time series data of bacterial competition experiments at a dilution rate of 0.75 per day and at constant temperature (20°C). A and B represent the time series data of *Acinetobacter johnsonii* and *Pedobacter*. Vertical bars represent the standard deviation of triplicate samples.

Table 1. Calculations of the corresponsing Lyapunov exponents from the time series data.

	Figures	*Pedobacter* (λ)	*Acinetobacter* (λ)	*Tetrahymena* (λ)
One species systems	1A	-	Not enough data points available	-
	1B	-	**0.25 ± 0.03**	-
	1C	**0.23 ± 0.05**	-	-
	1D	**0.22 ± 0.05**	-	-
Two species systems	2A	Not enough data points available	Not enough data points available	-
	2B	**0.26 ± 0.01**	**0.09 ± 0.01**	-
	3A	-	**0.25 ± 0.01**	**0.25 ± 0.03**
	3B	-	-	*Tetrahymena* went to extinction
	3C	**0.26 ± 0.04**	-	**0.26 ± 0.07**
	3D	Not enough data points available	-	Not enough data points available
Three species systems	4A	**0.28 ± 0.03**	**0.45 ± 0.14**	**0.14 ± 0.01**
	4B	**0.29 ± 0.06**	**0.21 ± 0.03**	**0.05 ± 0.02**
	4C	**0.08 ± 0.02**	**0.28 ± 0.02**	**0.17 ± 0.03**

One-prey-one-predator time series data. Now, we added a trophic level by introducing the ciliate *Tetrahymena* to the experimental system. The ciliate *Tetrahymena* was grazing on one bacterial strain (either *Pedobacter* or *Acinetobacter*). *Tetrahymena* could establish higher abundances grazing on the morphological stable strain *Pedobacter* with values fluctuating round about 4.0 x 10^3 cells ml^{-1}, than grazing on the morphological heterogenous strain *Acinetobacter* (Fig. 3). At best, the abundances were four times lower, fluctuating at low levels round about 1.0 x 10^3 *Tetrahymena* cells ml^{-1} (see Fig. 3A). In a parallel experiment (see Fig. 3B) *Tetrahymena* could not survive and went to extinction at day 34. This was due to the establishment of extreme large filaments with length up to more than 250.0 µm (Chapter II). Here, we focus on the interpretation of the dynamic behaviour of the time series data. For every experiment a positive Lyapunov exponent was estimated for the predator *Tetrahymena* and its bacterial prey (Table 1). In addition, a quantitative effect is only visible for the experiments with *Pedobacter*. In single species systems

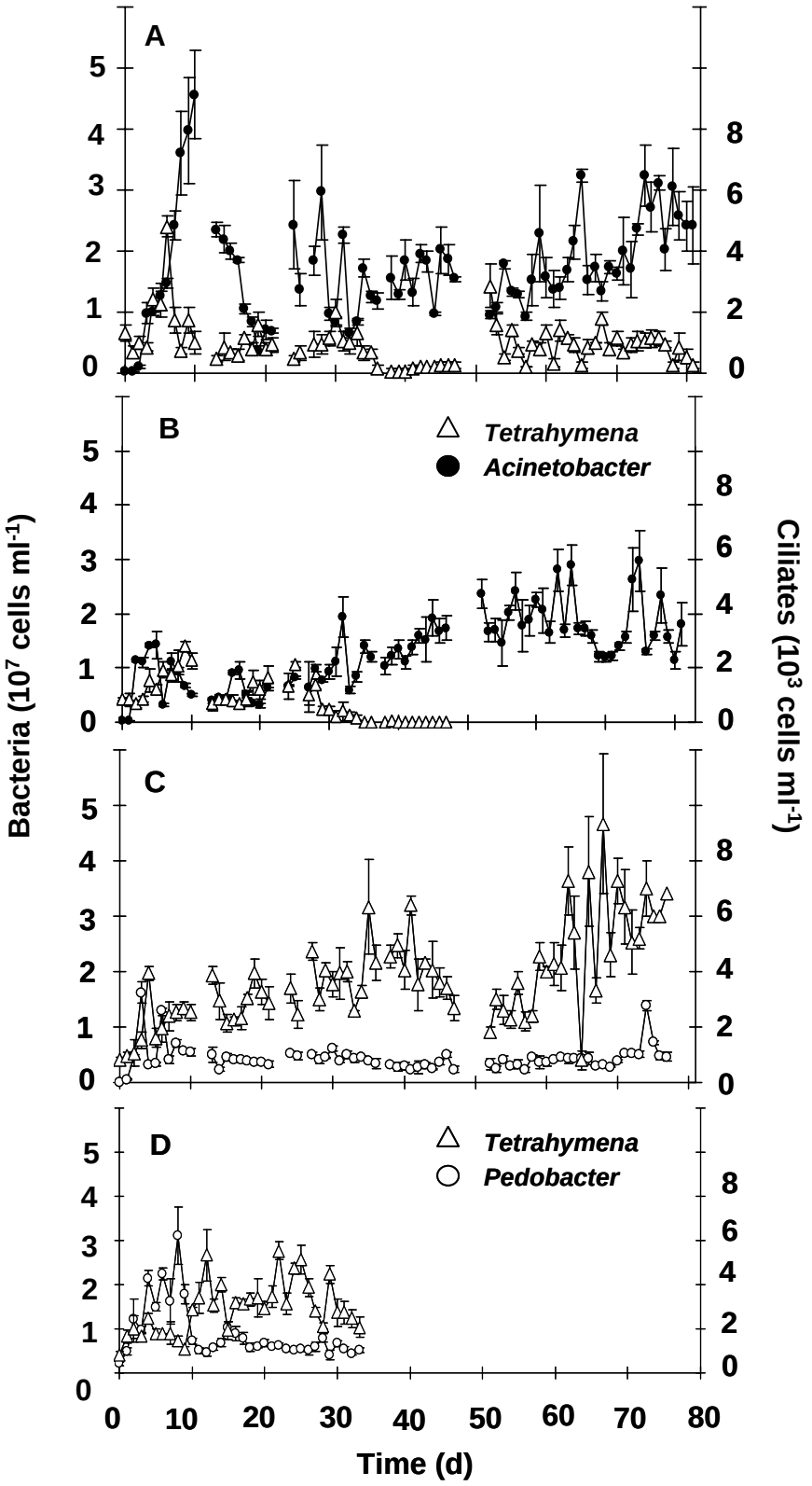

Fig. 3. Time series data of predator-prey chemostat experiments at a dilution rate of 0.75 per day and at constant temperature (20°C). A and B represent the time series data of *Acinetobacter* and *Tetrahymena.* C and D represent the time series data of *Pedobacter* and*Tetrahymena*. Vertical bars represent the standard deviation of triplicate samples.

Pedobacter established abudances round about 4.0 x 10^7 cells ml^{-1}. In two species predator-prey interactions the established abundances are four times lower and were fluctuating about 1.0 x 10^7 cells ml^{-1}. *Acinetobacter* made no qualitative or quantitative differences between predation pressure or monoxenic culture. *Acinetobacter* was able to compensate the grazing activity of *Tetrahymena* by the enhanced formations of long chain-like growth forms, adhering numerous cells.

Two-prey-one-predator time series data. In the next step, two bacteria competed for the present resources and were exposed to grazing pressure by the ciliate *Tetrahymena pyriformis.* The coexistence of all three species was possible at irregular fluctuating population dynamics (Fig. 4). The calculated Lyapunonv exponents for the bacterial populations were at least slightly positive in each experiment and ranged between 0.08 ± 0.02 and 0.45 ± 0.14 (Table 1). For the *Tetrahymena* abundances, the species enrichment had a kind of stabilizing effect because their Lyapunov exponents were lower (λ between 0.05 and 0.17) in comparison to the values in the one-prey-one-predator chemostats (λ between 0.25 and 0.26).

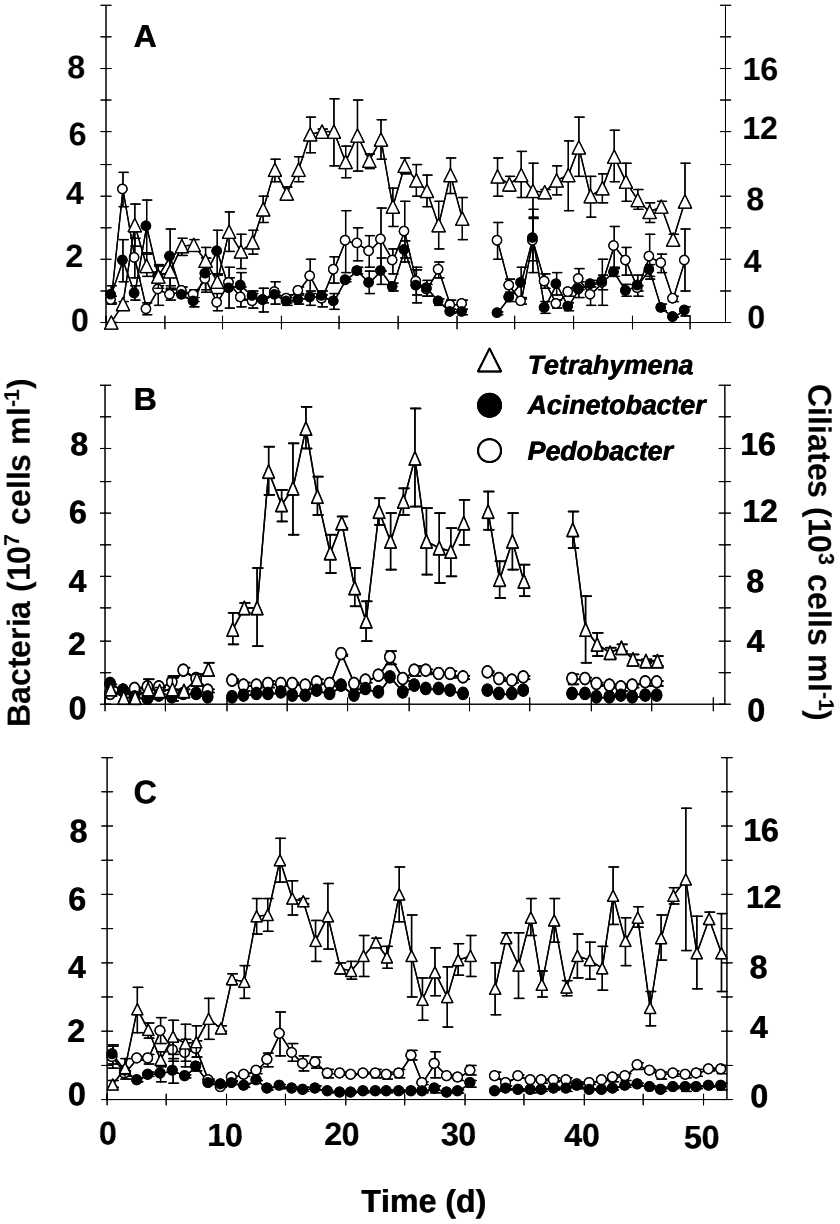

Fig. 4. Time series data of three species predator-prey chemostat experiments at a dilution rate of 0.75 per day and at constant temperature (20°C). A, B and C represent the times series data of replicate experiments. Vertical bars represent the standard deviation of triplicate samples.

1. 4 Discussion

Our experiments ran without any external disturbances, therefore keeping the external conditions as constant as possible. The microbial food web showed obviously no tendency to establish a stable equilibrium in the course of time. Without any external disturbances the microbial food web was dominated by chaotic population fluctuations. Beside the positive values of the Lyapunov

exponents, a second indication for chaotic population dynamics is a strong sensitivity towards the initial conditions (Hastings *et al.* 1993). A small change in the initial population size has a large impact on the final population size (Ellner & Turchin 2005). This was detected in our experiments for the bacterial single species as well as for the bacterial competition experiments. All chemostat experiments ran at the same conditions like ancestral genotype, temperature regime (20°C ± 0.3) and nutrient in- and outflow (0.75 per day ± 0.004), only the inoculation densities varied for each experiment. This let to significant variations in the mean abundances of replicate experiments in the course of time (Fig. 1). Striking examples to this issue were the competition experiments (Fig. 2). In one experiment, *Acinetobacter* was the better competitor with higher mean values in their mean abundances, in a replicate experiment it was vice versa. The coexistence per se, of both bacterial strains is probably due to the non-selective nutrient medium (proteose peptone and yeast extract, namely PPY 100). It obviously supplies enough energy and nutrients for the different requirements of both strains. Furthermore, both bacterial strains exhibited almost identical temperature dependent growth rates at 20°C with a value about 2.0 per day for *Acinetobacter* and *Pedobacter* in PPY 100 nutrient medium (Supplementary Material, Fig. 3). Therefore, both bacterial strains seemed to have equal competitive abilities. We conclude that the inoculation densities played a pivotal role for the quantitative differences in the chemostats.

Microevolutionary processes might be one explanation for this phenomenon. Differently evolving bacterial clones with a different mean fitness have already been described in chemostat experiments with *Escherichia coli* (Lenski & Travisano 1994; Maharjan *et al.* 2007). Ferenci stated in (Ferenci 2008): “Contrary to common belief, the chemostat environment is never in “steady state”, within generations populations become heterogeneous and evolving bacteria adopt alternative, parallel fitness strategies.” Continuously changing properties could be for example the membrane surface properties (shifting membrane protein composition like transporters or channels) or changes in the metabolism like changing enzymatic activities (Notley-McRobb *et al.* 2003; Maharjan *et al.* 2007). This may support different fitness levels, time lags and therefore unpredictable population dynamics of a monoclonal ancestor for longer time periods. Two of the monoxenic chemostat experiments, which

had a positive Lyapunov exponent lasted 80 days, encompassing about 120 generations of both bacterial strains. We can conclude that continuous intrinsic adaptive plasticity led to feedback mechanisms which caused the observed irregularities. This was supported by the predator-prey dynamics of *Acinetobacter* and *Tetrahymena* (Fig. 3B). A random mutational event led to the formation of inedible filamentous growth forms. Due to this, *Tetrahymena* went to extinction at day 34 (Chapter II).

A mixture of two potential prey resources had a stabilising effect on the population dynamics of *Tetrahymena* because the corresponding Lyapunov exponents ranged closer to zero (Table 1). This finding goes in line with the theoretical studies of (Fussmann & Heber 2002), where food chains have a broader tendency to exhibit chaotic dynamics (about 20.5 %) than food webs (about 6.4 %). Our experimental systems support May's (1974) theoretical findings: Two different bacterial strains (morphological heterogenous or morphological stable) showed an affinity to exhibit chaotic fluctuations over time at highly controllable chemostat experiments. This was approved by positive Lyapunov exponents and by the sensitivity towards the initial inoculation densities.

Time delays of self-regulating and adaptive abilities have already been proposed as important driver for irregular dynamics (Canale *et al.* 1973). Intrinsically driven chaotic behaviour might play an important role for natural ecosystem communities. Unintentional, irregular variations in the time series data beyond quarter out-of-phase predator-prey cycles have already been detected in several one-prey-one-predator systems (Ashby 1976; Chao *et al.* 1977; Swift *et al.* 1982). Or these phenomena were simply disregarded from the time series data (Grover 1988; Fussmann *et al.* 2000; Corno & Jürgens 2006). Our findings suggest that intrinsic chaos may be underestimated for the relevance in long-term behaviours of population dynamics. Chaos offers self-organization abilities which might play an important role for coexistence of species in every ecosystem (Petchey 2000; Sprott *et al.* 2005).

1. 5 References

Ashby R.E. (1976). LONG TERM VARIATIONS IN A PROTOZOAN CHEMOSTAT CULTURE. *Journal of Experimental Marine Biology and Ecology*, 24, 227-235.

Becks L., Hilker F.M., Malchow H., Jurgens K. & Arndt H. (2005). EXPERIMENTAL DEMONSTRATION OF CHAOS IN A MICROBIAL FOOD WEB. *Nature*, 435, 1226-1229.

Benincà E., Huisman J., Heerkloss R., Johnk K.D., Branco P., Van Nes E.H., Scheffer M. & Ellner S.P. (2008). CHAOS IN A LONG-TERM EXPERIMENT WITH A PLANKTON COMMUNITY. *Nature*, 451, 822-U7.

Bjornstad O.N. & Grenfell B.T. (2001). NOISY CLOCKWORK: TIME SERIES ANALYSIS OF POPULATION FLUCTUATIONS IN ANIMALS. *Science*, 293, 638-643.

Cadotte M.W., Drake J.A. & Fukami T. (2005). CONSTRUCTING NATURE: LABORATORY MODELS AS NECESSARY TOOLS FOR INVESTIGATING COMPLEX ECOLOGICAL COMMUNITIES. *Advances in Ecological Research, Vol. 37: Population Dynamics and Laboratory Ecology*, 37, 333-353.

Canale R.P., Lustig T.D., Kehrberg.Pm & Salo J.E. (1973). EXPERIMENTAL AND MATHEMATICAL MODELING STUDIES OF PROTOZOAN PREDATION ON BACTERIA. *Biotechnology and Bioengineering*, 15, 707-728.

Chao L., Levin B.R. & Stewart F.M. (1977). A COMPLEX COMMUNITY IN A SIMPLE HABITAT: AN EXPERIMENTAL STUDY WITH BACTERIA AND PHAGE. *Ecology*, 58, 369-378.

Corno G. & Jürgens K. (2006). DIRECT AND INDIRECT EFFECTS OF PROTIST PREDATION ON POPULATION SIZE STRUCTURE OF A BACTERIAL STRAIN WITH HIGH PHENOTYPIC PLASTICITY. *Applied and Environmental Microbiology*, 72, 78-86.

Costantino R.F., Desharnais R.A., Cushing J.M. & Dennis B. (1997). CHAOTIC DYNAMICS IN AN INSECT POPULATION. *Science*, 275, 389-391.

Eckmann J.P. & Ruelle D. (1985). ERGODIC-THEORY OF CHAOS AND STRANGE ATTRACTORS. *Reviews of Modern Physics*, 57, 617-656.

Ellner S.P. & Turchin P. (2005). WHEN CAN NOISE INDUCE CHAOS AND WHY DOES IT MATTER: A CRITIQUE. *Oikos*, 111, 620-631.

Ferenci T. (2008). BACTERIAL PHYSIOLOGY, REGULATION AND MUTATIONAL ADAPTATION IN A CHEMOSTAT ENVIRONMENT. *Advances in Microbial Physiology, Vol 53*, 53, 169-228.

Fussmann G.F., Ellner S.P., Shertzer K.W. & Hairston N.G. (2000). CROSSING THE HOPF BIFURCATION IN A LIVE PREDATOR-PREY SYSTEM. *Science*, 290, 1358-1360.

Fussmann G.F. & Heber G. (2002). FOOD WEB COMPLEXITY AND CHAOTIC POPULATION DYNAMICS. *Ecology Letters*, 5, 394-401.

Graham D.W., Knapp C.W., Van Vleck E.S., Bloor K., Lane T.B. & Graham C.E. (2007). EXPERIMENTAL DEMONSTRATION OF CHAOTIC INSTABILITY IN BIOLOGICAL NITRIFICATION. *Isme Journal*, 1, 385-393.

Gross T., Ebenhoh W. & Feudel U. (2005). LONG FOOD CHAINS ARE IN GENERAL CHAOTIC. *Oikos*, 109, 135-144.

Grover J.P. (1988). DYNAMICS OF COMPETITION IN A VARIABLE ENVIRONMENT: EXPERIMENTS WITH TWO DIATOM SPECIES. *Ecology*, 69, 408-417.

Hastings A. (2004). TRANSIENTS: THE KEY TO LONG-TERM ECOLOGICAL UNDERSTANDING? *Trends in Ecology & Evolution*, 19, 39-45.

Hastings A., Hom C.L., Ellner S., Turchin P. & Godfray H.C.J. (1993). CHAOS IN ECOLOGY - IS MOTHER-NATURE A STRANGE ATTRACTOR. *Annual Review of Ecology and Systematics*, 24, 1-33.

Hegger R., Kantz H. & Schreiber T. (1999). PRACTICAL IMPLEMENTATION OF NONLINEAR TIME SERIES METHODS: THE TISEAN PACKAGE. *Chaos*, 9, 413-435.

Huisman J. & Weissing F.J. (2001). BIOLOGICAL CONDITIONS FOR OSCILLATIONS AND CHAOS GENERATED BY MULTISPECIES COMPETITION. *Ecology*, 82, 2682-2695.

Jessup C.M., Kassen R., Forde S.E., Kerr B., Buckling A., Rainey P.B. & Bohannan B.J.M. (2004). BIG QUESTIONS, SMALL WORLDS: MICROBIAL MODEL SYSTEMS IN ECOLOGY. *Trends in Ecology & Evolution*, 19, 189-197.

Kaitala V., Ylikarjula J., Ranta E. & Lundberg P. (1997). POPULATION DYNAMICS AND THE COLOUR OF ENVIRONMENTAL NOISE. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 264, 943-948.

Lenski R.E. & Travisano M. (1994). DYNAMICS OF ADAPTATION AND DIVERSIFICATION - A 10,000-GENERATION EXPERIMENT WITH BACTERIAL-POPULATIONS. *Proceedings of the National Academy of Sciences of the United States of America*, 91, 6808-6814.

Lorenz E.N. (1963). DETERMINISTIC NONPERIODIC FLOW. *Journal of the Atmospheric Sciences*, 20, 130-141.

Maharjan R.P., Seeto S. & Ferenci T. (2007). DIVERGENCE AND REDUNDANCY OF TRANSPORT AND METABOLIC RATE-YIELD STRATEGIES IN A SINGLE ESCHERICHIA COLI POPULATION. *Journal of Bacteriology*, 189, 2350-2358.

Maruyama F., Yamaguchi N., Kenzaka T., Tani K. & Nasu M. (2004). SIMPLIFIED SAMPLE PREPARATION USING FRAME SPOTTING METHOD FOR DIRECT COUNTING OF TOTAL BACTERIA BY FLUORESCENCE MICROSCOPY. *Journal of Microbiological Methods*, 59, 427-431.

May R.M. (1974). BIOLOGICAL POPULATIONS WITH NONOVERLAPPING GENERATIONS - STABLE POINTS, STABLE CYCLES, AND CHAOS. *Science*, 186, 645-647.

May R.M. & Oster G.F. (1976). BIFURCATIONS AND DYNAMIC COMPLEXITY IN SIMPLE ECOLOGICAL MODELS. *American Naturalist*, 110, 573-599.

McCauley E., Nisbet R.M., Murdoch W.W., de Roos A.M. & Gurney W.S.C. (1999). LARGE-AMPLITUDE CYCLES OF DAPHNIA AND ITS ALGAL PREY IN ENRICHED ENVIRONMENTS. *Nature*, 402, 653-656.

Notley-McRobb L., Seeto S. & Ferenci T. (2003). THE INFLUENCE OF CELLULAR PHYSIOLOGY ON THE INITIATION OF MUTATIONAL PATHWAYS IN ESCHERICHIA COLI POPULATIONS. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 270, 843-848.

Petchey O.L. (2000). PREY DIVERSITY, PREY COMPOSITION, AND PREDATOR POPULATION DYNAMICS IN EXPERIMENTAL MICROCOSMS. *Journal of Animal Ecology*, 69, 874-882.

Rohani P., Miramontes O. & Keeling M.J. (2004). THE COLOUR OF NOISE IN SHORT ECOLOGICAL TIME SERIES DATA. *Mathematical Medicine and Biology-a Journal of the Ima*, 21, 63-72.

Rosenstein M.T., Collins J.J. & De Luca C.J. (1993). A PRACTICAL METHOD FOR CALCULATING LARGEST LYAPUNOV EXPONENTS FROM SMALL DATA SETS. *Physica D-Nonlinear Phenomena*, 65, 117-134.

Schönhuber W., Fuchs B., Juretschko S. & Amann R. (1997). IMPROVED SENSITIVITY OF WHOLE-CELL HYBRIDIZATION BY THE COMBINATION OF HORSERADISH PEROXIDASE-LABELED OLIGONUCLEOTIDES AND TYRAMIDE SIGNAL AMPLIFICATION. *Applied and Environmental Microbiology*, 63, 3268-3273.

Sprott J.C., Vano J.A., Wildenberg J.C., Anderson M.B. & Noel J.K. (2005). COEXISTENCE AND CHAOS IN COMPLEX ECOLOGIES. *Physics Letters A*, 335, 207-212.

Swift S.T., Najita I.Y., Ohtaguchi K. & Fredrickson A.G. (1982). SOME PHYSIOLOGICAL-ASPECTS OF THE AUTECOLOGY OF THE SUSPENSION-FEEDING PROTOZOAN TETRAHYMENA-PYRIFORMIS. *Microbial Ecology*, 8, 201-215.

Tel T., Nishikawa T., Motter A.E., Grebogi C. & Toroczkai Z. (2004). UNIVERSALITY IN ACTIVE CHAOS. *Chaos*, 14, 72-78.

Tsuchiya H.M., Jost J.L., Drake J.F. & Fredrick.Ag (1972). PREDATOR-PREY INTERACTIONS OF DICTYOSTELIUM-DISCOIDEUM AND ESCHERICHIA-COLI IN CONTINUOUS CULTURE. *Journal of Bacteriology*, 110, 1147-&.

Turchin P. (1995). CHAOS IN MICROTINE POPULATIONS. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 262, 357-361.

Upadhyay R.K. & Rai V.K. (1997). WHY CHAOS IS RARELY OBSERVED IN NATURAL POPULATIONS. *Chaos Solitons & Fractals*, 8, 1933-1939.

Vasseur D.A. & Yodzis P. (2004). THE COLOR OF ENVIRONMENTAL NOISE. *Ecology*, 85, 1146-1152.

Chapter II

Microevolution and inducible defence strategies of *Acinetobacter johnsonii* towards ciliate predation

2. 1 Introduction

Predator-prey interactions determine the trophic energy transfer in ecosystems. This includes all trophic levels ranging from microbial to macroinvertebrate food webs (Agrawal *et al.* 1999; van der Stap *et al.* 2007; Corno *et al.* 2008). Regarding aquatic microbial food webs, it is well known that predation by protists may alter many characteristics of food webs like composition, abundances and even morphotypes of prey species (Hahn & Höfle 2001; Jürgens & Matz 2002; Massana & Jürgens 2003; Pernthaler 2005). Protist grazing can not only shift the abundances but also the size distribution of bacterioplankton in marine and freshwater towards larger growth forms (Hahn & Höfle 1999; Matz & Kjelleberg 2005; Corno & Jürgens 2006; van der Stap *et al.* 2008). Little is known about the role of ciliate predation for microevolutionary processes. Ciliates are besides heterotrophic flagellates the most important predators of bacteria in aquatic ecosystems (Simek *et al.* 1994; Sherr & Sherr 2002).

Bacteria can establish relatively short generation times and therefore can incorporate a comparatively high mutation rate of the genome within a given time. Bacteria may increase their mutation rate under stress factors like heat-shocks or predation (Spiers et al. 2000). Lateral gene transfer units like plasmids, transposons and integrons are an additional source for the rapid evolution of bacteria. This might explain the huge diversity in some bacterial species for example in *Pseudomonas* with more than 267 different Operational Taxonomic Units (OTUs) (Stanier *et al.* 1966; Spiers *et al.* 2000). Evolutionary processes in bacteria occur constantly in heterogeneous environments as well as in homogeneous environments (Rainey & Travisano 1998; Maharjan *et al.* 2006). Chemostat experiments provide a useful tool to study long-term adaptation in microbial populations (Lenski & Travisano 1994). Adaptation in single species

systems has been investigated for example in *E. coli* under nutrient limitation (Helling *et al.* 1987; Rosenzweig *et al.* 1994; Notley-McRobb *et al.* 2003; Maharjan *et al.* 2007) or the temperature-specific fitness of *E. coli* (Bennett *et al.* 1990; Leroi *et al.* 1994). Natural communities are much more complex. Interactions among different species like interspecific competition, parasite-host interactions and predator-prey dynamics are common in almost every habitat.

It was shown that there is a co-evolution in bacteria-phage systems (Lenski & Levin 1985; Bohannan & Lenski 2000; Yoshida *et al.* 2007). Surprisingly, there is only little information about the potentially strong evolutionary impact of predator-prey interactions. We performed chemostat and batch experiments in a common homogenous environment to examine the impact of the ciliate *Tetrahymena pyriformis* on phenotypic properties of the bacterium *Acinetobacter johnsonii*. Up to now, morphological plasticity of bacteria in response to grazing was investigated nearly exclusively with strains showing a clear switch between single-cell stages and colony formation. We chose a more natural situation selecting a bacterial strain which is polymorphic in the presence and absence of predation. To include possible effects of microevolutionary processes (Lenski & Travisano 1994), we carried out long term experiments in continuous cultures for 80 days which comprise about 160 generations of *Acinetobacter*.

2. 2 Methods

Experimental organisms. A polymorphic strain (monoclonal culture) of *Acinetobacter johnsonii* (γ-Proteobacteria; kindly provided as an isolate by Michael Steinert, University of Braunschweig) was used as a model prey organism. To test whether competition changes the morphology of *Acinetobacter johnsonii*, another bacterial strain *Pedobacter sp.* (Cytophaga Flexibacter group, stable morphology; isolated by Kristin Beck, kindly provided by Klaus Jürgens, Institute for Baltic Research, IOW, Warnemünde) was introduced. An axenic culture of the ciliate *Tetrahymena pyriformis* (strain CCAP 1630/1W; Culture Collection of Algae and Protists, Windermere, U.K.) was used as the model organism for a ciliate grazer. All experiments were inoculated from overnight cultures (LB medium) out of a

deep-frozen (-80°C) stock. *Tetrahymena pyriformis* grew in 50 ml culture flasks containing PPY (proteose peptone, yeast extract) medium. This stock was stored at 20 °C in the dark which was refreshed fortnightly.

Food-selection experiments. Batch experiments were conducted in 50 ml centrifuge tubes (Falcon, Germany) at 20 ± 0.3°C. Eight experiments were run in parallel, each with a volume of 20 ml. In addition, we started three control experiments containing 18 ml of PPY 100 (100-times diluted) with 2 ml of the over night culture of *Acinetobacter johnsonii*. Initial abundance of *Acinetobacter johnsonii* in all food-selection experiments was 1.5 x 10^7 cells ml^{-1}. Grazing experiments were inoculated with *Tetrahymena pyriformis* (initial abundance ~3.2 x 10^4 ml^{-1}) taken from a well-grown stock culture. Abundances and morphology were determined at 0 min, 60 min, 120 min, 180 min and finally at 24 hours.

Long-term chemostat experiments. Chemostat experiments were carried out in single glass reactors with an average fluid content of 190 ml and were run at a dilution rate of 0.75 per day at 20 ± 0.3°C in the dark. The dilution rate was established by automated syringe pumps (Cavro XLP 6000, Modular Syringe Pump, TECAN, Crailsheim, Germany). All parts of the chemostat systems and inflowing air were sterilized prior to the experiments. Robot (RSP 9000 Cavro, TECAN, Crailsheim, Germany controlled triplicate sampling (0.5 ml each, altogether 1.5 ml) took place every 24 hours into microcentrifuge tubes. Four independent chemostat experiments were carried out under the same external conditions (nutrient supply: PPY hundred times diluted; 20°C). Two chemostats were inoculated with *Acinetobacter johnsonii* (initial abundance 2-4 x 10^5 cells ml^{-1}) and *Tetrahymena pyriformis* (initial abundance 8-10 x 10^2 cells ml^{-1}). The other two chemostats served as controls with one containing only *Acinetobacter johnsonii* (as a single species system). The second control chemostat was inoculated with *Acinetobacter johnsonii* and the competitor *Pedobacter sp.* All chemostat experiments ran in parallel and were inoculated from the same overnight grown cultures. The experiments lasted 80 days and population densities were determined daily. Additionally, we studied the morphology of *Acinetobacter*

johnsonii at five selected intervals over the course of six successive days (days 0-5; 15-20; 30-35; 55-60; 75-80).

Quantitative analysis. All samples were fixed 1:1 in PBS-buffer (phosphate buffered saline, pH 7.4) containing 4% formaldehyde and about 0.01 g/l SDS (sodium dodecyl sulphate). For the determination of the abundances, the samples were stained with the fluorescent dye CYBR Green I (Invitrogen, Karlsruhe, Germany, dilution with aqua dest. 1:5000). Staining occurred according to the frame spotting method (Maruyama et al. 2004). The two bacterial strains (each at least 900 cells) and *Tetrahymena pyriformis* (all cells in 15 µl probe volume) abundances were enumerated by epifluorescence microscopy (Zeiss Axiophot, Zeiss filter set 43; BP 550/25, FT 570, BP 605/70, 1250-times optical magnification in case of bacteria, 125-times magnification for *Tetrahymena pyriformis* enumeration). Cell size measurements (length and width) of *Acinetobacter johnsonii* were done microscopically to the nearest 0.2 µm (Zeiss Axiovision software). At least 100 cells of *Acinetobacter johnsonii* were measured per sample. For the calculation of the biovolume of *Acinetobacter johnsonii,* a cylinder with two spherical caps was assumed (Heldal et al. 1985) using the following equation: $V = \pi * w^2 * (l - w/3)/4$, where w = width (µm) and l = length (µm).

To check for contamination during the long-term chemostat experiments, oligonucleotide rRNA-targets were used to identify *Acinetobacter johnsonii* cells in chemostat samples. More specifically, we used the oligonucleotide probe Aca (sequence: 5´-ATC CTC TCC CAT ACT CTA-3`; (Wagner et al. 1994)). ACA-oligonucleotides were labelled with horseradish-peroxidase (5´-HRP). We used a simplified protocol for the CARD FISH (Catalysed Reporter Deposition-Fluorescence in Situ Hybridisation) after Schönhuber et al. (1997) where the embedding step and the inactivation of the endogenous peroxidase step was omitted.

2. 3 Results

Food-selection experiments. *Acinetobacter* formed various cell size classes ranging from volumes of 3 μm^3 to 30 μm^3 within a clonal culture (Fig. 1). Without grazing pressure, *Acinetobacter* grew to a 5.3 fold higher abundance (8.0 x 10^7 cells ml^{-1}) at the end of the experiment (Fig. 1A). *Acinetobacter* established only slight changes in the population size structure. During the whole experiment, 60 - 85 % of the growing population consisted of the smallest size fraction (< 3 μm^3). The remaining part consisted of cells with a volume of up to 12 μm^3. Chains larger than 12 μm^3 appeared too, but where more or less negligible (less than 2 % of the population) at every sampling unit (Fig. 1B).

High grazing pressure by *Tetrahymena* with 3.2 x 10^4 Individuals ml^{-1} reduced the *Acinetobacter* population after 24 hours almost tenfold to 1.6 x 10^6 cells ml^{-1} (Fig. 1A). Grazing by *Tetrahymena* induced a shift in the morphology of *Acinetobacter* (Fig. 1C). After 24 hours, the smallest size fraction (< 3 μm^3) almost disappeared. The next larger size class (3-6 μm^3, this resembles a length of about 1.5-8 µm) remained fairly constant and larger growth forms became dominant. The minimum and maximum width ranged from 0.8 to 1.4 µm in each experiment and was independent of the length of chains and single cells. After 24 hours, about 30 % of the *Acinetobacter* population established chains larger than 12 μm^3.

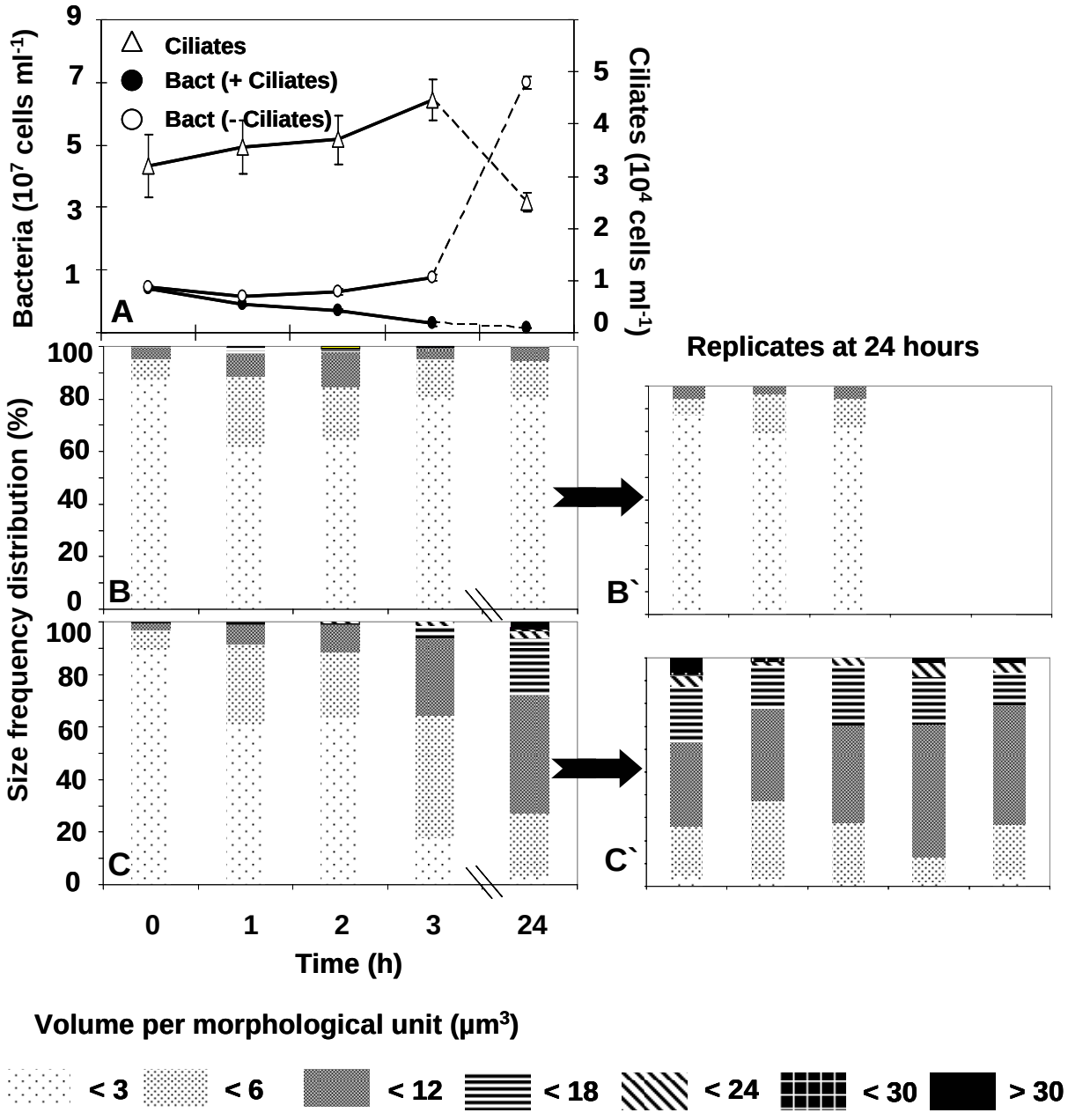

Fig. 1. Summary of the short term batch experiments. Abundances in A (mean values, in serveral cases the error bars are smaller than the symbols) and the corresponding size frequency distributions of *Acinetobacter johnsonii* in non-grazed experiments (B, three replicates) and in grazing experiments with the ciliate *Tetrahymena pyriformis* (C, five replicates). Replicates are shown for the measurements at 24 hours (B` and C`, right hand side).

Long-term chemostat experiments. In the long-term monoxenic culture (Fig. 2A) *Acinetobacter* reached a mean abundance of 1.7 x 10^7 cells ml^{-1} between days 15 and 80. The predator independent morphology of *Acinetobacter* was very heterogeneous ranging from single cells and short chains (< 6 μm^3) to very large chains with a biovolume of larger than 30 μm^3. The distribution of the different cell size classes was relatively homogenous from day 15 on, about 40 % of the population belonged to single cells and small chains (size class < 6 μm^3). The next larger size class (< 12 μm^3) contributed between 20 and 30 % to the biovolume.

In the competition experiment with *Acinetobacter* and *Pedobacter* (Fig. 2B), the morphology of *Acinetobacter* stayed constant after a short transient phase at the beginning of the experiment (days 0-5). About 40 % of the population belonged to forms with a volume smaller than 6 μm^3. 30 % of the population consisted of forms with medium sized chains up to 12 μm^3. The remaining part of the population (about 30 %) consisted of larger growth forms. The mean abundances of *Acinetobacter* were rather stable from day 15 on, with 7.0 x 10^6 cells ml^{-1} (Fig. 2B, right hand side).

In one of the grazing experiments with *Tetrahymena* (Fig. 2C), the size frequency distribution of the *Acinetobacter* population increased stepwise. From days 15 to 20 onwards a succession to larger growth forms during the whole course of the experiment was detectable. At the end of the experiment (days 75-80), 50 % of the population consisted of extremely large filaments and chains larger than 30 μm^3. Whereas single cells and small chains (< 6 μm^3, the preferred food resource) composed a minor part of the population (5-20 %). Because of predator-prey interactions, the abundances of both species were fluctuating over time (Fig. 2C, right hand side).

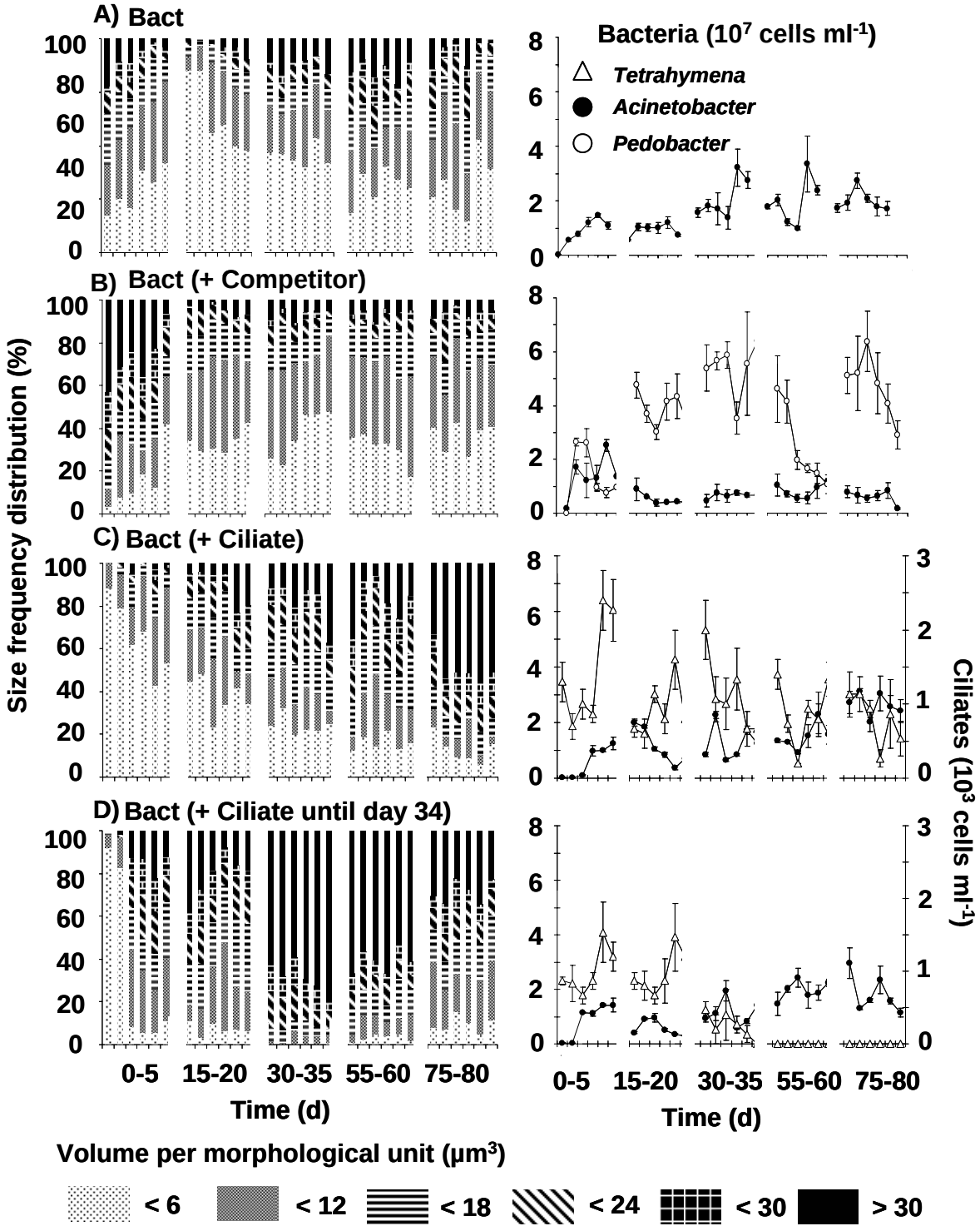

Fig. 2. On the left hand side, fraction of the different volumes (µm^3) of the *Acinetobacter* population per day in four chemostat experiments in a single species system (A) in a two species competition system with *Acinetobacter* and *Pedobacter* B). Two species predator-prey systems with *Acinetobacter* and *Tetrahymena* in C) and D). On the right hand side are the corresponding abundances per ml (mean values of triplicate samples).

In a parallel experimental onset (Fig. 2D), the influence of *Tetrahymena* grazing on the morphology of *Acinetobacter* was already evident after the first five days. Single cells and small filaments up to 6 μm^3 were strongly reduced from day two on. From day 30 to 35 more than 60 % of the *Acinetobacter* population consisted of filaments larger than 30 μm^3, whereas single cells and small filaments (< 6 μm^3) were not present any more. *Tetrahymena* went to extinction at day 34. At day 34 onwards, the system consisted of a monoxenic culture of *Acinetobacter*. 20 days after predation pressure, at days 55 and 60, no general change in the size structure of the *Acinetobacter* population occurred. Only the fraction of single cells and small filaments (< 6 μm^3) appeared again at low concentrations (1-5 % of the biovolume). There was a trend detectable in the remaining *Acinetobacter* population towards smaller cell sizes at days 75 – 80 (40 days after the release from predation).

The biovolumes of *Acinetobacter* were the lowest in the competition experiment (Fig. 3). The presence of predators induced the establishment of high biovolumes of *Acinetobacter* due to the presence of extreme growth forms like filaments or chains with a biovolume larger than 30 μm^3. The fraction of very large growth forms (> 30 μm^3) differed qualitatively and quantitatively in grazing and non-grazing experiments. The chemostats with *Acinetobacter* alone and *Acinetobacter* in competition with *Pedobacter* had the lowest fraction of growth forms larger than 30 μm^3 with a mean value of 6.7 % and 11.0 % of the whole population (Fig. 2A, B). For the experiments with grazers, at least 28.8 % of the population consisted of growth forms larger than 30 μm^3. For one chemostat, the largest fraction of extreme growth forms occurred at the end of the experiments between days 75 and 80 (Fig. 2C). In experiment D with the extinction of *Tetrahymena*, the fraction of extreme growth forms was relatively high during the whole course of the experiment with peaks at time steps from 30 to 35 days and from 55 to 60 days. In this chemostat 55.4 % of the population was composed of filaments larger than 30 μm^3 (Fig. 2D). Furthermore, the contribution of size classes > 70 μm^3 (extreme large filaments) was exceptionally high (Supplementary Material, Fig. 4).

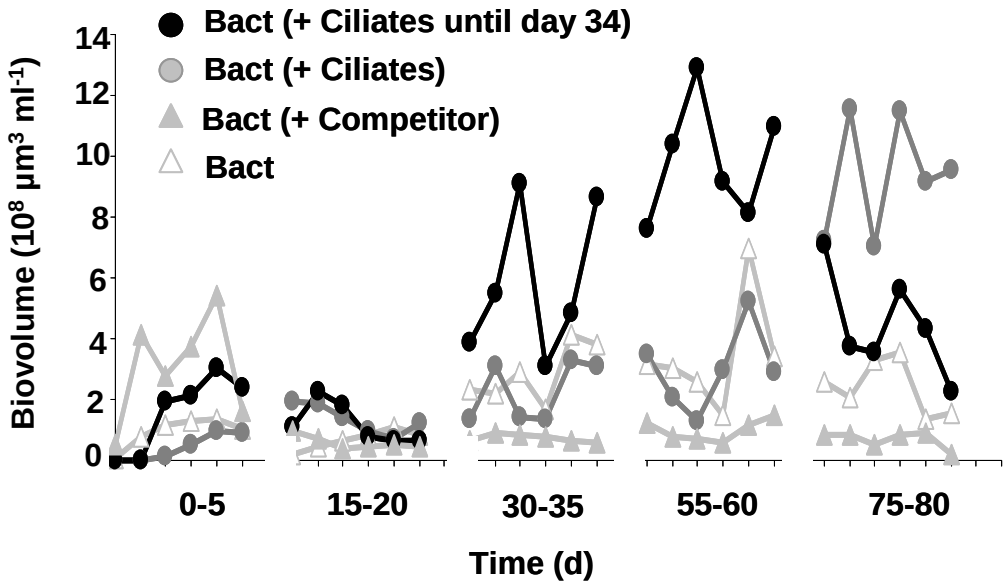

Fig. 3. Biovolumes per ml of *Acinetobacter johnsonii* in four replicate chemostat experiments. In two chemostats *T. pyriformis* is grazing on *Acinetobacter* in comparison to a single species chemostat and a two species chemostat in competition with *Pedobacter.*

Figure 4 gives examples for the different morphologies observed in each chemostat experiment at day 35 (left hand side) and at the end of the experiment at day 80 (right hand side). At steady state conditions (between days 35 and 80), there was always a mixture of different cell sizes in the single species system present (Fig. 4A). *Acinetobacter* was able to form chains of various cell sizes ranging from single cells to chains from 1.5 to 170.0 µm of length. In the two-species competition chemostat (Fig. 4B), *Acinetobacter* single cells and chains had a length of minimum 1.8 to maximum 168.5 µm. In one of the two grazing experiments with *Tetrahymena* (Fig. 4C), the *Acinetobacter* cells and chains ranged between 1.7 to a maximum length of 264.8 µm. In the other grazing experiment (Fig. 4D), *Tetrahymena* went to extinction at day 34. In this chemostat, *Acinetobacter* showed a different morphology compared to all other chemostats. Here, *Acinetobacter* didn't form chains but filamentous growth forms (Fig. 4D). The length of this filamentous morphotype ranged between 3.7 and 223.7 µm. The width of the measured *Acinetobacter* cells was in the same range (between 0.8 to 1.4 µm) as in all chemostat experiments.

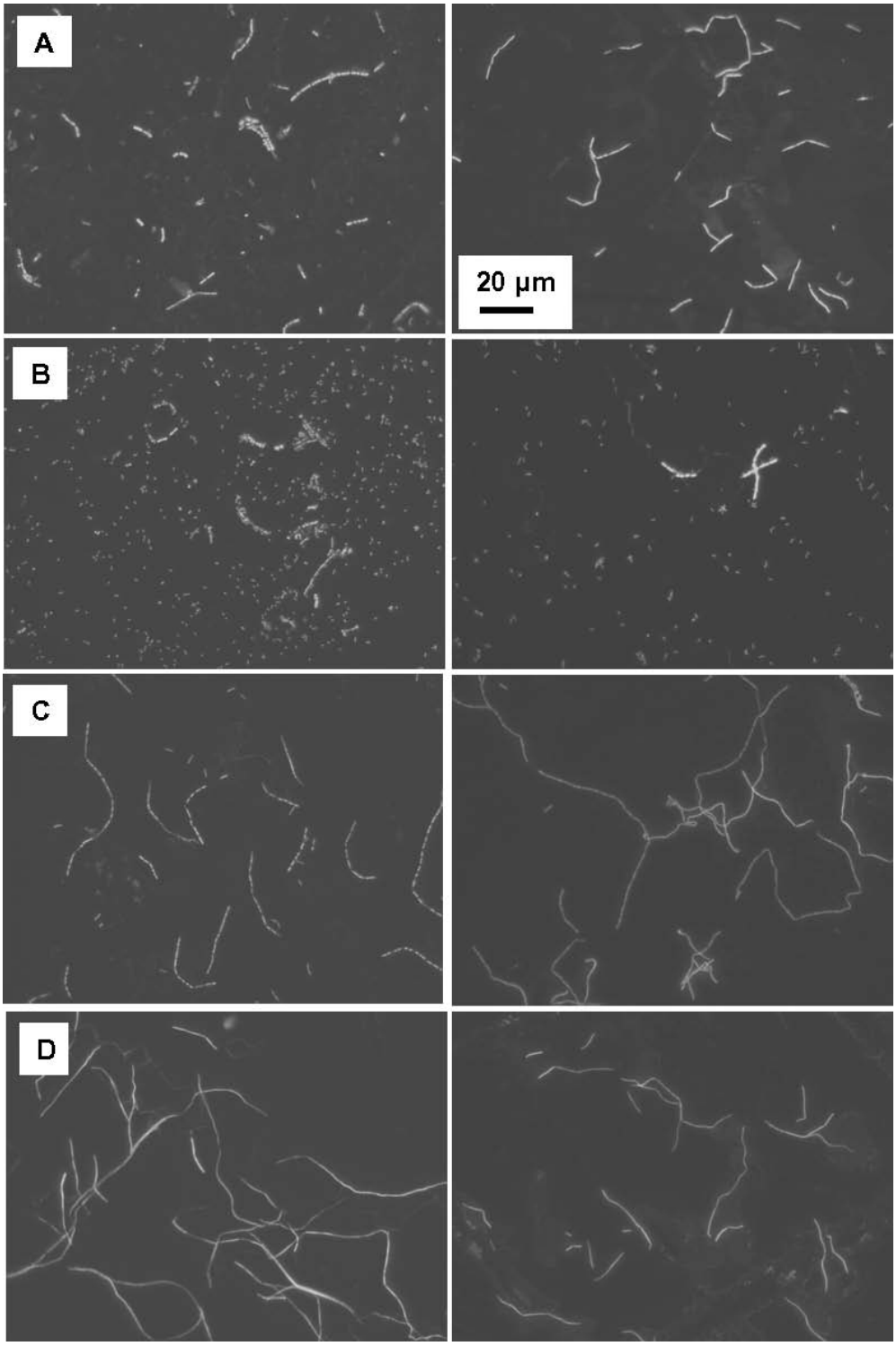

Fig. 4. Photographs of CYBR-Green stained cells of *Acinetobacter johnsonii* in chemostat culture at day 35 (left panel) and at day 80 (right panel). Where A) is single species system with *Acinetobacter*, B) is a two species competition system with *Acinetobacter* and *Pedobacter*,C) and D) are two species predator-prey systems with *Acinetobacter* and *Tetrahymena*. The scale bar in panel A) refers to all pictures.

2. 4 Discussion

Predation by bacterivorous flagellates was found to be a strong trigger for morphological changes in bacterial communities (Simek *et al.* 1994; Hahn & Höfle 1999; Jürgens & Matz 2002; Massana & Jürgens 2003). Several bacterial strains are able to form inedible growth forms in the presence of predators. These morphological shifts were shown to be reversible and are summarized under the term phenotypic plasticity (Hahn & Höfle 1999; Corno & Jürgens 2006). Examples regarding these phenomena considered bacterial strains which performed an overall switch between single cells and adaptive growth forms.

In the present study, we used a bacterial strain which shows a large variety of morphotypes for clonal cultures of *Acinetobacter johnsonii* from single cells smaller than < 3 μm^3 to large chains which exceeds a volume of > 30 μm^3. Introducing predatory ciliates in short-term food-selection and long-term chemostat experiments enhanced the frequencies of large morphotypes. In short-term batch experiments, the shift of morphotypes of *Acinetobacter* was mainly due to size-selective grazing by *Tetrahymena*. The results suggested that *Tetrahymena* preferably fed on single cells and small chains of *Acinetobacter* with a biovolume smaller than 6 μm^3 corresponding to a length of 1.5 to 8.0 µm. This is known to form the size range of filaments edible by *Tetrahymena* (Young 2006).

As long-term chemostat experiments indicated, the size distribution of morphotypes of *Acinetobacter* did not change under conditions of competition with *Pedobacter*, even though *Acinetobacter* obviously suffered from starvation as indicated by their low biovolumes persisting in the chemostats (Fig. 3). On the other hand, predation had a rather activating effect on the biovolumes per ml, especially when *Acinetobacter* established a huge amount of extreme large growth forms (more than 50 % of > 30 μm^3). Despite the large losses due to grazing, the biovolumes at grazing pressure were in the same range as in non-grazed monoxenic cultures of *Acinetobacter*. This stimulation of growth activity due to predation is a well-known phenomenon (Johannes 1965; Hahn *et al.* 1999).

One chemostat culture of *Acinetobacter* showed a comparatively slow and stepwise shift towards inedible morphotypes during the whole course of the

experiment. On average, the *Acinetobacter* population became more and more grazing resistant. Similar to (Garland & Kelly 2006) where they described this phenomenon for the evolution of adaptive phenotypic plasticity by means of positive directional selection over generations towards heat tolerance of one genotype.

For the replicate experiment a much faster shift to extremely large growth forms occurred (detectable already at day two). The overall morphotype of this *Acinetobacter* population was remarkably different from the other experiments. The intersections between each cell were only hardly visible within these growths forms (Fig. 3C and D). Filaments outcompeted chain like morphotypes at an early stage of the experiment. Filamentous growth forms led to the complete extinction of *Tetrahymena* at day 34. The establishment of filamentous growth forms of *Acinetobacter* should be genetically fixed since filamentous forms stayed in the chemostat until the end of the experiment (for about 90 *Acinetobacter* generations) in the absence of *Tetrahymena*.

The capacity of bacterial divergence under constant chemostat conditions is well-known for *Escherichia coli* (Maharjan *et al.* 2006). Microevolutionary phenomenona were recently observed for predator-prey systems consisting of rotifers and algae (Yoshida *et al.* 2007; Jones *et al.* 2009; Becks *et al.* 2010). Therein, defended and undefended genotypes oscillations counterbalanced each other, so that prey densities remained fairly constant while predator densities oscillated.

We could show in the present study that such changes in genotypes towards grazing resistant forms may easily occur. The grazing pressure by *Tetrahymena* should have supported the switch towards the grazing-resistant form. It would be desirable for future studies to identify responsible mutations in the genome as it was possible for *E. coli* (Notley-McRobb *et al.* 2003; Maharjan *et al.* 2006; Ferenci 2008).

2. 5 References

Agrawal A.A., Laforsch C. & Tollrian R. (1999). TRANSGENERATIONAL INDUCTION OF DEFENCES IN ANIMALS AND PLANTS. *Nature*, 401, 60-63.

Becks L., Ellner S.P., Jones L.E. & Hairston N.G. (2010). REDUCTION OF ADAPTIVE GENETIC DIVERSITY RADICALLY ALTERS ECO-EVOLUTIONARY COMMUNITY DYNAMICS. *Ecology Letters*, 13, 989-997.

Bennett A.F., Dao K.M. & Lenski R.E. (1990). RAPID EVOLUTION IN RESPONSE TO HIGH-TEMPERATURE SELECTION. *Nature*, 346, 79-81.

Bohannan B.J.M. & Lenski R.E. (2000). LINKING GENETIC CHANGE TO COMMUNITY EVOLUTION: INSIGHTS FROM STUDIES OF BACTERIA AND BACTERIOPHAGE. *Ecology Letters*, 3, 362-377.

Corno G., Caravati E., Callieri C. & Bertoni R. (2008). EFFECTS OF PREDATION PRESSURE ON BACTERIAL ABUNDANCE, DIVERSITY, AND SIZE-STRUCTURE DISTRIBUTION IN AN OLIGOTROPHIC SYSTEM. *Journal of Limnology*, 67, 107-119.

Corno G. & Jürgens K. (2006). DIRECT AND INDIRECT EFFECTS OF PROTIST PREDATION ON POPULATION SIZE STRUCTURE OF A BACTERIAL STRAIN WITH HIGH PHENOTYPIC PLASTICITY. *Applied and Environmental Microbiology*, 72, 78-86.

Ferenci T. (2008). BACTERIAL PHYSIOLOGY, REGULATION AND MUTATIONAL ADAPTATION IN A CHEMOSTAT ENVIRONMENT. *Advances in Microbial Physiology, Vol 53*, 53, 169-228.

Garland T. & Kelly S.A. (2006). PHENOTYPIC PLASTICITY AND EXPERIMENTAL EVOLUTION. *Journal of Experimental Biology*, 209, 2344-2361.

Hahn M.W. & Höfle M.G. (1999). FLAGELLATE PREDATION ON A BACTERIAL MODEL COMMUNITY: INTERPLAY OF SIZE-SELECTIVE GRAZING, SPECIFIC BACTERIAL CELL SIZE, AND BACTERIAL COMMUNITY COMPOSITION. *Applied and Environmental Microbiology*, 65, 4863-4872.

Hahn M.W. & Höfle M.G. (2001). GRAZING OF PROTOZOA AND ITS EFFECT ON POPULATIONS OF AQUATIC BACTERIA. *Fems Microbiology Ecology*, 35, 113-121.

Hahn M.W., Moore E.R.B. & Höfle M.G. (1999). BACTERIAL FILAMENT FORMATION, A DEFENSE MECHANISM AGAINST FLAGELLATE GRAZING, IS GROWTH RATE CONTROLLED IN BACTERIA OF DIFFERENT PHYLA. *Applied and Environmental Microbiology*, 65, 25-35.

Heldal M., Norland S. & Tumyr O. (1985). X-RAY MICROANALYTIC METHOD FOR MEASUREMENT OF DRY-MATTER AND ELEMENTAL CONTENT OF INDIVIDUAL BACTERIA. *Applied and Environmental Microbiology*, 50, 1251-1257.

Helling R.B., Vargas C.N. & Adams J. (1987). EVOLUTION OF ESCHERICHIA-COLI DURING GROWTH IN A CONSTANT ENVIRONMENT. *Genetics*, 116, 349-358.

Johannes R.E. (1965). INFLUENCE OF MARINE PROTOZOA ON NUTRIENT REGENERATION. *Limnology and Oceanography*, 10, 434-442.

Jones L.E., Becks L., Ellner S.P., Hairston N.G., Yoshida T. & Fussmann G.F. (2009). RAPID CONTEMPORARY EVOLUTION AND CLONAL FOOD WEB DYNAMICS. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 364, 1579-1591.

Jürgens K. & Matz C. (2002). PREDATION AS A SHAPING FORCE FOR THE PHENOTYPIC AND GENOTYPIC COMPOSITION OF PLANKTONIC BACTERIA. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology*, 81, 413-434.

Lenski R.E. & Levin B.R. (1985). CONSTRAINTS ON THE COEVOLUTION OF BACTERIA AND VIRULENT PHAGE - A MODEL, SOME EXPERIMENTS, AND PREDICTIONS FOR NATURAL COMMUNITIES. *American Naturalist*, 125, 585-602.

Lenski R.E. & Travisano M. (1994). DYNAMICS OF ADAPTATION AND DIVERSIFICATION - A 10,000-GENERATION EXPERIMENT WITH BACTERIAL-POPULATIONS. *Proceedings of the National Academy of Sciences of the United States of America*, 91, 6808-6814.

Leroi A.M., Lenski R.E. & Bennett A.F. (1994). EVOLUTIONARY ADAPTATION TO TEMPERATURE .3. ADAPTATION OF ESCHERICHIA-COLI TO A TEMPORALLY VARYING ENVIRONMENT. *Evolution*, 48, 1222-1229.

Maharjan R., Seeto S., Notley-McRobb L. & Ferenci T. (2006). CLONAL ADAPTIVE RADIATION IN A CONSTANT ENVIRONMENT. *Science*, 313, 514-517.

Maharjan R.P., Seeto S. & Ferenci T. (2007). DIVERGENCE AND REDUNDANCY OF TRANSPORT AND METABOLIC RATE-YIELD STRATEGIES IN A SINGLE ESCHERICHIA COLI POPULATION. *Journal of Bacteriology*, 189, 2350-2358.

Maruyama F., Yamaguchi N., Kenzaka T., Tani K. & Nasu M. (2004). SIMPLIFIED SAMPLE PREPARATION USING FRAME SPOTTING METHOD FOR DIRECT COUNTING OF TOTAL BACTERIA BY FLUORESCENCE MICROSCOPY. *Journal of Microbiological Methods*, 59, 427-431.

Massana R. & Jürgens K. (2003). COMPOSITION AND POPULATION DYNAMICS OF PLANKTONIC BACTERIA AND BACTERIVOROUS FLAGELLATES IN SEAWATER CHEMOSTAT CULTURES. *Aquatic Microbial Ecology*, 32, 11-22.

Matz C. & Kjelleberg S. (2005). OFF THE HOOK - HOW BACTERIA SURVIVE PROTOZOAN GRAZING. *Trends in Microbiology*, 13, 302-307.

Notley-McRobb L., Seeto S. & Ferenci T. (2003). THE INFLUENCE OF CELLULAR PHYSIOLOGY ON THE INITIATION OF MUTATIONAL PATHWAYS IN ESCHERICHIA COLI POPULATIONS. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 270, 843-848.

Pernthaler J. (2005). PREDATION ON PROKARYOTES IN THE WATER COLUMN AND ITS ECOLOGICAL IMPLICATIONS. *Nature Reviews Microbiology*, 3, 537-546.

Rainey P.B. & Travisano M. (1998). ADAPTIVE RADIATION IN A HETEROGENEOUS ENVIRONMENT. *Nature*, 394, 69-72.

Rosenzweig R.F., Sharp R.R., Treves D.S. & Adams J. (1994). MICROBIAL EVOLUTION IN A SIMPLE UNSTRUCTURED ENVIRONMENT - GENETIC DIFFERENTIATION IN ESCHERICHIA-COLI. *Genetics*, 137, 903-917.

Schönhuber W., Fuchs B., Juretschko S. & Amann R. (1997). IMPROVED SENSITIVITY OF WHOLE-CELL HYBRIDIZATION BY THE COMBINATION OF HORSERADISH PEROXIDASE-LABELED OLIGONUCLEOTIDES AND TYRAMIDE SIGNAL AMPLIFICATION. *Applied and Environmental Microbiology*, 63, 3268-3273.

Sherr E.B. & Sherr B.F. (2002). SIGNIFICANCE OF PREDATION BY PROTISTS IN AQUATIC MICROBIAL FOOD WEBS. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology*, 81, 293-308.

Simek K., Vrba J. & Hartman P. (1994). SIZE-SELECTIVE FEEDING BY CYCLIDIUM SP ON BACTERIOPLANKTON AND VARIOUS SIZES OF CULTURED BACTERIA. *Fems Microbiology Ecology*, 14, 157-167.

Spiers A.J., Buckling A. & Rainey P.B. (2000). THE CAUSES OF PSEUDOMONAS DIVERSITY. *Microbiology-Uk*, 146, 2345-2350.

Stanier R.Y., Palleron.Nj & Doudorof.M (1966). AEROBIC PSEUDOMONADS - A TAXONOMIC STUDY. *Journal of General Microbiology*, 43, 159-&.

van der Stap I., Vos M. & Mooij W.M. (2007). INDUCIBLE DEFENSES AND ROTIFER FOOD CHAIN DYNAMICS. *Hydrobiologia*, 593, 103-110.

van der Stap I., Vos M., Tollrian R. & Mooij W.M. (2008). INDUCIBLE DEFENSES, COMPETITION AND SHARED PREDATION IN PLANKTONIC FOOD CHAINS. *Oecologia*, 157, 697-705.

Wagner M., Erhart R., Manz W., Amann R., Lemmer H., Wedi D. & Schleifer K.H. (1994). DEVELOPMENT OF AN RIBOSOMAL-RNA-TARGETED OLIGONUCLEOTIDE PROBE SPECIFIC FOR THE GENUS ACINETOBACTER AND ITS APPLICATION FOR IN-SITU MONITORING IN ACTIVATED-SLUDGE. *Applied and Environmental Microbiology*, 60, 792-800.

Yoshida T., Ellner S.P., Jones L.E., Bohannan B.J.M., Lenski R.E. & Hairston N.G. (2007). CRYPTIC POPULATION DYNAMICS: RAPID EVOLUTION MASKS TROPHIC INTERACTIONS. *Plos Biology*, 5, 1868-1879.

Young K.D. (2006). THE SELECTIVE VALUE OF BACTERIAL SHAPE. *Microbiology and Molecular Biology Reviews*, 70, 660-703.

Chapter III

The influence of temperature on the population dynamics of a microbial microcosm – model analyses and chemostat experiments

3. 1 Introduction

Predator-prey interactions are triggered by external parameters like nutrient availability and different temperature regimes (Montagnes & Weisse 2000; Weisse *et al.* 2002; Gächter & Weisse 2006; Viergutz *et al.* 2007). Temperature fluctuations are common in most freshwater environments and can exhibit irregular patterns (Montagnes & Weisse 2000). In the background of climate change and rising frequency of weather extremes, increased maximum temperatures were predicted (Easterling *et al.* 2000; Walther *et al.* 2002). In The river Rhine the daily mean temperature changes up to 10°C during one month (data not shown). In smaller scales e.g. microhabitats even higher temperature fluctuations are possible, for example at the surface of a freshwater system. In field experiments, it is not possible to study the influence of temperature fluctuations in isolation because of uncontrollable variations external conditions like food web composition and nutrient availability. Therefore, experiments in the laboratory are necessary to study the influence of temperature on intrinsic population dynamics in a noise-reduced environment (Cadotte *et al.* 2005). Laboratory experiments were conducted in continuous culture in so called chemostats where different extrinsic parameters like temperature and dilution rate can be controlled. We use a defined microbial food web at controllable experimental conditions to study systematically the impact of daily changing temperatures between 16.7 and 29.7°C on the intrinsic population dynamics over time (Jessup *et al.* 2004).

For the first time, chaotic temperature fluctuations at different dilution rates were applied in order to disentangle their impacts on the general nature of the intrinsic population dynamics in a three species microbial food web. Chaotically changing temperatures were also studied in a corresponding mathematical model system. In a simplified model based on Takeuchi & Adachi (1983) temperature dependent growth rates were introduced and different

scenario analyses were performed. This gave a first idea of how the intrinsic population dynamics could be altered due to chaotic temperature fluctuations. The microbial food web consisted of three species a bacterivorous ciliate *Tetrahymena pyriformis* and two bacterial prey organisms *Acinetobacter johnsonii* and *Pedobacter sp*.

Detailed studies of the intrinsic dynamical behaviour of the present microbial food web displayed complex intrinsic population dynamics over time (Chapter I). Even simple microbial systems can have the potential for chaotic behaviour without any external stimulus (May 1974; Becks *et al.* 2005). It is a great challenge to interpret intrinsic, irregular, unforeseeable – though deterministic - population fluctuations at two external triggers: dilution rate and temperature.

3. 2 Methods

Experimental set up. Chemostat experiments were carried out in one-stage glass reactors with an average fluid content of 190 ml. Chemostats ran at different dilution rates per day (0.45, 0.5 and 0.75), this served as a bifurcation parameter similar to mathematical model analyses. All chemostats were placed in a polystyrene isolated water bath at dim light. Temperatures in the water bath of the chemostats were adjusted by a temperature control unit (JULABO FC600, Seelbach, Germany) which allowed the establishment of daily changing temperatures (16.9 - 29.7 ± 0.3°C) as well as stable temperatures (20 ± 0.3°C). Chaotically changing temperatures between 16.9 and 29.7°C were established by fitting temperatures on a chaotic time series of *Tetrahymena pyriformis* (Becks et al. (2005): figure 1d, p. 1227, Supplementary Material, Fig. 5). The value of the corresponding Lyapunov exponent was positive (0.22 ± 0.08) which is indicative of a chaotic time series.

The experimental design of the chemostats required sterile conditions. Therefore, all parts of the chemostat systems including medium reservoirs (10 l of PPY 100: 0.2 gl^{-1} proteose peptone, Fluka, Munich, Germany, 0.025 gl^{-1} yeast extract, Sigma, Steinheim, Germany) were sterilized prior to the experiments. Furthermore, sterile air was used for the gentle mixing of each chemostat.

Each chemostat was inoculated with a suspension of three species under sterile conditions: As predator, the ciliate *Tetrahymena pyriformis* (axenic culture, provided by the Culture Collection of Algae and Protists, Wintermere, U.K., strain CCAP 1630/1W), the bacterial prey, *Pedobacter sp.* (cytophaga flexibacter group, 2 µm x 1 µm, isolated by Kristin Beck (2000) from Lake Schöhsee and kindly provided by Klaus Jürgens, Baltic Sea Research Institute, IOW, Warnemünde, Germany) and *Acinetobacter johnsonii* (γ-proteobacteria; kindly provided as an isolate by Michael Steinert, University of Braunschweig, Germany). The bacteria were inoculated from overnight cultures (LB: 0.1 gl^{-1} tryptone, DIFCO, Michigan, USA; 0.05 gl^{-1} yeast extract; 0.1 gl^{-1} NaCl, Merck, Darmstadt, Germany) of a deep-frozen (-80 °C) stock. Enumeration and qualitative analyses were done as already described in Chapter I and II.

Theoretical analysis. The intrinsic dynamic behaviour of a two-prey-one-predator food web under chemostat conditions can be described by three differential equations (1 - 3) after Takeuchi & Adachi (1983) modified by Becks et al. (2005):

$$dx_1/dt = x_1 (b_1 - x_1 - \alpha x_2 - \varepsilon z - F) \qquad (1)$$

$$dx_2/dt = x_2 (b_2 - \beta x_1 - x_2 - \mu z - F) \qquad (2)$$

$$dz/dt = z (d\varepsilon x_1 + d\mu x_2 - F) \qquad (3)$$

x_1 and x_2 denote the densities of the two prey, b_1 and b_2 are parameters of the intrinsic growth rates of both bacteria. $\alpha > 0$ and $\beta > 0$ describe the competition between the two prey species. $\varepsilon > 0$ and $\mu > 0$ are coefficients of decrease of each prey species due to predation. z denotes the density of the predator population, within d is a coefficient of the amount of energy that can be used for growth. F is the dilution rate per day of the chemostat system. The intrinsic rate of decrease of the predator is equal to the dilution rate F. This assumption can be made because there is no other loss factor (e.g. cannibalism) for the predator.

In cooperation with Otto Richter (University of Braunschweig), the growth rates (r(T)) for each individual (b_1, b_2 and d) were modelled as a temperature dependent parameter according to the following function :

$$r(T) = h(T - T_{\min})\ r_{\max}\left(\frac{T_{\max} - T}{T_{\max} - T_{opt}}\right)^{x} \exp\left(x\frac{T - T_{opt}}{T_{\max} - T_{opt}}\right) \qquad (4)$$

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

and $$W = (Q_{10} - 1)(T_{\max} - T_{opt})$$

Numerical analyses were done using the equations (1-4) and the parameter estimates are given in Table 1. Numerical analyses were carried out with the software Modelmaker 4 (ModelKinetix, Wallingford, United Kingdom). The dilution rate (with 0.1 < F < 1.0) was used as bifurcation parameter for the different dynamic behaviours of the system to calculate the possible dynamic behaviour of the system. The population dynamics were analysed at constant temperature (20°C, time steps 1-400) at chaotic temperature fluctuations (the double sequence of Fig. x in the appendix, 16.9 - 29.7°C, time steps 400 - 460) and again at constant temperature (20°C, time steps 461-1000), altogether 1000 time steps.

Table 1: Values of the different parameter for the numerical analyses.

Parameter	**Value**	**Intend**
rmax1	2	max. growth rate of x1
rmax2	2	max. growth rate of x2
rmax3	1.5	max. growth rate of z
tmax	40	lethal temperature of x1 and x2
tmax1	35	lethal temperature of z
tmin	4	min. temp for growth x1 and x2
tmin1	10	min. temp for growth z
topt	26	optimal temp for x1 and x2
topt1	29	optimal temp for z
Q_{10}	2	metabolic factor
x_1 t(0)	0.2	initial population density
x_2 t(0)	0.2	initial population density
z t(0)	0.06	initial population density
α	1.0	competition coefficient x1
ε	4	selectivity for x1
β	1.5	competition coefficient x2
μ	1.0	prey preference
F	0.1 – 1.0	dilution rate (bifurcation parameter)

3. 3 Results

Temperature fluctuations - model analyses. Numerical analyses were carried out to study the principal behavior of a two-prey-one-predator system at two different temperature scenarios. The different temperature conditions were a first step at 20°C (time steps 0-400), then chaotic temperature fluctuations between 16.9 - 29.7°C and again 20°C (at 460-1000 time steps, third temperature condition). Sensitivity analyses revealed that coexistence of all tree species was possible at dilution rates of F = 0.7-1.0. Therein, the intrinsic behavior at constant temperature conditions (20°C) showed three different kinds of possible dynamics over time: Stable equilibrium (point attractor in phase space), stable limit cycles (ring attractor in phase space) and irregular fluctuations (strange attractor in phase space).

Between F = 0.86-1.0 (Fig. 1A) the model reaches a stable equilibrium after a short transient time at constant temperature (20°C). In the second step, during the chaotic temperature fluctuations, the system was highly disturbed. Aperiodic population dynamics are clearly visible in the time series data and in the corresponding phase space diagram (on the right hand side, where the abundances of the predator z were plottet against the prey x1). The irregular trajectories in the phase space (red lines) can be well distinguished from the point-attractor (marked in black) at constant temperature. In the third step the system reached its stable equilibrium (point attractor) again at constant temperature (20°C).

Between F = 0.81 - 0.85 (Fig. 1B) the system reaches stable limit cycles at constant temperature (20°C) during the second phase (chaotically changing temperatures) the population dynamics were highly disturbed. This is visible in the time series data and in the phase space diagram with the irregular trajectories (red line) around the ring attractor (black line). At constant temperature (20°C, time steps 460 - 1000), it reaches a stable limit cycle again.

Between F = 0.77 - 0.81 (Fig. 1C) the population dynamics of the three species system exhibited aperiodic cycles (black lines). The phase with chaotically changing temperatures was visible and affected the aperiodic population dynamic (phase space: strange attractor) as well as stable equilibrium (phase space: point attractor) and stable limit cycles (phase space: ring attractor). The

abundances fluctuate along to the temperature variations. Temperatures fluctuations affect the growth rates, which became manifest in increasing or decreasing abundances.

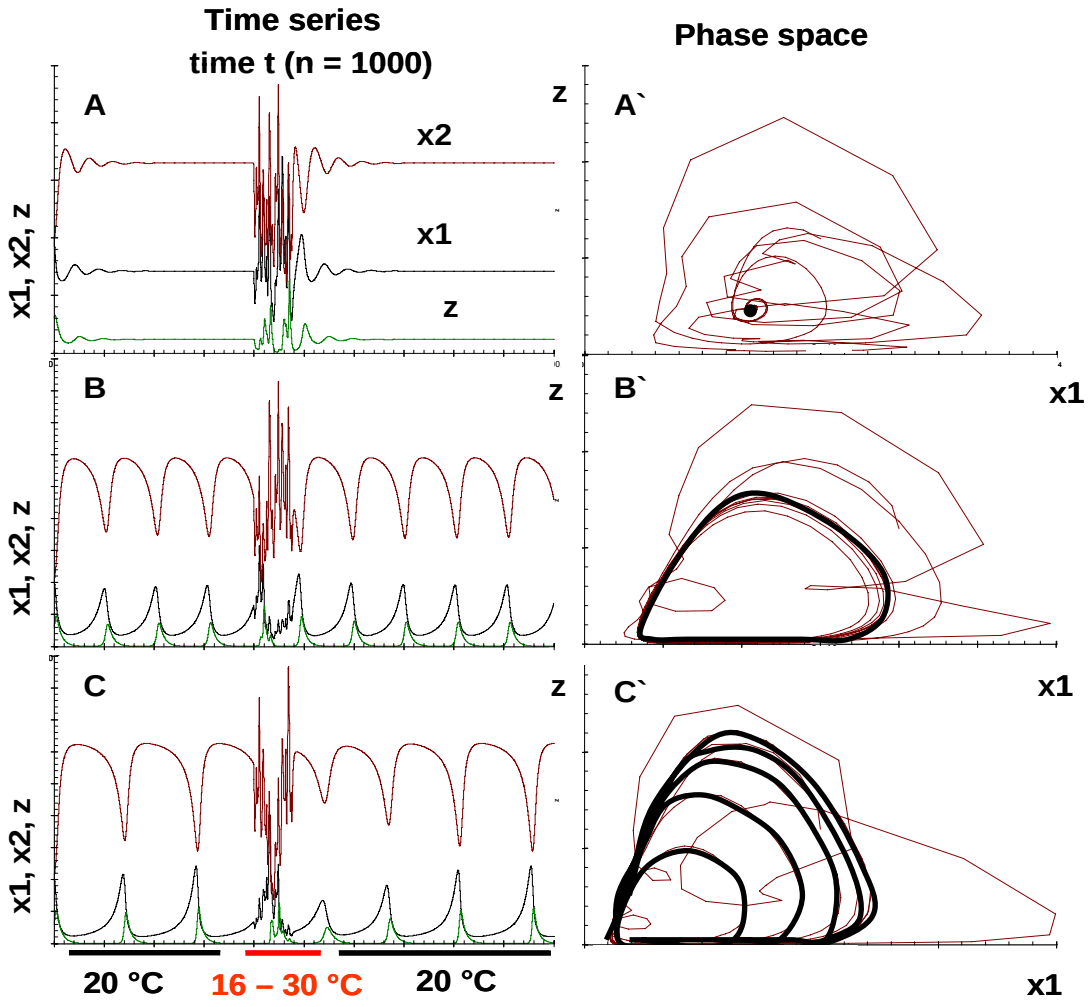

Fig. 1. Time series (left hand side) and corresponding phase space diagrams (right hand side, solid black lines underline the dynamical behaviour of the system at constant temperature) of the numerical analyses at different dilution rates and different temperature scenarios.

Temperature fluctuations - chemostat experiments. At first, a constant temperature (20°C, days 1-34, Fig. 2) was set, then daily changing temperatures between 16.9-29.7°C from days 35-72 and finally again constant conditions were established (20°C, days 73-102). Population dynamics were in general aperiodic at all dilution rates as well as for each temperature scenario. For the bacterial mean abundances (Fig. 3A), no clear trend was detectable

between each temperature step. Whereas, a slight increase of the mean *Tetrahymena* abundances at the dilution rates of 0.45, 0.5 and 0.75 were detectable (Fig. 5B). In the first temperature step (days 0-34, 20°C), the mean abundances ranged around 1 x 10^3 cells ml^{-1} for every established dilution rate. During the next temperature step (days 35-72, 16.9-29.7°C), the mean abundance of *Tetrahymena* increased to about 1.2 -1.5 x 10^3 cells ml^{-1} for each dilution rate. The final temperature step, back to constant temperature conditions at 20°C, led to an additional increase of the mean abundances of *Tetrahymena* ranging around 1.5-1.8 10^3 cells ml^{-1} for the dilution rates of 0.45 and 0.75 per day. This trend was also visible in the corresponding phase space diagrams (Fig. 4) of the time series data, where the *Tetrahymena* abundances were plotted against the abundances of *Acinetobacter* at that time. Each attractor moved progressively towards higher abundances of *Tetrahymena*.

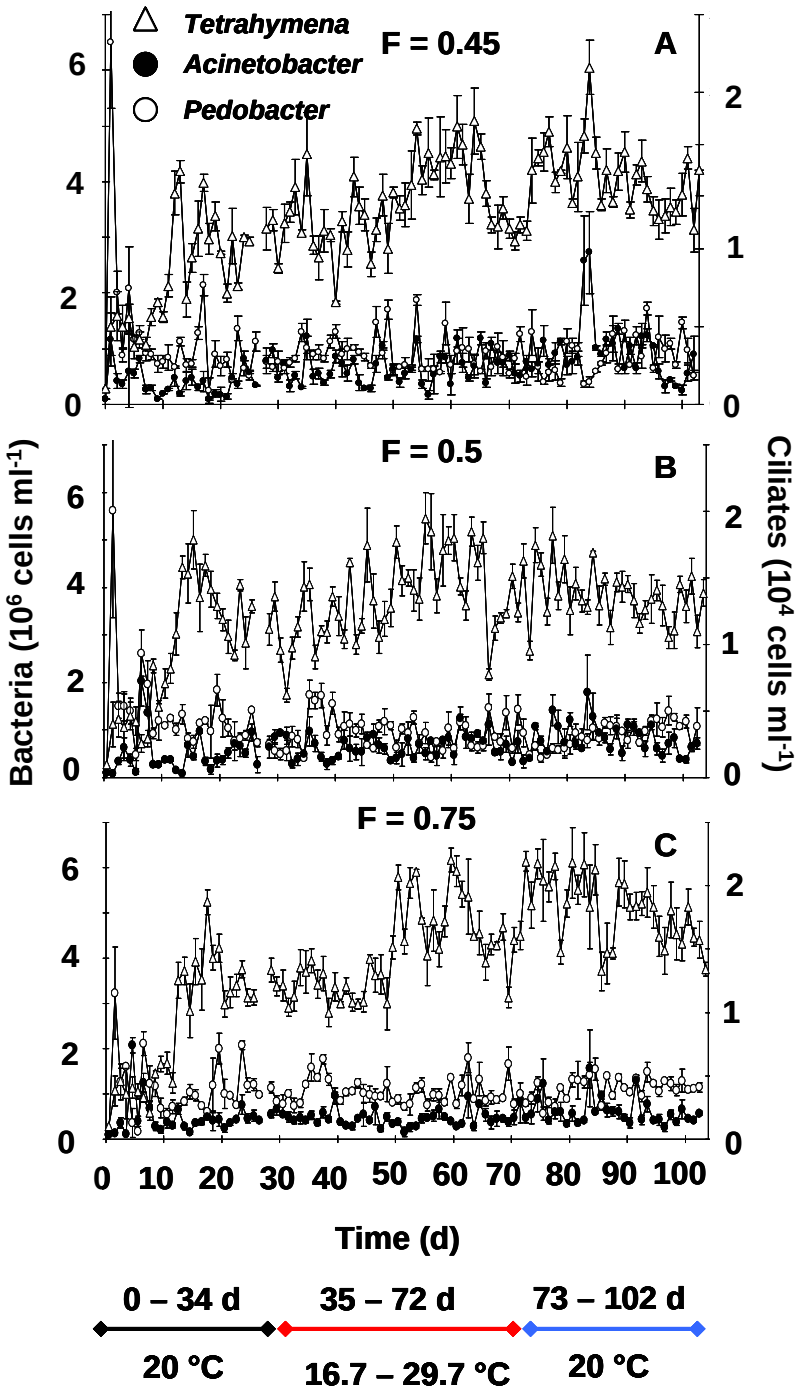

Fig. 2. Times series data of three species predator-prey chemostat experiments at dilution rates of 0.45 (A), 0.5 (B), 0.75 (C) at three different temperature steps: days 0-34 (20.0°C), days 35-72 (daily changing between 16.7 and 29.7°C), days 73-102 (20.0°C) Vertical bars re esent the standard deviation of triplicate samples.

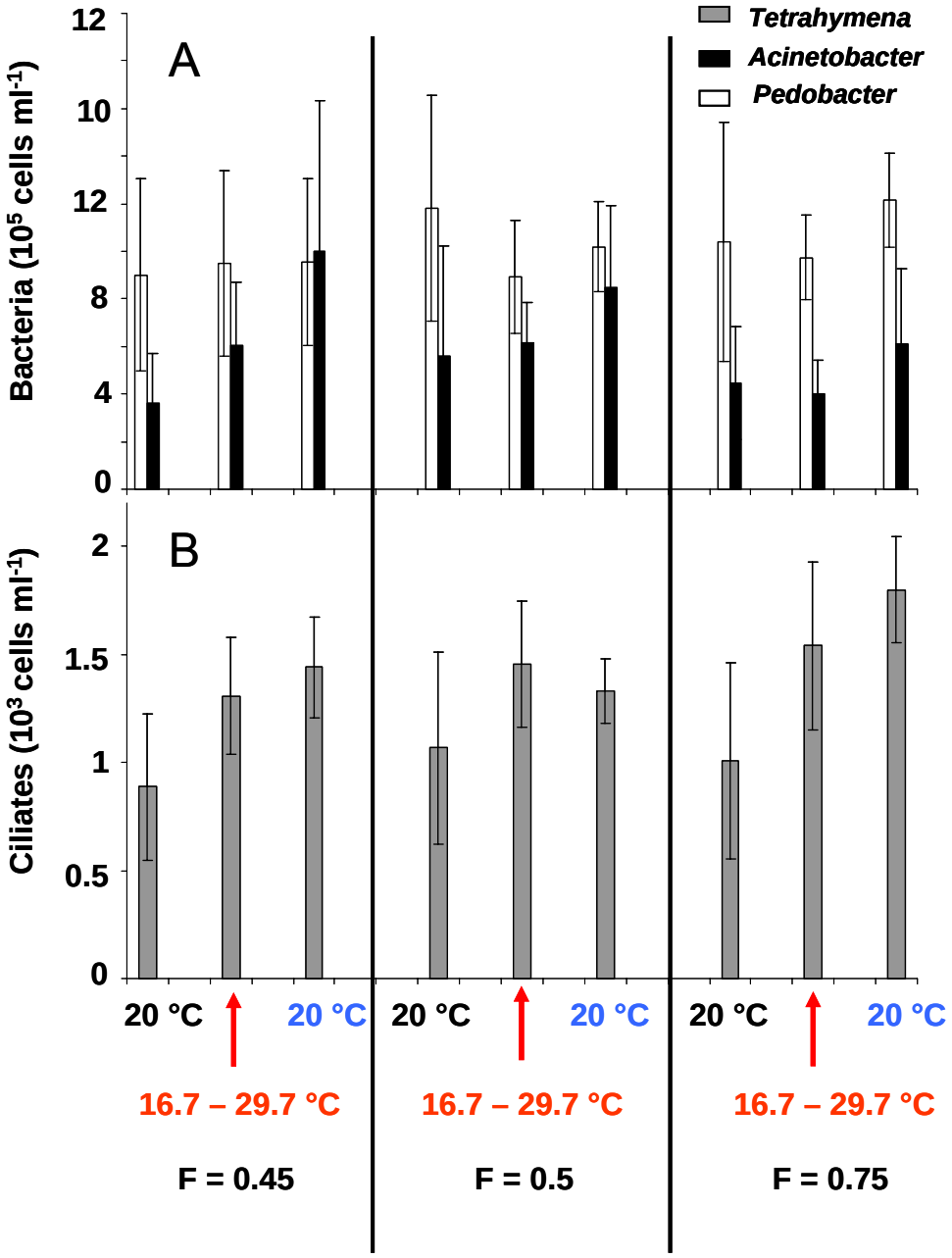

Fig. 3. Mean abundances and standart deviation of each species from days 10 to 30 at different dilution rates of the chemostat experiments (Fig. 2). A shows the abundances of both bacterial strains, B shows the abundances of the ciliate.

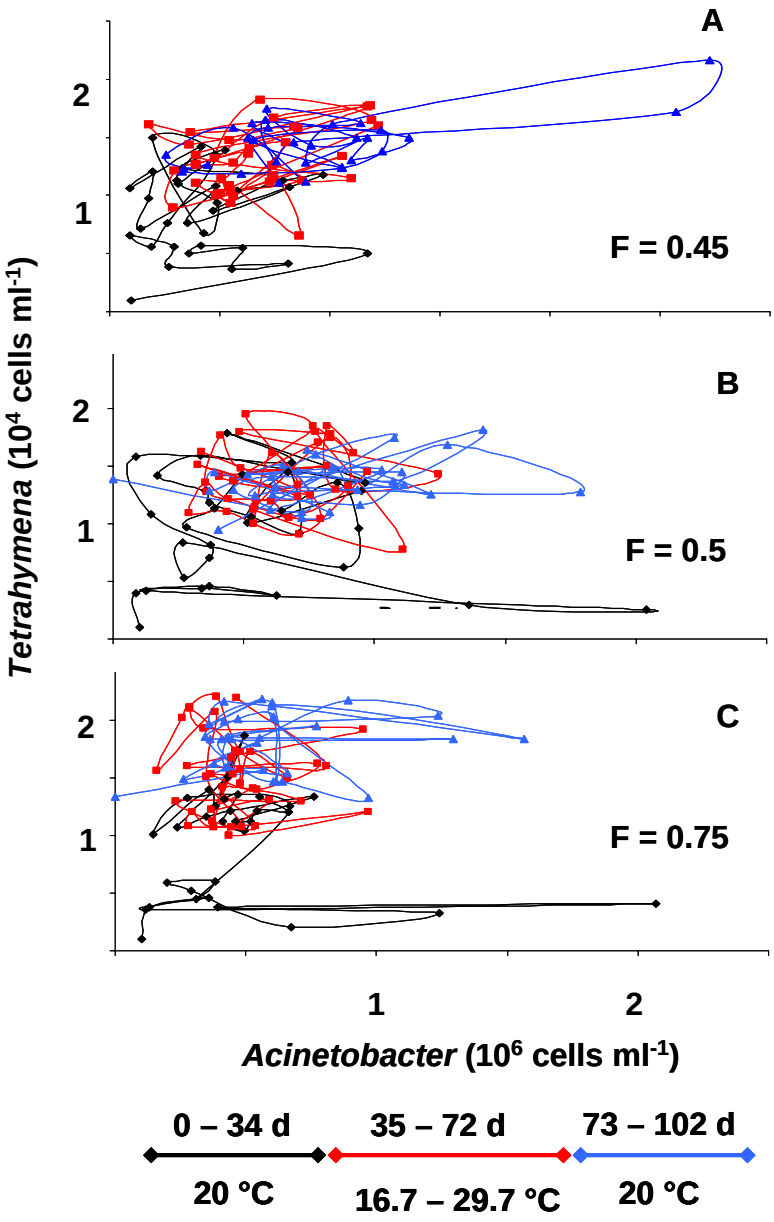

Fig. 4. Phase space diagrams of the three species predator-prey chemostat experiments at dilution rates of 0.45 (A), 0.5 (B), 0.75 (C) at three different temperature regimes: days 0-34 (20.0°C, black line), days 35-72 (chaotically changing between 16.7 and 29.7°C, red line), days 73-102 (20.0°C, blue line). The *Tretrahymena* abundances were plottet against the *Acinetobacter* abundances at that time.

3. 4 Discussion

For the first time, chaotically varying temperatures were applied on a well defined microbial food web at three different dilution rates. The interpretation of the time series data is a great challenge, since the intrinsic population dynamics at constant external conditions exhibited already aperiodic oscillations (Chapter I). However, the combination of theoretical and experimental studies should have shed some light on the underlying mechanisms.

Numerical analyses with a simplified mathematical model showed that intrinsic population dynamics can be highly affected by fluctuating temperatures (Fig. 1). Sensitivity analyses showed different intrinsic population dynamics like stable equilibrium, stable limit cycles as well as irregular population dynamics within a narrow range of coexistence (F = 0.7-1) at constant temperature.

Unlike to these model predictions, corresponding chemostat experiments in the lab revealed, a broader range of coexistence at least between F = 0.1-1.2 (data partly not shown and Supplementary Material, Fig. 6) at constant temperature conditions. This phenomenon may be explained by the morphological heterogeneous bacterial strain *Acinetobacter*. This bacterial strain is able to form grazing resistant morphotypes (Chapter II) which might have stabilised the predator-prey dynamics (Vos *et al.* 2004; van der Stap *et al.* 2009). This been theoretically confirmed by David Heckmann, a bachlor stundent who worked in our working group in 2009 (Supplementary Material, Fig 2). Numerical analyses were carried out with a simplified mathematical model, were predator triggered morphological changes of one bacterial strain was not implemented. This might explain the differences in the range of coexistence between model predictions and laboratory experiments made within this study.

As I have shown in Chapter I, the intrinsic dynamic behaviour of the present microbial food web had a broad tendency to exhibit irregular population dynamics. This phenomenon could not be changed by using the dilution rate as a bifurcation parameter. Unlike to model predictions, stable limit cycles or stable equlibria could not be detected yet. Becks et al. (2005) found for bacterial preys without any kind of phenotypic plasticity three different kinds of population

dynamics like stable limit cycles at F = 0.45, chaos at F = 0.5 and stable equilibrium at F = 0.75.

It is rather difficult to disentangle the impact of fluctuating temperatures from intrinsically driven irregular population fluctuations. Nevertheless, the different temperature regimes had an activating impact on productivity of *Tetrahymena* (Fig. 3 and 4). This phenomenon may be due to changing environments like temperature fluctuations as well as changing nutrient availability. Changing external trigger caused changing selection pressures which may stimulate adaptation processes (Rainey & Travisano 1998; Spiers *et al.* 2000; Beaumont *et al.* 2009). As I have already shown for *Acinetobacter* (Chapter II), microevolutionary processes might also play an important role for the population of *Tetrahymena*.

Analyses of the morphology of *Acinetobacter* in a three species chemostat experiment at an intermediate dilution rate of 0.5 per day (Supplementary Material, Fig. 7A) revealed that there occurred daily changes in the size frequency distribution of *Acinetobacter* (Supplementary Material Fig. 7B). The changes occurred almost regularly with a period of three days, where the fraction of volumes larger than 6 μm^3 fluctuated between 40 and 20 %. The morphological adaptations towards larger growth forms of *Acinetobacter* were not as strong as for two species predator-prey chemostat experiments (Chapter II). For the present three species microbial food web, two selection pressures affected *Acinetobacter* simultaneously: Grazing and competition. Grazing by *Tetrahymena* and competition with *Pedobacter*, might shift the morphology of *Acinetobacter* at the same time. Competition with *Pedobacter* for the present resources might lead to a limitated nutrient availability. It is known for an other *Acinetobacter* strain that starvation resulted in single celled coccoid morphotypes (James *et al.* 1995). Therefore, a trade-off between grazing resistant, large morphotypes and single-celled competitors for nutrients might be contributed to the heterogonous morphology of *Acinetobacter* in the three species microbial food web.

We can conclude, that intrinsic irregular population dynamics were only slightly affected by daily temperature fluctuations around 16.7 and 29.7°C. Interactions within the populations like predation and competition had obviously a stronger

effect on the dynamics than external triggers like dilution rate and temperature fluctuations.

3. 5 References

Beaumont H.J.E., Gallie J., Kost C., Ferguson G.C. & Rainey P.B. (2009). EXPERIMENTAL EVOLUTION OF BET HEDGING. *Nature*, 462, 90-97.

Becks L., Hilker F.M., Malchow H., Jurgens K. & Arndt H. (2005). EXPERIMENTAL DEMONSTRATION OF CHAOS IN A MICROBIAL FOOD WEB. *Nature*, 435, 1226-1229.

Cadotte M.W., Drake J.A. & Fukami T. (2005). CONSTRUCTING NATURE: LABORATORY MODELS AS NECESSARY TOOLS FOR INVESTIGATING COMPLEX ECOLOGICAL COMMUNITIES. *Advances in Ecological Research, Vol. 37: Population Dynamics and Laboratory Ecology*, 37, 333-353.

Easterling D.R., Meehl G.A., Parmesan C., Changnon S.A., Karl T.R. & Mearns L.O. (2000). CLIMATE EXTREMES: OBSERVATIONS, MODELING, AND IMPACTS. *Science*, 289, 2068-2074.

Gächter E. & Weisse T. (2006). LOCAL ADAPTATION AMONG GEOGRAPHICALLY DISTANT CLONES OF THE COSMOPOLITAN FRESHWATER CILIATE MESERES CORLISSI. I. TEMPERATURE RESPONSE. *Aquatic Microbial Ecology*, 45, 291-300.

James G.A., Korber D.R., Caldwell D.E. & Costerton J.W. (1995). DIGITAL IMAGE-ANALYSIS OF GROWTH AND STARVATION RESPONSES OF A SURFACE-COLONIZING *ACINETOBACTER SP. Journal of Bacteriology*, 177, 907-915.

Jessup C.M., Kassen R., Forde S.E., Kerr B., Buckling A., Rainey P.B. & Bohannan B.J.M. (2004). BIG QUESTIONS, SMALL WORLDS: MICROBIAL MODEL SYSTEMS IN ECOLOGY. *Trends in Ecology & Evolution*, 19, 189-197.

May R.M. (1974). BIOLOGICAL POPULATIONS WITH NONOVERLAPPING GENERATIONS - STABLE POINTS, STABLE CYCLES, AND CHAOS. *Science*, 186, 645-647.

Modelmaker 4 for Windows. (2001-2003). *ModelKinetix, Wallingford, United Kingdom*

Montagnes D.J.S. & Weisse T. (2000). FLUCTUATING TEMPERATURES AFFECT GROWTH AND PRODUCTION RATES OF PLANKTONIC CILIATES. *Aquatic Microbial Ecology*, 21, 97-102.

Rainey P.B. & Travisano M. (1998). ADAPTIVE RADIATION IN A HETEROGENEOUS ENVIRONMENT. *Nature*, 394, 69-72.

Spiers A.J., Buckling A. & Rainey P.B. (2000). THE CAUSES OF PSEUDOMONAS DIVERSITY. *Microbiology-Uk*, 146, 2345-2350.

Takeuchi, Y. and N. Aadachi. (1983). EXISTENCE AND BIFURCATION OF STABLE EQUILIBRIUM IN TWO-PREY-ONE-PREDATOR COMMUNITIES. *Bulletin of Mathematical Biology*. 45, 877-900.

van der Stap I., Vos M., Kooi B.W., Mulling B.T.M., van Donk E. & Mooij W.M. (2009). ALGAL DEFENSES, POPULATION STABILITY, AND THE RISK OF HERBIVORE EXTINCTIONS: A CHEMOSTAT MODEL AND EXPERIMENT. *Ecological Research*, 24, 1145-1153.

Viergutz C., Kathol M., Norf H., Arndt H. & Weitere M. (2007). CONTROL OF MICROBIAL COMMUNITIES BY THE MACROFAUNA: A SENSITIVE INTERACTION IN THE CONTEXT OF EXTREME SUMMER TEMPERATURES? *Oecologia*, 151, 115-124.

Vos M., Kooi B.W., DeAngelis D.L. & Mooij W.M. (2004). INDUCIBLE DEFENCES AND THE PARADOX OF ENRICHMENT. *Oikos*, 105, 471-480.

Walther G.R., Post E., Convey P., Menzel A., Parmesan C., Beebee T.J.C., Fromentin J.M., Hoegh-Guldberg O. & Bairlein F. (2002). ECOLOGICAL RESPONSES TO RECENT CLIMATE CHANGE. *Nature*, 416, 389-395.

Weisse T., Stadler P., Lindstrom E.S., Kimmance S.A. & Montagnes D.J.S. (2002). INTERACTIVE EFFECT OF TEMPERATURE AND FOOD CONCENTRATION ON GROWTH RATE: A TEST CASE USING THE SMALL FRESHWATER CILIATE *UROTRICHA FARCTA. Limnology and Oceanography*, 47, 1447-1455.

Supplementary Material

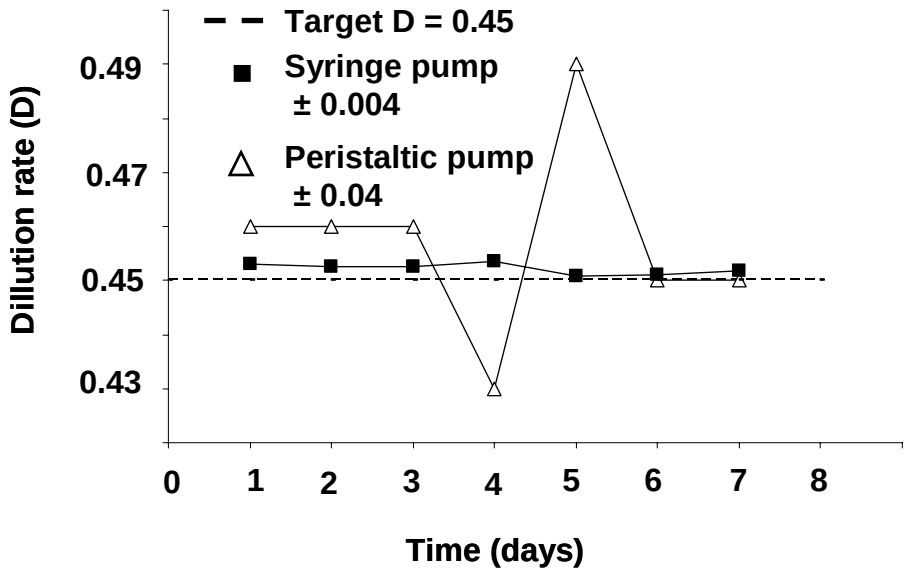

Fig. 1. Comparison between syringe pump (black squares) and peristaltic pump (open triangles) during chemostat experiments. The target dilution rate is 0.45 per day (dashed line).

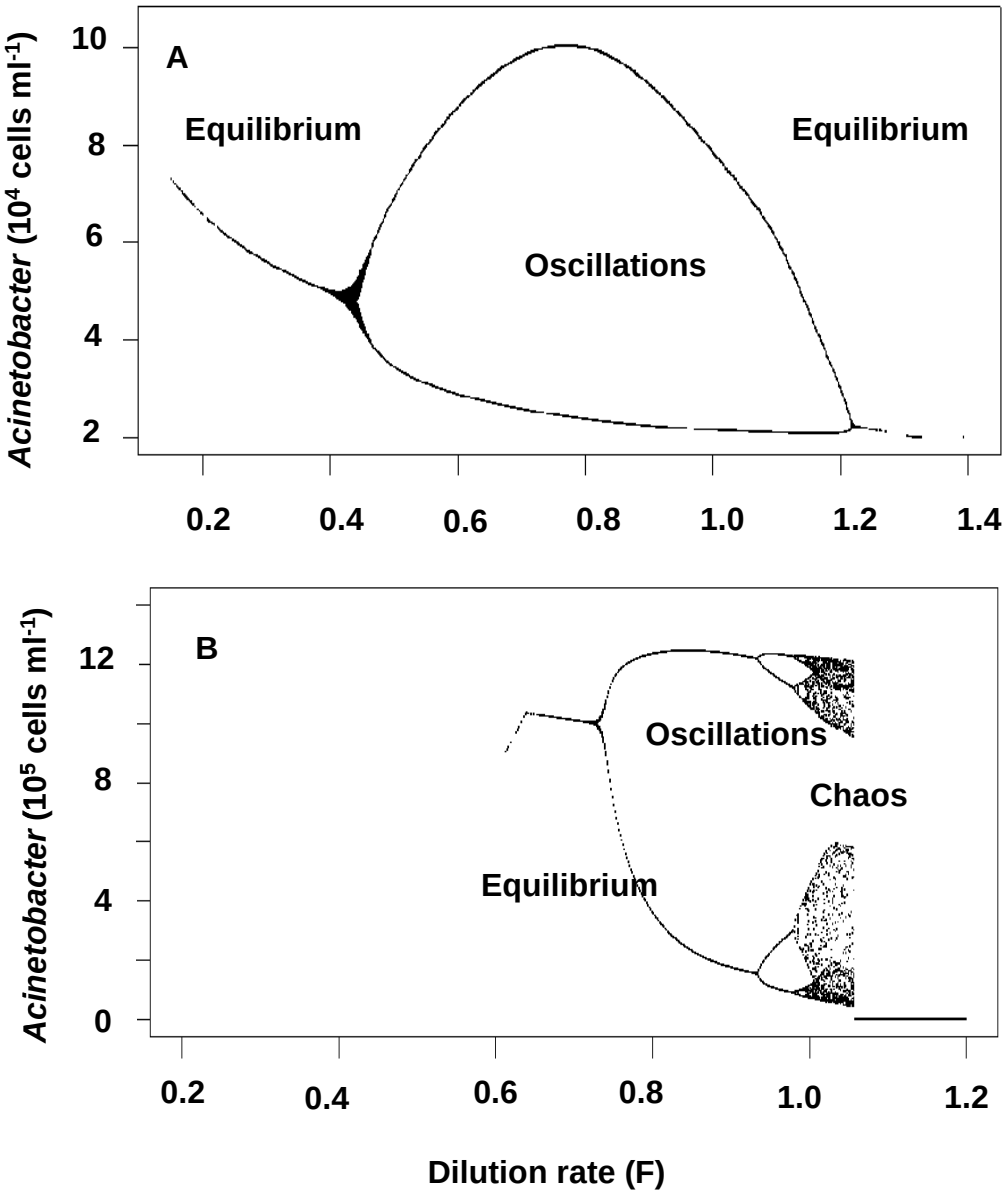

Fig. 2. Bifurcation diagrams of mathematical models with grazing resistance (A) and without grazing resistance (B) where coexistence of all three species was possible. Local minima and maxima of the bacterial abundances were plotted against the dilution rates. Population dynamics shift from equilibrium to oscillations and back to equilibrium (A) or to chaos (B) (Model analyses were done by David Heckmann a bachelor student in 2009).

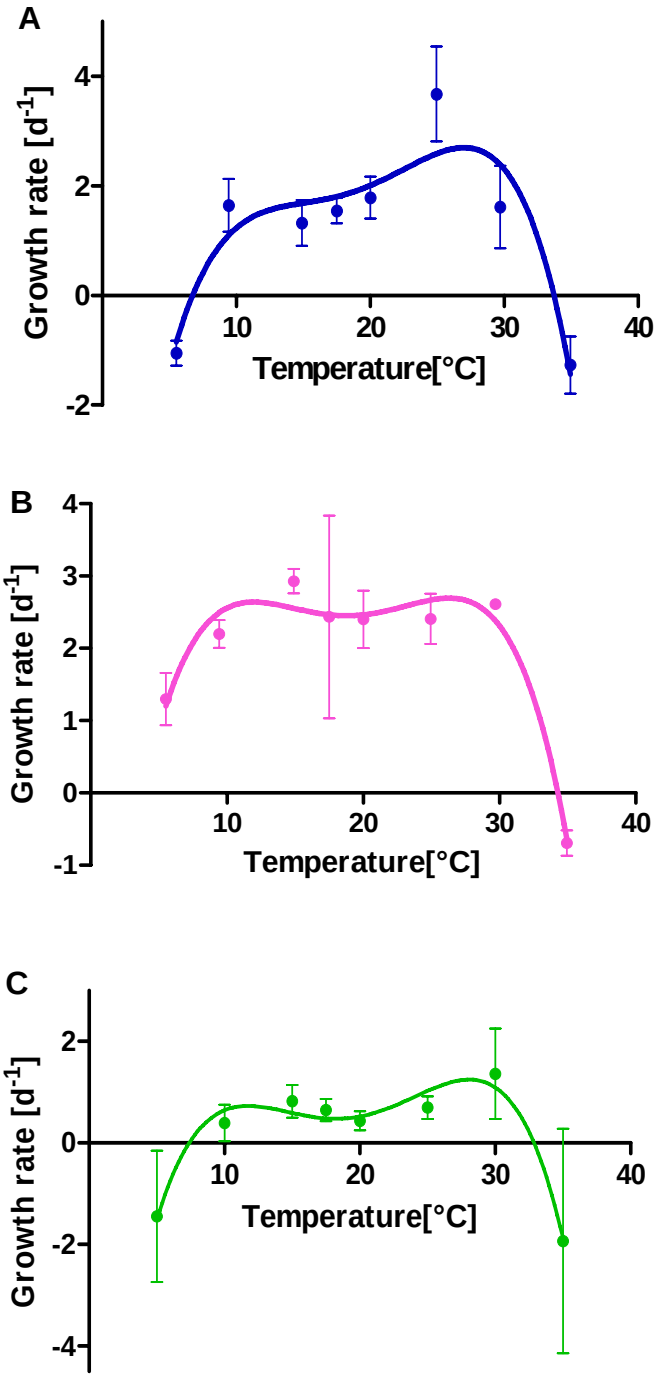

Fig. 3. Temperature dependent growth rates of *Acinetobacter* (A), *Pedobacter* (B) and *Tetrahymena* (C) (Experimental work was done by my colleague Mar Monsonís Nomdedeu).

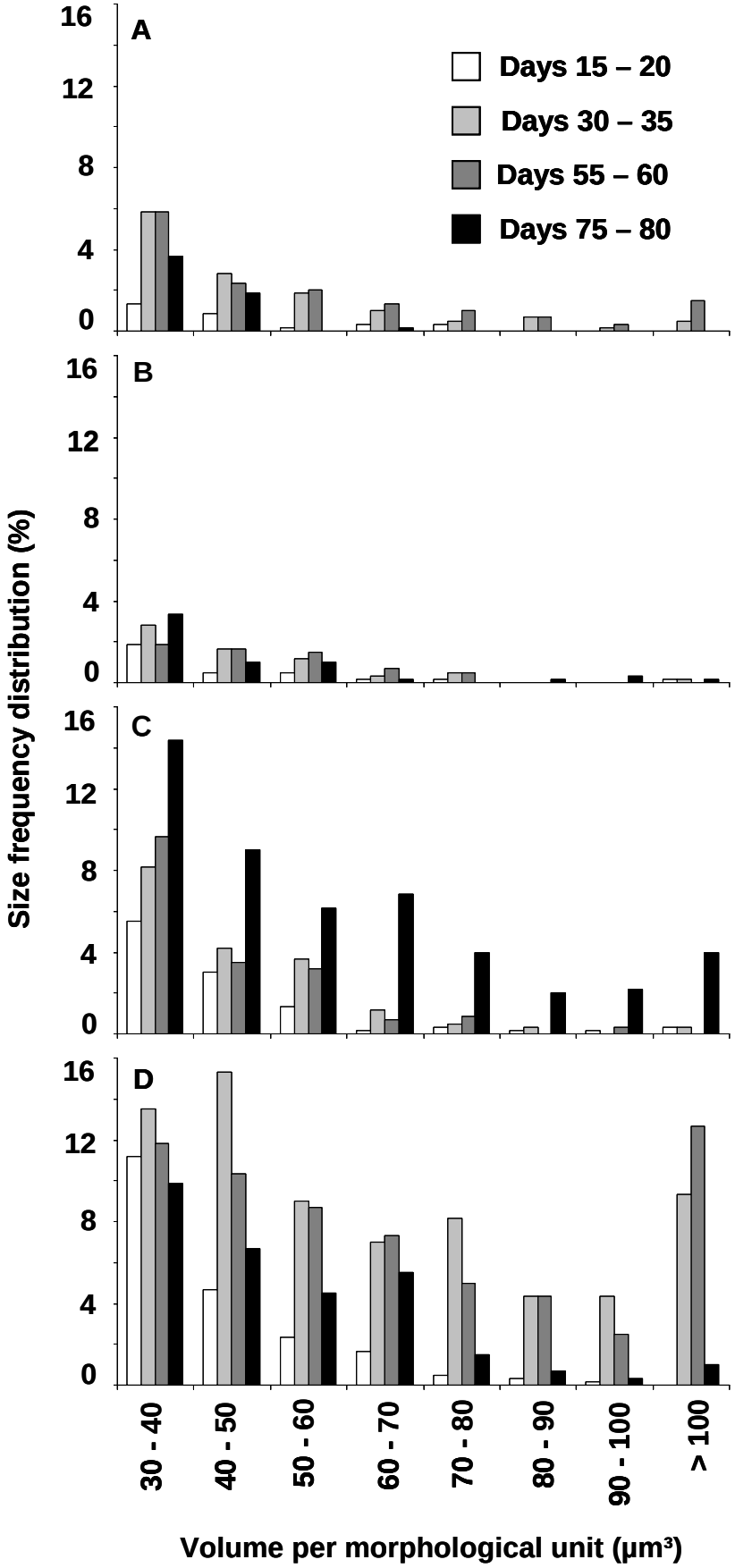

Fig. 4. Size frequency distribution of cells with a volume of 30 to > 100 µm³ of each chemostat experiment at four time periods in a single species system (A) in a two species competition system with *Acinetobacter* and *Pedobacter* (B), C and D are two species predator-prey systems with *Acinetobacter* and *Tetrahymena.*

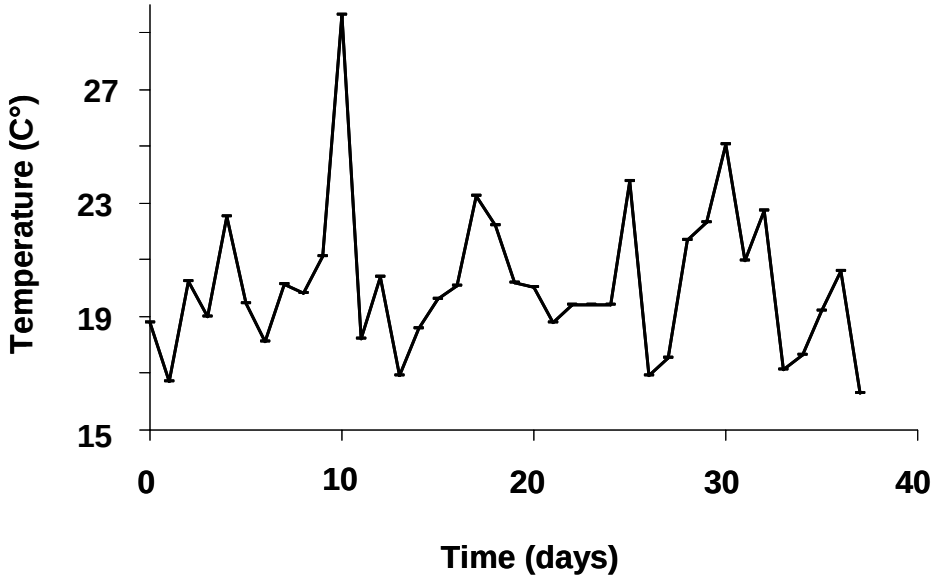

Fig. 5. Chaotically changing temperatures between 16.9 and 29.7°C were established by fitting temperatures on a chaotic time series of *Tetrahymena pyriformis* (Becks et al. (2005): figure 1d, p. 1227).

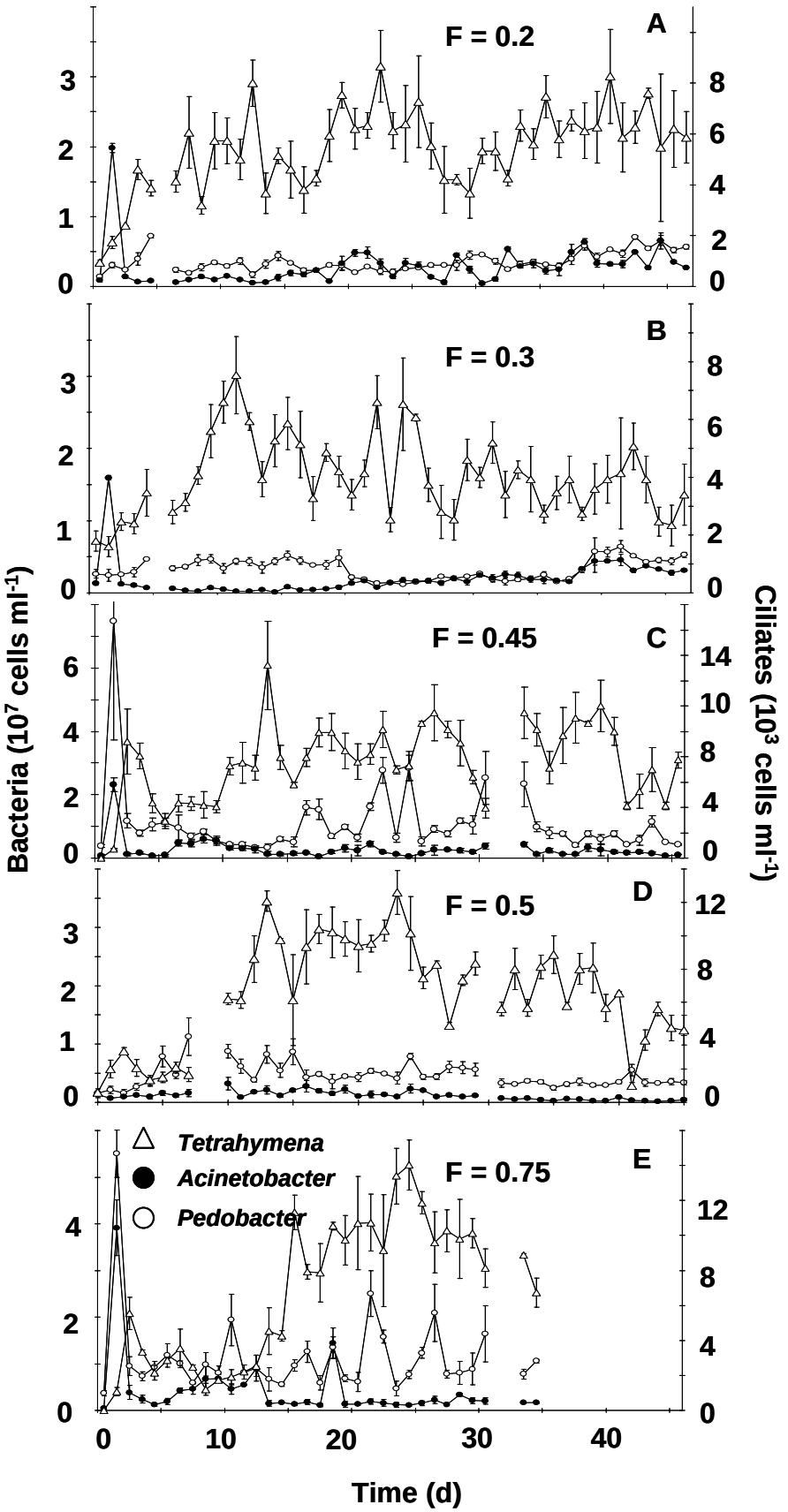

Fig. 6. Times series data of three species predator-prey chemostat experiments at dilution rates of 0.2 (A), 0.3 (B), 0.45 (C), 0.5 (D) and 0.75 (E) per day at constant temperature (20°C). Vertical bars represent the standard deviation of triplicate samples taken every 24 hours separately from each chemostat.

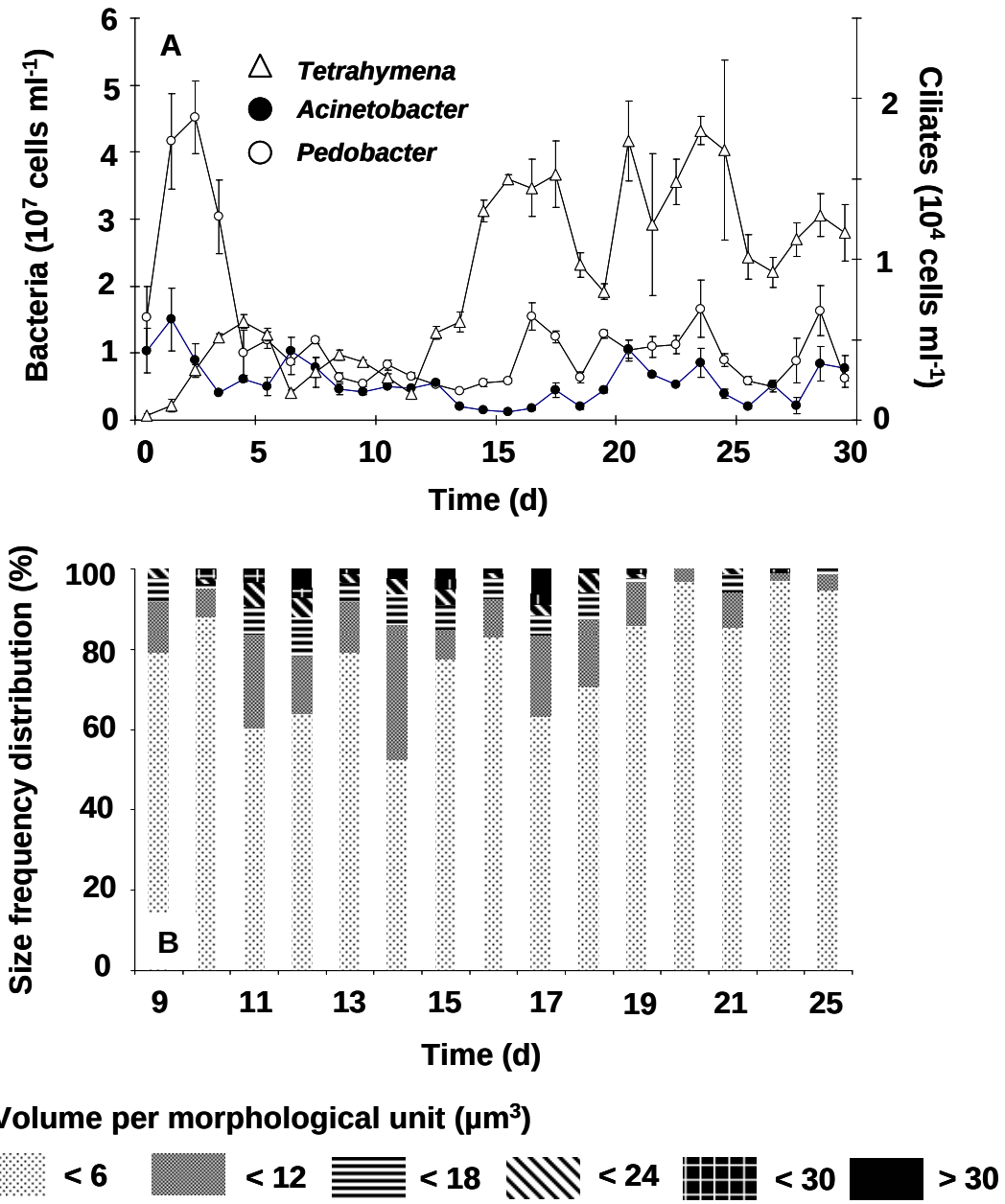

Fig. 7. Time series data (A) of three species predator-prey chemostat experiment at a dilution rate of 0.5 per day and at constant temperature (20°C). Vertical bars represent the standard deviation of triplicate samples. Fraction of the different volumes (µm^3) of the *Acinetobacter* population of chemostat experiment (A) of days 9-25.

Abstract

Population dynamics are triggered by intrinsic and extrinsic parameters. Extrinsic parameters can be distinguished in biotic factors and abiotic factors. Abiotic factors include amongst others temperature, light and habitat structure. The most important biotic factors are for example competition for the present resources (nutrients and space) and predator-prey dynamics. For ecologists it is rather difficult to distinguish density dependent (purely intrinsic population dynamics) from the influences of environmental factors (extrinsic factors).

A simplified predator-prey system was designed to examine in detail the impact of intrinsic and extrinsic parameters on the population dynamics over time. Therefore, my colleagues and me established a highly controllable and to the greatest possible extent automated experimental setup for chemostat experiments. Inaccuracies like fluctuations in the dilution rate (nutrient inflow), unintended temperature fluctuations and the risk of contaminations with other bacteria or fungi could be minimized. The first aim of the present work was to study purely intrinsic driven population dynamics. The microbial model system consisted of three species, two different bacterial strains (*Acinetobacter johnsonii* and *Pedobacter sp.*) and a ciliate predator (*Tetrahymena pyriformis*).

Here, it is shown for the first time that intrinsically driven aperiodic population dynamics may play an important role for the coexistence of species. Microbial interactions showed, with increasing complexity from single-species systems to a three-species system in highly controllable chemostat experiments, the tendency to exhibit intrinsic chaotic population dynamics. Therefore, chaotic population dynamics can arise from strictly deterministic and density-dependent mechanisms. Indicators for a chaotic behaviour were 1) sensitivity of dynamics towards initial conditions, 2) continuous variability of abundances under constant environmental conditions and 3) positive values of corresponding Lyapunov exponents.

Adaptive phenotypic plasticity and microevolution are the most powerful mechanisms of bacteria to reduce the risk of predation by protists. Changes in the morphology of the bacteria *Acinetobacter johnsonii* (gamma-proteobacteria) under grazing pressure by the ciliate *Tetrahymena pyriformis* were studied in short-term food-selection experiments and in long-term chemostat experiments.

Strong selection pressures like predation may be a strong driver of microevolutionary processes. Numerical model analyses and corresponding three-species chemostat experiments revealed an increasing range of coexistence if one bacterial prey exhibited predation triggered shifts towards larger growth forms, using the dilution rate as an extrinsic bifurcation parameter.

Temperature fluctuations are common in almost every natural habitat. Temperature is one of the most important extrinsic factor influencing the overall intrinsic biological processes like metabolic growth, respiration, ingestion, reproduction. The influence of temperature gained more and more attention ever since the awareness of the global climate change. The response to global warming in ecosystems is a complex interplay of populations and their temperature dependent reaction norms. For the first time, chaotic temperature fluctuations were chosen as an extrinsic trigger, which may influence the intrinsic behavior of the microbial two-prey-one-predator system. It is likely that chaotic temperature fluctuations drive intrinsic population dynamics into aperiodic fluctuations. In the present study, aperiodic population dynamics dominated at both trophic levels at different external triggers like dilution rate and temperature. Intrinsically driven irregularities in population dynamics may play a more important role in real ecosystems within the trophic cascades as expected until now.

Kurzzusammenfassung

Organismen interagieren innerhalb und zwischen den verschiedenen trophischen Ebenen, beispielsweise durch Konkurrenz oder durch Räuber-Beutebeziehungen. Daraus ergibt sich ein dynamisches Verhalten, welches eine entscheidende Rolle in der Koexistenz der Arten und für das Funktionieren eines Ökosystems spielt. Die Analyse der Populationsdynamiken innerhalb eines Ökosystems ist sehr komplex, weil sich extrinsische (biotische und abiotische Faktoren) und intrinsische (dichteabhängige) Dynamiken überlagern.

In der vorliegenden Arbeit geht es darum, die verschiedenen Faktoren im Einzelnen zu analysieren und deren Bedeutung für die Interaktionen des gesamten Systems abzuschätzen. Im Vorfeld wurden entsprechende mathematische Modelluntersuchungen durchgeführt, die Arbeitshypothesen für die experimentellen Arbeiten lieferten. Um extrinsische und intrinsische Dynamiken systematisch untersuchen zu können, wurden Experimente im Labor durchgeführt. Als Modellsystem wurde ein vereinfachtes aquatisches mikrobielles Nahrungsnetz untersucht bestehend aus zwei unterschiedlichen Bakterien-Stämmen (*Acinetobacter johnsonii* und *Pedobacter sp*.) und einem räuberischen Ciliaten (*Tetrahymena pyriformis*). Innerhalb dieser Arbeit ist es gelungen, den experimentellen Aufbau für Chemostate, die unter sehr konstanten Bedingungen analysiert werden sollen, zu optimieren. Ungewollte externe Schwankungen, wie beispielsweise in der Durchflussrate, bei der Probennahme und in den Temperaturbedingungen konnten auf ein absolutes Minimum reduziert werden. Mit diesem experimentellen Aufbau konnte gezeigt werden, dass die intrinsischen, dichteabhängigen Populationsdynamiken ohne externe Einflüsse bereits sehr komplex sind und auf ein chaotisches Verhalten schließen lassen.

Erstmalig haben Modelluntersuchungen und entsprechende Experimente im Labor gezeigt, dass induzierbare Fraßschutzmechanismen maßgeblich dazu beitragen können, dass der Bereich der Koexistenz der drei Arten sich erweitert, wenn die Durchflussrate als extrinsischer Parameter, welcher einen Einfluss auf die Wachstumsrate der einzelnen Organismen hat, verändert wird.

Schwankungen in der Temperatur sind in nahezu allen Ökosystemen weit verbreitet. Diese reichen von kurzzeitigen, täglichen Fluktuationen bis hin zu jahreszeitlichen Temperatureffekten. Wenn man eine globale Erderwärmung in Betracht zieht, bekommt die Analyse des Temperatureinflusses eine noch größere Bedeutung. Innerhalb dieser Arbeit wurden zum ersten Mal Analysen mit der Wirkung von extrinsischen, chaotischen Temperaturschwankungen auf das dynamische Verhalten eines mikrobiellen Nahrungsnetzes durchgeführt. Die Ergebnisse lieferten Hinweise darauf, dass intrinsisch bedingtes chaotisches Verhalten durch chaotische Temperaturschwankungen beeinflusst werden kann. Das untersuchte mikrobielle Nahrungsnetz ist charakterisiert durch Räuber-Beute Dynamiken, Fraßschutzmechanismen und Konkurrenz. Die Betrachtung dieser Faktoren im Einzelnen und im Zusammenspiel zeigte, dass aperiodische Populationsdynamiken in dem vereinfachten Modellsystem dominierten, auch wenn die externen Parameter, wie Temperatur und Durchflussrate verändert wurden. Diese grundsätzliche dynamische Eigenschaft hat vermutlich einen größeren Einfluss in der Natur auf die verschiedenen Glieder eines Ökosystems als bisher angenommen.

Köln, den 03. Oktober 2010

Erklärung

Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit - einschliesslich Abbildungen und Tabellen - die anderen Werken im Wortlaut oder dem Sinn nach entnommen habe, in jedem Einzellfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie noch nicht veröffentlicht worden ist sowie, dass ich solche Veröffentlichung 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.

_____

Christine Willen

Lebenslauf

Zu meiner Person:

Christine Willen
Melatener Weg 31
50825 Köln
Mobil: 0176 25539292
E-Mail: cwillen@uni-koeln.de

Geburtsdatum, -ort:	06. Juni 1982, Wildeshausen
Staatsangehörigkeit:	deutsch
Familienstand:	ledig, nicht ortsgebunden

Schulausbildung:

07/1994 bis 06/2001	Copernicus Gymnasium in Löningen Abschluss Abitur (Leistungskurse: Französisch und Biologie; Durchschnittsnote: 2,8)

Studium und Promotion:

	Abschluss der Promotion voraussichtlich 11/2010
Seit 09/2007	Promotionsstudium und wissenschaftliche Mitarbeiterin am Zoologischen Institut der Universität zu Köln im Rahmen des von der DFG geförderten Forschungsprojektes „The impact of climate variability on aquatic ecosystems“. Ausrichtung von dem Kurs: Modellierung in der Ökologie. Teilnahme an Fachtagungen (DGL und DGP von 2008-2010) mit forschungsbezogenen Vortrag oder Posterpräsentation
03/2006 bis 02/2007	Diplomarbeit am Zoologisches Institut der Universität zu Köln mit dem Thema: Influence of chaotic temperature fluctuations on an experimental microbial food web (Gesamtnote: sehr gut)
10/2001 bis 02/2007	Studium der Biologie an der Universität zu Köln mit den Schwerpunkten Zoologie, Entwicklungsbiologie und Biochemie

Studienbegleitende Tätigkeiten:

Seit 10/2007	ehrenamtliche Mitarbeit beim Kölner Studentenmagazin „Meins“
03/2007 bis 05/2007	studentische Hilfskraft am Zoologischen Institut der Universität zu Köln
Seit 03/2006	ehrenamtliche Mitarbeit beim Kölner Hochschulradio „Kölncampus“
WS 2003/2004	ehrenamtliche Co-Tutorin einer Einführungsgruppe für Studienanfänger im Fach Biologie an der Universität zu Köln

Sonstige Kenntnisse:

Sprachkenntnisse:	Englisch: sehr gute Kenntnisse Französisch: ausgebaute Grundkenntnisse
EDV:	sehr gute Kenntnisse der MS Office Programme Gute Kenntnisse in: ModelMaker (Modellierungs Software), Tisean (Datenanalyse Software)
Führerschein:	Klasse 3
Hobbys:	Sport (Judo, Tae Bo) Wissenschaftsjournalismus Musik hören, reisen