ÖAW
Österreichische Akademie
der Wissenschaften

Austrian Academy of Sciences

Annual Report 2008

Institute for Limnology

REPORTING PERIOD:	1.1.2008 – 31.12.2008
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Content

Mission Statement

Our mission is to investigate and advance scientific understanding of freshwater ecosystems, and to disseminate this knowledge through scholarly publication and education. As an ecological discipline, limnology is the comprehensive science of the biological properties of inland waters in their physical-chemical environment. We use aquatic model organisms, reaching from bacteria to fish species, to investigate patterns and processes of general ecological and evolutionary relevance. We are especially interested in understanding the proximate and ultimate factors responsible for the distribution, interactions, and specific adaptations of freshwater organisms. Our emphasis is on process oriented basic research, but our research results are also used for consulting and in the search for solutions in the management and protection of inland waters. Our present research provides the basis for the sustainable use and protection of the water quality of our fresh waters. We provide training for national and international graduate students and post-doctoral fellows within various research projects and the International Post-Graduate training programmes in Limnology. Our current research focuses on investigating the origin and maintenance of diversity of aquatic organisms and communities at various levels, ranging from molecules (genes and proteins) over organisms and populations to communities.

1. Scientific Activity 2008

1.1. Zusammenfassung des wissenschaftlichen Berichts 2008

Die Forschungs- und Lehraktivitäten des Instituts wurden mit dem Beginn der Umbauarbeiten am Hauptgebäude im Frühjahr 2008 erheblich behindert und mussten im Sommer für sechs Wochen während der Übersiedlung in die Ausweichquartiere und Adaptierung der neuen Laborgebäude weitgehend eingestellt werden. In Folge dieser schwierigen Situation hat die Teilnahme von Gastforschern und graduierten Studierenden an unseren Forschungsaktivitäten im Vorjahr gegenüber früheren Jahren abgenommen. Trotz dieser Widrigkeiten setzte das Institut seine Untersuchungen innerhalb des gegenwärtigen Schwerpunktes fort, der *Erforschung des Ausmaßes, der Enstehung und der ökologischen Bedeutung der intraspezifischen Diversität aquatischer Mikro- und Makroorganismen*. Mit zehn auf Akademieposten angestellten Wissenschaftlern kann das Institut nicht alle Süßwassertypen, Taxa und Diversitätsebenen im Einzelnen untersuchen. Unser Vorgehen besteht darin, mittels Modellorganismen innerhalb der funktionellen Guilden und Schlüsseltaxa die Diversität in Seen vor allem auf den Ebenen von den Genen bis zur Gemeinschaft zu untersuchen. Wir konzentrieren uns dabei auf einige wenige Habitate, um den Ursprung, das Ausmaß und die Veränderung der Diversität in ausgewählten Taxa zu untersuchen. In den vier Arbeitsgrupen, die die Mikrobielle Ökologie i.w.S. bearbeiten, werden die verschiedenen Ebenen der Diversität überwiegend in unterschiedlichen, sich ergänzenden Forschungsprojekten untersucht. Die im Jahre 2008 erzielten Ergebnisse dieser vier Arbeitsgruppen werden am Beginn des nächsten Kapitels vorgestellt.

Die AG Molekulare und cytogenetische Evolution asexueller aquatischer Organismen verfolgt einen ähnlichen strategischen Forschungsansatz wie die mikrobielle Ökologie, während die verwandte AG Experimentelle evolutionäre Ökologie die Bedeutung der asexuellen bzw. sexuellen Fortpflanzung mit Rotatorien (Rädertierchen) in einem einzigen, umfassenden Projekt untersucht. Mit der Einrichtung dieser beiden Arbeitsgruppen, die das „Paradoxon der sexuellen Fortpflanzung" ('paradox of sex') von verschiedenen Aspekten und mit unterschiedlichen Organismen untersuchen, hat das Institut seine theoretische Ausrichtung innerhalb der aquatischen Diversitätsforschung verstärkt. Die eher anwendungsorientierte Biodiversitätsforschung wird vor allem innerhalb der AGs Fischökologie und Paläolimnologie berücksichtigt. Erstere trägt zur Erhalt der natürlichen biologischen Ressourcen in Österreich bei, indem der Zustand der einheimischen Reinankenarten in den Salzkammergutseen und das Ausmaß ihrer Hybridisierung mit einer eingeführten Art untersucht werden. Die Kenntnis der spät-pleniglacialen Klimaänderungen, die innerhalb der paläolimnologischen Forschung erworben wurde, ist von offenkundiger Bedeutung für zukünftige Szenarien, wie sie innerhalb der nationalen und globalen Erforschung der Klimaänderungen entworfen werden. Die verwandte, langfristige Forschung des Instituts zur Algenökologie wurde mit der Pensionierung des verantwortlichen Wissenschafters, Martin Dokulil, zum Jahresende 2008 abgeschlossen.

Das Projekt 'Initiative of River Ecology in Sri Lanka: from Science to Application (IRESA)', das über ein reines limnologisches Forschungsprojekt hinausgeht, wurde 2008 fortgeführt. Wesentliche Ergebnisse werden in diesem Bericht im Vergleich zwischen kontinentalen, im gemäßigten Klima liegenden, und tropischen Bächen berichtet. Weiters wurden wesentliche Lehr- und Forschungstätigkeiten des Instituts innerhalb der Internationalen Post-Graduierten Programme Limnologie (IPGL) fortgesetzt. Der IPGL-Kurs hat den Diplom- (Masters) Studiengang "Environmental Sciences with specialization in Limnology and Wetland Ecosystems" neu gestaltet und organisierte einen zweiten internationalen Workshop zum Thema "Bridging research, technology & development: sustainable water management in Eastern Africa", der von 140 Fachleuten besucht wurde. Die Lehraktivitäten innerhalb der IPGL-Programme mussten wegen des Umbaus des Hauptgebäudes in Mondsee im vergangenen Jahr in die Forschungsstation des Wassercluster nach Lunz verlagert werden.

Zu den Höhepunkten der Grundlagenforschung des Instituts im Jahre 2008 zählten

- die Charakterisierung des Mikroviridin-Genclusters, der die ribosomale Synthese des bioaktiven Peptids Mikroviridin in der Cyanobakteriengattung *Planktothrix* kodiert
- die Verbindung von ökologischer und phylogenetischer Diversität des freilebenden, heterotrophen Bakteriums *Polynucleobacter necessarius* und von weit verbreiteten, allgegenwärtigen Einzellern des Morphotyps *Spumella*
- die Entwicklung einer Methode für die quantitative Analyse heterotropher planktischer Einzeller und Mikroalgen aus konservierten Freilandproben, wobei die morphologische mit der Analyse der kleinen ribosomalen DNA-Untereinheit verbunden wurde
- die Entdeckung signifikanter Wechselwirkungen zwischen dem Habitat und dem Organismusstamm bei aquatischen Einzellern
- die Entwicklung eines automatischen Analysesystems für experimentelle Rotatorien-Populationen, das Fitness-Unterschiede zwischen obligaten und zyklischen parthenogenetischen Rotatorien aufdecken konnte
- die Veröffentlichung eines modelltheoretischen Artikels über den Langzeit-Erhalt der Parthenogenese in einen kleinen Fisch, dem Amazonenkärpfling

- der Nachweis. dass einheimische Reinaken eine höhere Fitness besitzen als aus dem Ostseeraum eingeführte Coregonen und deren Hybride
- die Rekonstruierung der Wassertemperaturen in den Alpen am Ende der letzten Eiszeit über einen Zeitraum von vor ca. 19000 bis 13000 Jahren
- die aus einem Modell abgeleitete Voraussage möglicher Auswirkungen kurzfrister Temperaturänderungen, die im Zuge der Klimaänderungen wahrscheinlich sind, auf den Kohlenstofffluss in kleinen Wasserkörpern
- die Entflechtung der Bedeutung von Nahrungsquantität und Nahrungsqualität in gemäßigten und tropischen Bächen

Die Forschungsergebnisse wurden in 18 begutachteten Veröffentlichungen sowie in 40 mündlichen bzw. Posterbeiträgen auf nationalen und internationalen Fachtagungen vorgestellt.

1.2. Summary of the scientific report 2008

Research and teaching activities at Mondsee were seriously hampered with the beginning of the renovation and construction works of the main building in spring 2008, and virtually stopped for six weeks during summer when the building had to be evacuated and the interim laboratories and offices were adapted according to our needs. As a consequence of the difficult situation, the number of guest researchers and graduate students involved in our research activities declined in 2008, relative to former years. In spite of these obstacles, the Institute for Limnology continued its research within the current focus, i.e. investigating the ***extent, origin and ecological significance of intraspecific diversity of aquatic micro- and macroorganisms***. With its staff complement of 10 scientists, the Institute cannot cover all freshwater types, taxa and levels of diversity in detail. Our research philosophy is to use model organisms within functional guilds and key taxa to investigate the lake diversity mainly at the levels ranging from genes through communities. Similarly, we focus on a few study sites to investigate the origin, extent and change of diversity in specific taxa. In the four working groups dealing with Microbial Ecology in a broad sense, the various levels of diversity are investigated mostly in different research projects that are complementary to each other. The results obtained within each of the four WGs during 2008 are presented at the beginning of the next chapter.

The WG Molecular and Cytogenetic Evolution of Asexual Aquatic Organisms uses a similar strategy as in microbial ecology, while the related WG Experimental Evolutionary Ecology investigates the role of asexual vs. sexual reproduction with rotifers in a single, comprehensive project. With these two working groups, studying the ‘paradox of sex’ from different aspects and with several taxa, the Institute has strengthened its theoretical commitment within its research into aquatic biodiversity. The more practical, applied side of biodiversity research is primarily tackled within the WGs Fish Ecology and Paleolimnology. The former contributes to the conservation of natural biological resources in Austria, investigating the status of indigenous whitefish species in the Salzkammergut lakes and its extent of hybridization with an introduced species. Knowledge of late-Pleniglacial climatic changes gained within the paleolimnological research is of obvious relevance for future scenarios developed within the national and global climate change research. The related long-term research on Algal Ecology was finished with the retirement of the leading scientist, Martin Dokulil, at the end of 2008.

The project ‘Initiative of River Ecology in Sri Lanka: from Science to Application (IRESA)’, an outreach activity of the Institute, was continued 2008. Research results are presented in comparison to those previously obtained in continental temperate and tropical streams. Last but not least, the Institute continued its various training and research activities within the International Post-Graduate Training Programmes in Limnology (IPGL). IPGL reconstructed its M.Sc. programme "Environmental Sciences with specialization in Limnology and Wetland Ecosystems" and organized a second workshop on "Bridging research, technology & development: sustainable water management in Eastern Africa" that was attended by 140 experts. Teaching activites on lake limnology within IPGL, however, had to be relocated from Mondsee to the research station of the Wassercluster Lunz in Lower Austria because of the construction works at the main building in Mondsee.

Highlights of our basic research in 2008 were

- the characterization of the microvidin (mvd) gene cluster encoding the ribosomal synthesis of the common bioactive peptide microviridin in the cyanobacterium genus *Planktothrix*
- linking ecological and phylogenetic diversity of the free-living, heterotrophic bacterium *Polynucleobacter necessarius* and of widespread and ubiquitous protists of the *Spumella* morphotype
- developing a method for the quantitative analysis of planktonic heterotrophic protists and microalgae from preserved field samples combining morphological and small-subunit (SSU) rRNA gene sequence analysis
- detecting significant habitat x strain interaction in aquatic protists
- developing an automated analysis system for experimental rotifer populations that revealed fitness differences between obligate vs. cyclical parthenogenetic rotifers
- publishing a conceptual paper on the long-term persistence of parthenogenesis in a small fish, the Amazon molly
- demonstrating that indigenous Alpine whitefish have higher fitness than introduced Baltic whitefish and their hybrids
- reconstructing water temperatures in the Alps for the last glacial termination spanning the period from approximately 19,000 to 13,000 years ago
- predicting from a modeling approach the potential impact of short-term temperature fluctuations on carbon flux in small water bodies in the course of climate change
- disentangling the significance of food quantity vs. food quality in temperate and tropical streams

Research results were disseminated by 18 peer-reviewed publications and 40 oral and poster presentations at national and international scientific meetings.

1.3. Report on the scientific activity during 2008

Physiology and Molecular Ecology of secondary metabolite synthesis in algae

(**Rainer Kurmayer**[1], Guntram Christiansen, William Okello, Veronika Ostermaier, Nadja Straubinger, Josef Knoblechner, Maria Reischauer, Johanna Schmidt)

The production of toxins by algae in fresh and marine water affects the ecosystem at all trophic levels. Cyanobacteria are prolific producers of bioactive natural products, mostly belonging to the nonribosomal peptide and polyketide classes. For the vast majority of those toxins, usually defined as secondary metabolites (compounds that are not required for primary metabolism and growth) their function is unknown, and the understanding of their regulation and evolution in the ecosystem is poor. In this year we succeeded to

- elucidate and characterize the microvidin (mvd) gene cluster encoding the ribosomal synthesis of the common bioactive peptide microviridin
- estimate the distribution and abundance of non-toxic mutants of cyanobacteria in Alpine lakes
- estimate the influence of primary metabolism via growth on the variation in the net production of two non-ribosomal bioactive peptides

Elucidation and characterization of the microvidin (mvd) gene cluster

The *Planktothrix agardhii* strain CYA 126.8 produces five different families (microcystins, anabaenopeptins, cyanopeptolins, aeruginosides and microviridins) of small peptide natural products. Microviridins are strong eleastase inhibitors and are discussed to kill herbivorous crustaceans by preventing their regular molting. During the last years we could show that four of these structures are synthesized via nonribosomal peptide syntethases (NRPS). Within the project FWF-P18185 (Microevolution of toxin synthesis in cyanobacteria) we report that the microviridins, although similar in structure and composition to the other four NRPS families, are synthesized via an alternative biosynthetic pathway. A linear peptide precursor, which is ribosomally produced is subsequently modified by four different enzymes leading to the tri-cyclic microviridin.

We have isolated a gene operon consisting of six genes, which was subsequently insertionally inactivated leading to a microviridin deficient mutant of *Planktothrix agardhii*

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[1] Heads of working groups in bold face

126.8. *In vitro* characterization of three enzymes being part of the gene cluster shed light on the chronology of the maturation of the precursor to the final microviridin (Fig. 1).

Homology searches of cyanobacterial genomes from databases showed furthermore that this novel biosynthetic pathway is widely distributed among the phylum cyanobacteria.

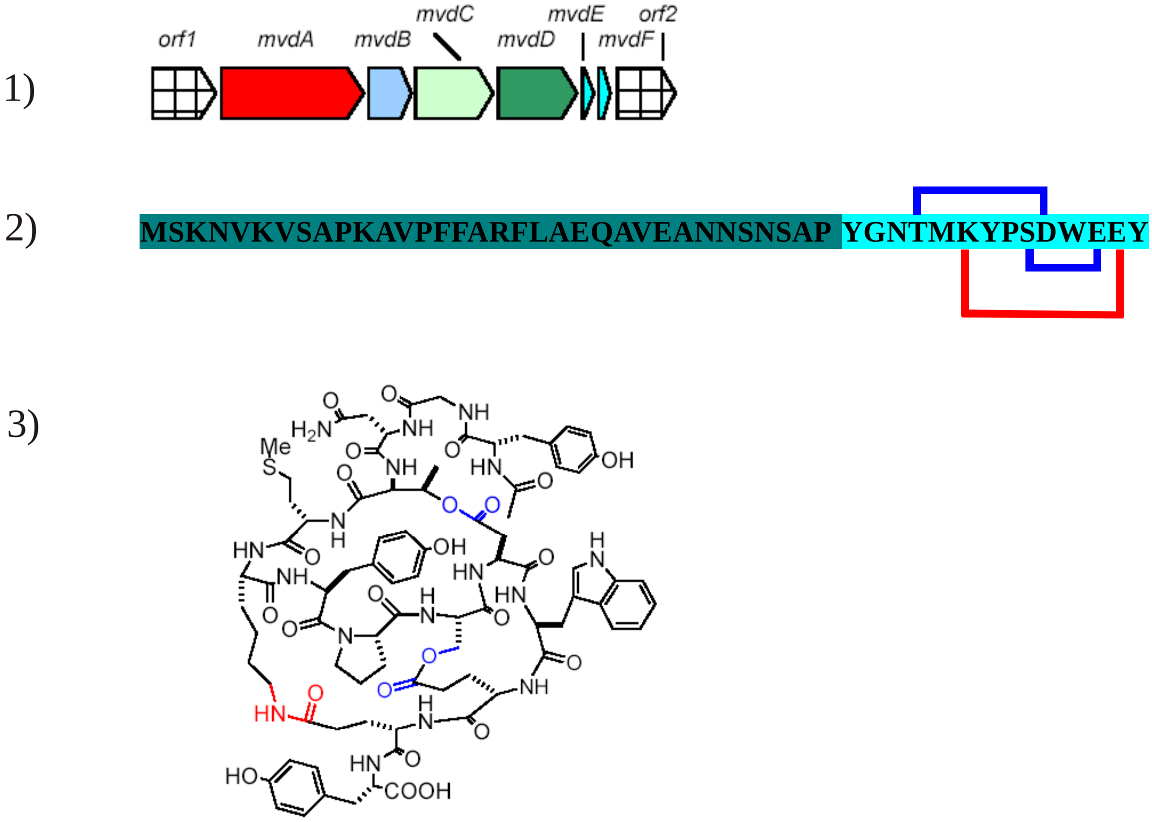

Fig. 1. Scheme of the microviridin (mvd) gene cluster encoding the synthesis of the protease inhibitor microviridin. In contrast to many other non-ribosomally peptides (like the toxic microcystins) this peptide is ribosomally synthesized as a prepeptide (48 amino acids, mvdE, mvdF) and then post-translationally modified by acetyltransferases (mvdB) and cyclized via enzymes catalysing the formation of an amide bond (mvdC) and two ester bonds (mvdD) (from Philmus et al. 2008).

Our research provides in depth insight into the synthesis of bioactive compounds that are generally addressed as toxins produced by cyanobacteria. During evolution cyanobacteria have evolved at least two independent pathways for the synthesis of cyclic peptides with both groups showing an inhibition of different enzyme classes (Protein phosphatases, proteases) in the nanomolar range.

Distribution and abundance of non-toxic mutants of cyanobacteria in lakes of the Alps

The dynamics of certain *Planktothrix* genotypes in field populations were described within the project FWF-P18185. We used real-time PCR to quantify the different microcystin synthetase gene cluster (mcyA and mcyD) genotypes (i.e. microcystin-producing and non-producing (inactive) strains) of *Planktothrix* in 12 lakes comprising a large (>10,000 fold) variation in the abundance of *Planktothrix*. This was achieved by first characterizing the mutation spectrum in the strains under investigation, and secondly by designing of specific primers for the TaqMan Assay. Altogether, four different mutations were assayed during July 2005 - July 2007. The results indicate a low proportion of non-toxic mutants within toxic populations (min 0%, mean 6.5% ± 1.1 (SE), max 52.3% of the total population) and a linear relationship between population density and inactive mcy genotypes (Fig. 2).

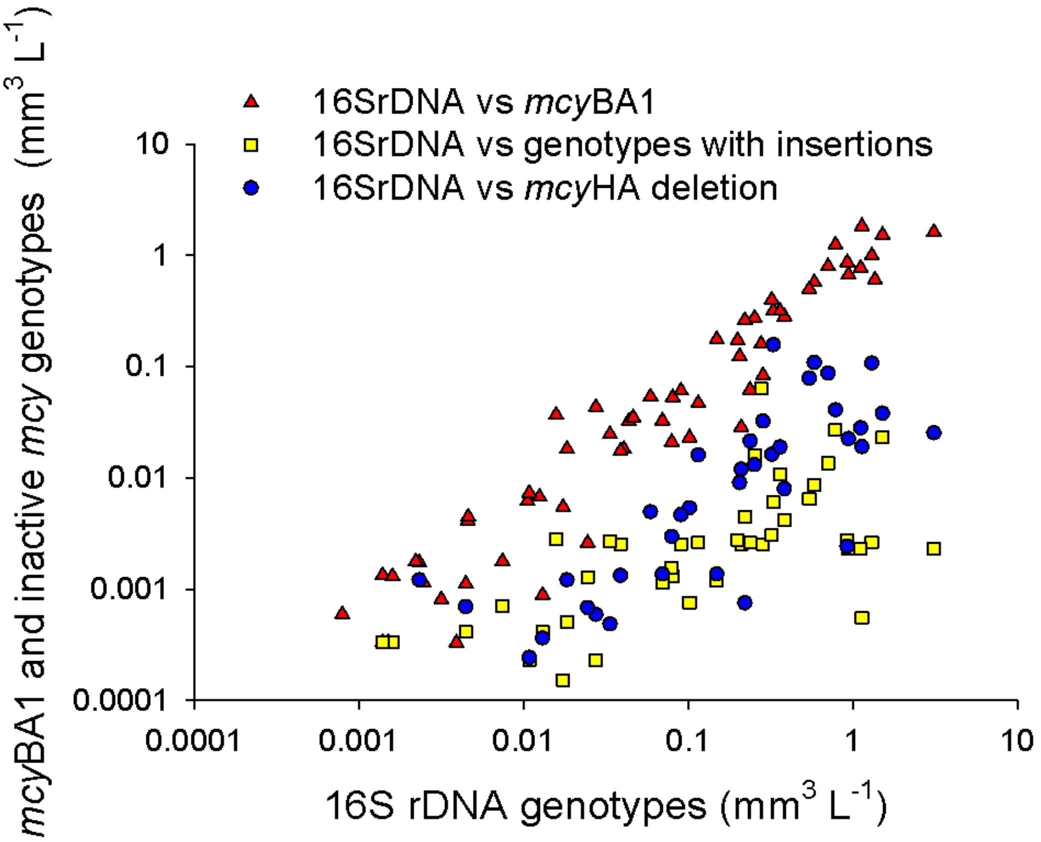

Fig. 2. Relationship between population cell density (as determined via 16S rDNA), *mcy* genotype abundance (*mcy*BA1, red triangles) and inactive *mcy* genotypes containing insertions (yellow squares) or deletions (blue circles; total n = 79) (from Ostermaier & Kurmayer 2009).

This study is the first that investigates the ecological success of both toxin-producing genotypes as well as genetically most closely related but inactive non-toxic congeners. While traditionally planktonic species are seen as major ecological units this work is attempting to identify subpopulations formed by specific genotypes that may influence the basis of ecological processes.

Variation in peptide net production and growth among strains of the toxic cyanobacterium *Planktothrix* spp.

In this study we have compared 49 strains of red-pigmented and green-pigmented Planktothrix spp. in growth rates as well as in their microcystin production rate and their production of closely related peptides (the anabaenopeptins;
Fig. 3). Excluding the strains lacking microcystin/anabaenopeptin, the microcystin and anabaenopeptin contents varied up to 14-fold and 12-fold, respectively. The variation in minimum and maximum peptide content (0.32 – 4.51 µg microcystin mg-1 dry weight; 0.85 – 10.32 µg anabaenopeptin mg-1 dry weight) exceeded the variation found for chlorophyll a (4.8 - 16.9 µg mg-1 dry weight). The extracellular proportions of microcystin (0 - 62%) and anabaenopeptin (0 - 58%) varied among strains, however, proportions of extracellular microcystin and anabaenopeptin were low, on average (< 10%). Among all strains cellular growth rates showed a 5-fold variation (0.07 – 0.33 doublings of dry weight day-1) and were independent of the pigmentation and the peptide net production rate.

In summary, we found that the microcystin production rate varies independently from the cellular growth rate among strains, implying that (i) the peptide net production rate is strongly influenced by genetic regulation mechanisms that differ among strains, and (ii) does not depend on the 16S rRNA machinery of the primary metabolism of the cell. During the ongoing FWF-project P20231 (Genetic determination of toxin content in cyanobacteria) the same strains are studied and analysed for the occurrence of mutations within the respective gene clusters and the resulting transcriptional regulation of microcystin net production.

Conclusions

Factors influencing the production of toxins such as microcystins in cyanobacteria have been investigated for years and it has been found out that environmental factors are able to induce moderate (usually by a factor of 3-4) changes in cellular microcystin content. Earlier work has concluded that microcystin production – although it is clearly a secondary metabolite – is tightly coupled to cell division, implying that microcystin production can be predicted from the growth rate. We argue that it is completely unclear how the microcystin production rate relates to the variation in growth rates among strains as well as to the variation in the production of other peptides. Instead we conclude that the frequently observed relationship between cell division and microcystin net production rate is rather the result of an accumulation of microcystins in specific cell compartments rather than due to a direct dependence of microcystin synthesis on the processes involved in the cell division cycle.

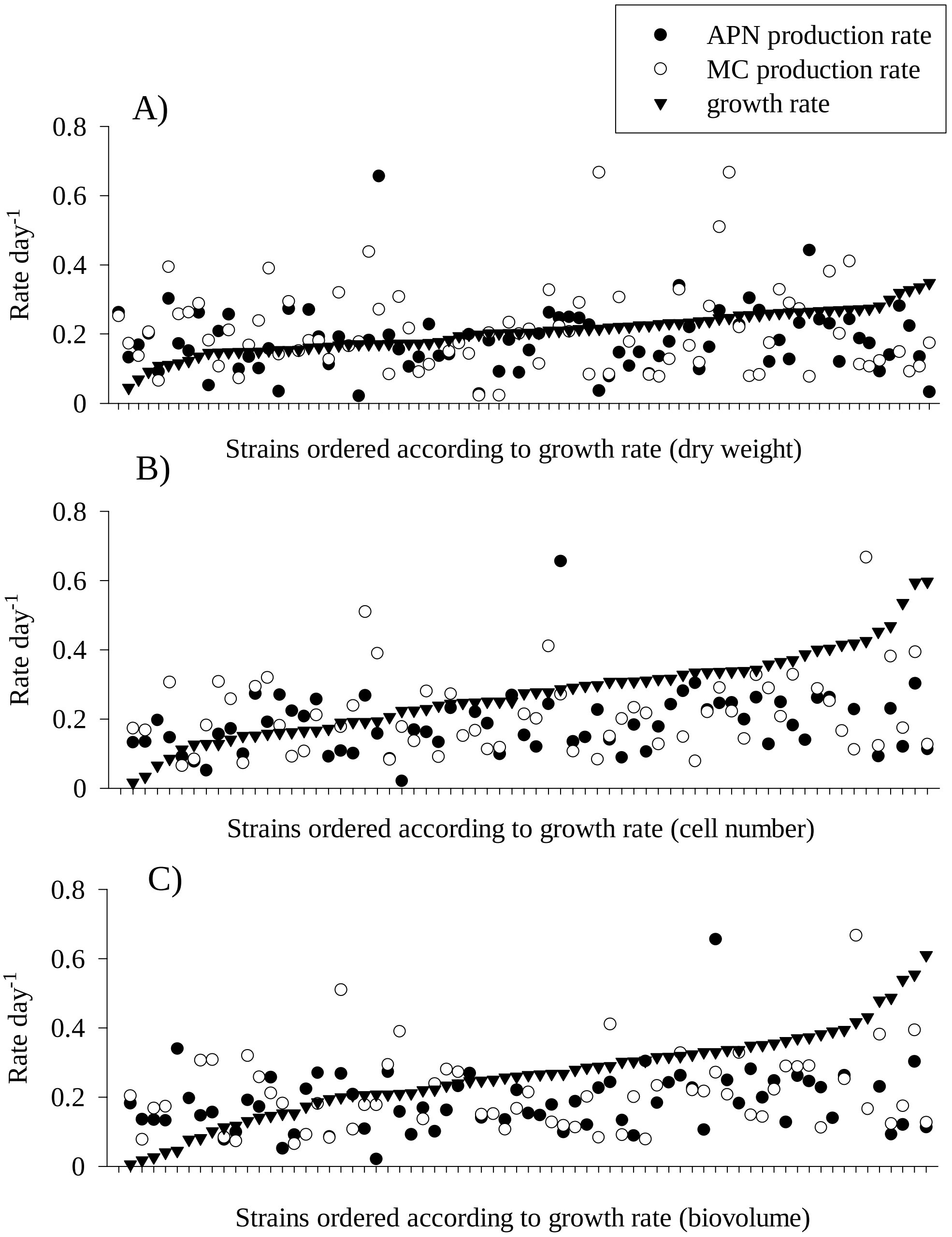

Fig. 3. Growth rates (black triangles) and corresponding peptide production rates (microcystin, open circles and anabaenopeptin, closed circles) for different *Planktothrix* strains measured under maximum growth rate conditions. Cell growth rates were estimated from dry weight (A), cell numbers (B) and by calculating biovolume from filaments assuming a cylindrical shape (C).

Environmental Microbiology

(**Martin Hahn**, Ulrike Brandt, Jitka Jezberova, Jan Jezbera, Thomas Scheuerl, Sophie Schärfl)

Bacteria and Archaea represent the genetically most diverse superkindoms. The exploration of prokaryotic diversity and its taxonomic description is still hampered by methodical (e.g., lack of sufficient cultivation methods) and conceptual obstacles. The current taxonomic species concept for prokaryotes (i.e. the Bacteriological Code) is not built on an evolutionary theory. Alternative concepts on how to define and delineate prokaryotic species are currently hotly debated, and intensive research on evolutionary mechanisms driving "speciation" in prokaryotes is going on in many research laboratories. One consequence of the shortfalls of the current taxonomic species concept for prokaryotes is a lack of comparability of prokaryotic and eukaryotic species taxa and another consequence is the frequent lack of congruence between prokaryotic species taxa and ecologically coherent taxa.

The long-term goals of the Environmental Microbiology group are to contribute to a better understanding of evolutionary forces driving diversification of prokaryotes. We use model groups (e.g. *Polynucleobacter* spp., lineages of freshwater *Actinobacteria*, and *Bacteroidetes*) for detailed studies on ecological and genetic diversification.

The research of the group Environmental Microbiology focused on three aspects during the reporting period:

- Linking ecological and phylogenetic diversity of *Polynucleobacter necessarius*
- Research on presence, diversity and distribution of actinorhodopsins in freshwater *Actinobacteria*
- Advancing the taxonomy of planktonic freshwater bacteria.

Ecological and phylogenetic diversity of *Polynucleobacter necessarius*

The overall goals of the investigations are to reveal the evolution of *Polynucleobacter* bacteria and to contribute to the current debate on speciation mechanisms in bacteria. The *Polynucleobacter* cluster represents a phylogenetically diverse group of *Betaproteobacteria* (Fig. 4). Bacteria of two different lifestyles, i.e. obligate endosymbionts of ciliates and obligately free-living strains inhabiting the water column of freshwater systems, are affiliated with this phylogenetic cluster. The cluster contains at least four species-like subgroups (previously designated PnecA, PnecB, PnecC, and PnecD), however, only one of these groups (PnecC) contained with *Polynucleobacter necessarius* a validly described species. This species was established for obligate endosymbiotic strains living in the cytoplasm of the ciliate *Euplotes aediculatus*. Interestingly, these endosymbiotic strains are closely related to free-living planktonic strains, however, according to the species description of *P. necessarius*, these free-living strains could not be assigned to this species.

Taxonomic revision of the genus *Polynucleobacter* and the species *P. necessarius*. In cooperation with Prof. E. Stackebrandt and Dr. E. Lang (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany), we investigated the phylogenetic relationship of closely related endosymbiotic and free-living PnecC strains. The obtained results clearly indicated that these closely related strains differing fundamentally in lifestyle have to be considered according to the Bacteriological Code as members of a single species. Therefore, we emended the genus and the species, and proposed to establish for the two groups of strains differing in lifestyle two separate subspecies, i.e. *Polynucleobacter necessarius* subsp. *necessarius* (obligate endosymbionts) and *P. nec.* subsp. *asymbioticus* (for planktonic strains affiliated with subcluster PnecC) (Hahn et al., Internat. Jour. System. Evolut. Microbiol., in press). Furthermore, the description of a second *Polynucleobacter* species representing subcluster PnecD was submitted (Hahn et al., submitted).

Ubiquity of *P. necessarius* subsp. *asymbioticus*. (Jezbera, Jezberova, Brandt and Hahn) In a survey of about 100 freshwater habitats located in a 2000 km^2 area covering almost the entire Salzkammergut Lake District the ubiquitous distribution of this subspecies was demonstrated by cultivation-independent methods (FISH, RLBH) and cultivation of strains. The investigated set of habitats encompassed several gradients (e.g., water chemistry, size of habitats, altitude, trophic status). Surprisingly, the subspecies was detected along all gradients, for instance it was detected (and isolated) in acidic (minimum pH 3.8), circumneutral, and alkaline (max. pH 8.5) lakes and ponds. Even in small ephemeral puddles (not fed by stagnant water bodies) the subspecies was detected. Statistical analyses on environmental parameters controlling the abundance of the subspecies in the different habitats are currently under way.

Revealing evolutionary and ecological mechanisms resulting in ubiquity of *P. necessarius* subsp. *asymbioticus*. Based on the Bacteriological Code the investigated subspecies was described. This taxonomic code does not really consider ecological traits and forces microbiologists to describe species taxa encompassing organisms potentially largely differing in their genetic makeup (only strains sharing less then 70% DNA-DNA homology can be assigned to different species). Evolutionary theory predicts that bacterial species described according to the Bacteriological Code typically encompass several “ecotypes” differing in ecological adaptation and occupying different ecological niches.

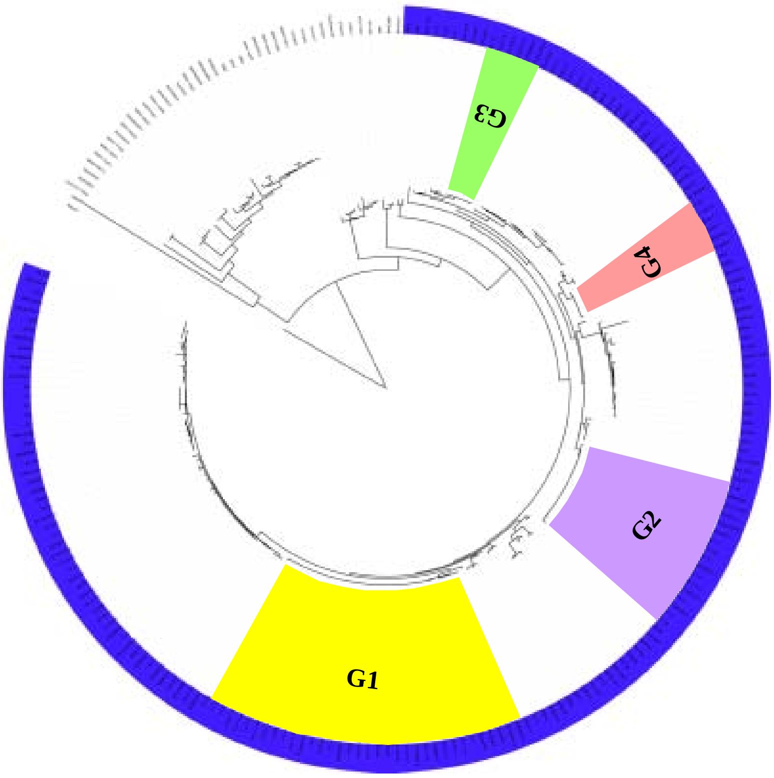

Fig. 4. Genetic diversity of *Polynucleobacter* spp. bacteria illustrated as a neighbour-joining tree calculated with 16S-23S ITS (internal transcribed spacer) sequences (500 bp). The tree represents the diversity within the *Polynucleobacter* culture collection established by the Hahn lab. Strains assigned to the species *P. necessarius* (Heckmann & Schmidt, 1987; Hahn et al., in press) are highlighted by blue color. Some of the potential "ecotypes" (designated G1, G2, G3 and G4) are highlighted by other colors. Note that strains representing the highlighted "ecotypes" are overrepresented in the tree due to specific sampling efforts. The tree is rooted by a *Ralstonia solarnacearum* sequence.

However, the evolution of such "ecotypes" is not yet understood, and knowledge on how to identify and delineate such "ecotypes" is largely missing. We aim on reconstruction of the evolution of the monophyletic species *P. necessarius*, and especially on reconstruction of the evolution of potential "ecotypes" within the species. In a first step, we aimed on identification of potential "ecotypes" by phylogenetic analysis of two markers (16S-23S rRNA ITS, and glnA). In a second step we tested by environmental mapping if identified "ecotype candidates" represent ecologically coherent taxa. For this purpose a reverse line blot hybridization method was developed, which enables the detection of potential "ecotypes" in environmental samples based on signature sequences in their 16S-23S ITS sequences (Fig. 5). This environmental mapping clearly supported the hypothesis of the existence of "ecotypes" within the species *P. necessarius*. We further tested this hypothesis by cross-transplantation experiments with pure cultures and natural populations. In one of these experiments, we transplanted subpopulations of three potential “ecotypes” from their acidic home habitat in a circumneutral garden pond (already inhabited by potential other “ecotypes”). This and other transplantation experiments clearly confirmed the ecological adaptations of the “ecotypes” predicted by the environmental mapping. Altogether, these investigations clearly demonstrated that the observed ubiquity of the species resulted from ecological differentiation.

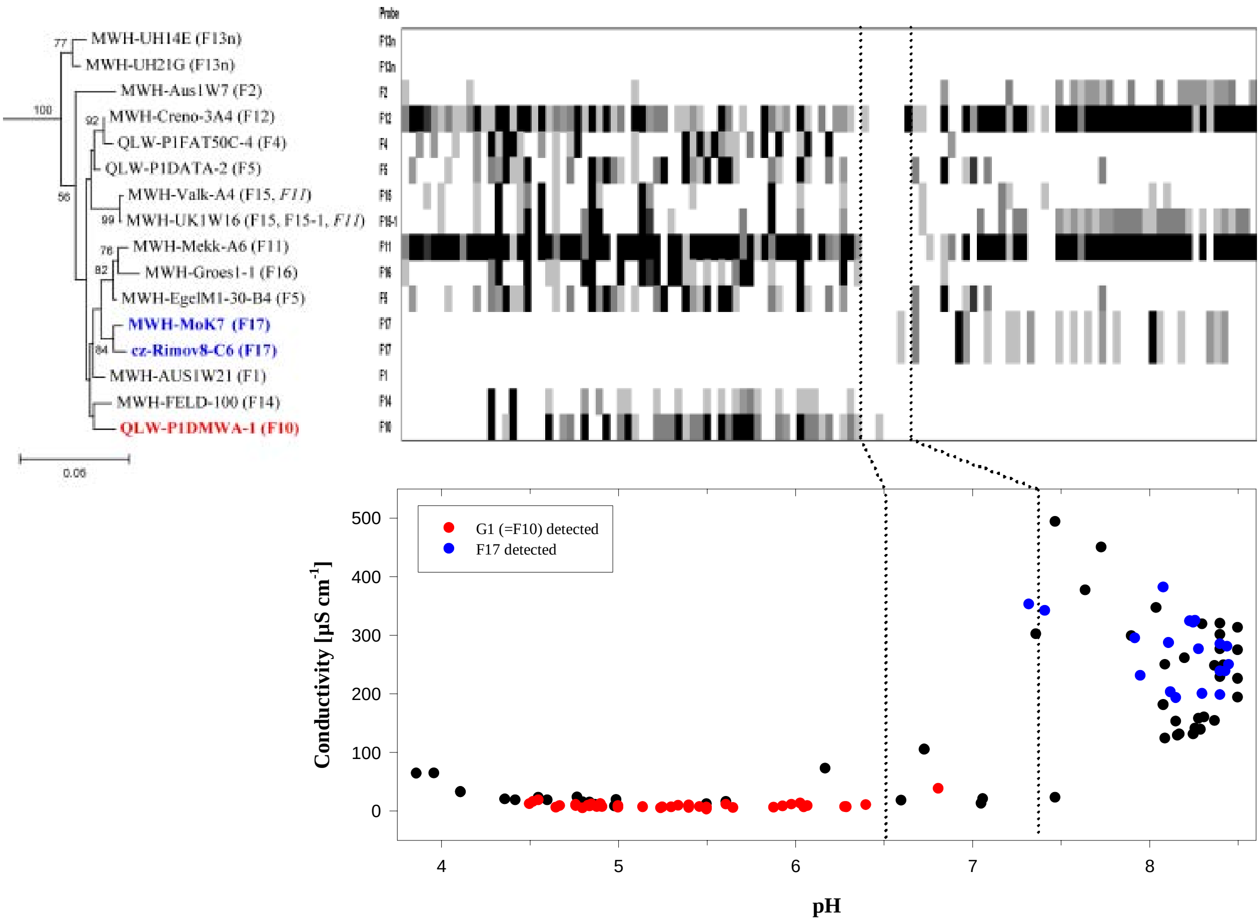

Fig. 5. Detection of “ecotype candidates” by reverse line blot hybridization in environmental samples representing habitats in the Salzkammergut Lake District as well as reference habitats outside this area. Environmental samples were sorted by increasing pH values (x axis of both plots). The upper plot depicts detection (black and gray rectangles) of “ecotype candidate” taxa shown in the phylogenetic tree. The lower plot depicts detection of “ecotype” G1 (red dots; red label in the tree) and “ecotype” F17 (blue dots; blue label in the tree). Habitats in which both “ecotypes” were not detected are depicted by black dots. Note that the subspecies *P. necessarius* subsp. *asymbioticus* was detected in all studied habitats by FISH and PCR assays.

In a third step, we will test alternative evolutionary models on mechanisms resulting in formation of “ecotypes”. Alternative models potentially possessing the highest explanatory power are the ecotype model and the metapopulation model, which differ in basic forces driving the evolution (selective sweeps versus population bottlenecks and gene flow restriction). These models shall be tested by using “ecotype” G1 (Figs. 4 and 5). We established a collection of 30 G1 strains isolated from several small habitats located in three distantly (up to 100 km) located areas. These strains are currently investigated by multi locus sequence analysis. The established sequence data will be used for testing the above-mentioned models.

Future research (submitted project proposal) shall focus on geneflow within and between *P. necessarius* populations and especially between “ecotype” populations.

Master thesis by T. Scheuerl.

Another aspect of our research on *Polynucleobacter* bacteria was the characterization of the ecological function(s) of these bacteria. Physiological experiments on substrate utilization were performed with one strain and compared with the prediction on physiological capabilities derived from the interpretation of the complete genome sequence of the same strain. Hypothesis based on the revealed physiological traits were tested by manipulation experiments with a natural *Polynucleobacter* population from which the investigated strain was isolated some years ago.

Actinorhodopsins in freshwater *Actinobacteria*

(cooperation with A. Sharma and W. F. Doolittle, Dalhousie University, Canada)

Actinorhodopsins are variants of proteorhodopsins, which represent transmembrane proteins acting as light-driven proton pumps potentially enabling bacteria to harvest light energy. Proteorhodopsins and actinorhodopsins are homologues of bacteriorhodopsins long known form *Archaea*. Proteorhodopsins were discovered in marine *Proteobacteria* in the year 2000. Meanwhile, it was demonstrated that a large fraction of marine bacterioplankton represented by diverse phylogenetic groups is encoding proteorhodopsins. This indicates that a large fraction of the “heterotrophic” marine bacterioplankton is able to harvest light energy. Recently, Sharma et al. concluded from the analyses of metagenomic data that freshwater *Actinobacteria* might encode variants of proteorhodopsins. We tested this hypothesis by screening of previously established pure and mixed cultures of freshwater *Actinobacteria*. This resulted in detection and characterization of two groups of actinorhodopsins, and indicated the presence of unexpected physiological traits in a large fraction of freshwater bacterioplankton (Sharma et al., in press).

Taxonomy of freshwater bacteria

(in cooperation with various external cooperation partners)

Taxonomic studies were performed, which resulted in the description of eight candidatus species affiliated with different lineages of freshwater *Actinobacteria* (Hahn, 2009; Jezbera et al., in press), and the revision of the genus *Polynucleobacter* and the species *P. necessarius* (Hahn et al., in press).

Diversity and ecology of Flagellates and Microalgae

(**Jens Boenigk**, Liselotte Eisl, Anneliese Wiedlroither, Barbara Findenegg, Steffen Jost, Ralph Medinger)

This working group is focussed on diversity, evolution and ecology of eukaryotic microorganisms with special emphasis on chrysophytes. During 2008, the analysis of distribution pattern concentrated on an Alpine lake gradient comprising 40 lakes ranging from the German border north of Salzburg to the central Alps.

Diversity: We developed and published a method for the quantitative analysis of planktonic protists and microalgae from preserved field samples combining morphological and small-subunit (SSU) rRNA gene sequence analysis. We linked a microscopic screening with PCR of single cells using field samples preserved with Lugol's iodine solution. Cells possessing a rigid cell wall were incubated with viscozyme and subsequently with proteinase K for cell disruption; this was unnecessary for fragile cells. The addition of sodium thiosulfate to the PCR tube considerably decreased the inhibiting effect of the fixative (iodine) on the PCR and thus allowed for successful single-cell PCR even of long DNA fragments (up to as many as 3,000 base pairs). We further applied the protocol to investigate the dominant SSU rRNA genotypes in distinct flagellate morphospecies originating from different samples. We hypothesized that despite the morphological similarity, protist morphospecies in different habitats or sampled during different seasons are represented by different genotypes. Our results indicate species-specific differences: the two species *Ochromonas* sp. and *Dinobryon divergens* were represented by several different genotypes each, and for the latter species, the dominating genotype differed with habitat. In contrast, *Dinobryon pediforme*, *Dinobryon bavaricum*, and *Synura sphagnicola* were exclusively represented by a single genotype each, and the respective genotype was the same in different samples. In summary, our results highlight the significance of molecular variation within protist morphospecies. (Projects FWF P18315 & Alpine Research Programme of the Austrian Academy of Sciences).

Complementory to the field investigations the historical literature was analysed for conceptual progress in species definitions and the specific effects of methodology on taxon resolution.

The ecology of the smallest eukaryotes, i.e., nanoflagellates, is a comparatively young but rapidly developing research area. Until recently, the minute size of these organisms and the related methodological limitations slowed scientific progress in important research questions such as flagellate megasystematics and flagellate species concept(s). In consequence, ecological key questions, such as that of flagellate biogeography and endemism, flagellate microdiversity, and functional differentiation, as well as (more generally) of a taxon-specific ecology are underdeveloped. I reviewed the impact of the historical development of nanoflagellate biology and of nanoflagellate classification of the development of the major ecological and ecophysiological research questions (Boenigk 2008).

In summary, the conceptual progress in flagellate (and protist) biology may currently exceed by far that of the past decades. The long research tradition, despite the too often lacking conceptual progress, should therefore not discourage, but in contrast stimulate future research on aspects related to the basic ‘units of biodiversity’ in the smallest but most abundant eukaryotes on earth. (P19706 and Alpine Research Programme of the Austrian Academy of Sciences)

Evolution - Theoretical basis of macroevolutionary differentiation

The evolution of phagotrophy within algal lineages is a key to understanding both the evolution of eukaryotes and the coexistence of mixotrophs and phagotrophs in aquatic habitats. We examined what conditions may have favoured the evolution of a mixotrophic flagellate into a heterotroph, thereby loosing its photosynthetic ability. Using a dynamic model of heterotrophic and mixotrophic flagellates and two types of prey (large and small bacteria), we examine the influence of DOC as a measure of trophic conditions, the photosynthetic growth rate of the mixotroph, and external limitations of photosynthesis on the coexistence and relative dominance of both types of flagellates. The results confirm former studies insofar that heterotrophs will dominate in eutrophic environments if photosynthesis is limited by light availability. Furthermore, our results suggest that, provided the photosynthetic advantage of the mixotroph is not too large, the heterotroph will be able to invade a community of mixotrophs and bacteria in a wide range of conditions and it will also dominate in poor environments. As carbon-limited conditions were presumably widespread and typical throughout history of the earth, such a scenario leading to purely heterotrophs within algal lineages under carbon-limited conditions may explain for the numerous transitions from phototrophic to mixotrophic and further to heterotrophic organisms within virtually all major algal lineages. Our model predicts a high probability for the evolution of phagotrophs in oligotrophic enviroments and thereby challenges former concepts which affiliated the evolution of phagotrophy with eutrophic or strongly light-limited enviroments only.

Microevolution and population structure

We analyzed the extent of ecophysiological and molecular homogeneity, or heterogeneity, of distinct protist populations and tested for correlations between molecular distance and ecophysiological adaptation in a widespread and ubiquitous protistan taxon. We selected heterotrophic nanoflagellates of the *Spumella* morphotype as a model organism, as these flagellates are widespread and among the dominant bacterivores in many microbial communities. We investigated the molecular microdiversity and the ecophysiological tolerances for a total of 13 strains originating from two freshwater samples. We further investigated the affiliation of different ecotypes with distinct small subunit ribosomal ribonucleic acid (SSU rRNA) genotypes, or molecular operational taxonomic units. These local population studies were further compared to the molecular and ecophysiological diversity of 28 strains originating from remote sampling sites. None of the investigated populations are homogeneous, but are rather heterogeneously composed of different ecotypes and genotypes, possibly corresponding to cryptic species. This population heterogeneity may partly explain the deviations between studies on single strains and populations in both laboratory and field studies. The molecular distance between the strains was correlated with the salinity and temperature adaptation of the respective strains, contradicting the assumption that SSU rRNA variation reflects accumulated neutral mutations. Independent of whether this correlation reflects adaptation above the (biological) species level or variation between asexually reproducing lineages, we demonstrate the unsuitability of the current classification system (species concept) for the investigated organisms, at least with respect to ecological and ecophysiological investigations (FWF project P18767).

Testing phylogenetic markers and developing a single cell multiplex PCR protocol

We investigated the suitability of different phylogenetic markers, specifically SSU, ITS1, ITS2, LSU and COX1 for analyzing chrysophyte populations. We extended the previously described method for quantitative analysis of planktonic protists and microalgae from field samples preserved with Lugol's iodine solution to a multiplex PCR approach covering nucleus encoded as well as mitochondrial genes. We focused on genes with a different phylogenetic resolution and specifically also on genes which are used for barcoding. This method will be applied to analyse chrysophyte population structure, population dynamics and microevolution in the field.

Ecology: The molecular diversity of protists and its ecophysiological significance is largely unknown. To assess the ecophysiological microdiversity, the tolerance of 54 clonal strains of the *Spumella* morphotype (Chrysomonadida) to salinity, pH and temperature was measured within the FWF project P 18315 and subsequently correlated to genetic distances. Results suggest that the morphospecies concept is inappropriate to reflect ecological differentiation in protists. Overall genotypic variation is neutral with respect to pH but highly meaningful with respect to temperature and salinity. On the level of sequence clusters, despite a potentially ubiquitous distribution, the mean ecophysiological adaptation as well as the centre of geographical extension was clearly different between the clades. Within clades we found a correlation between molecular and geographic distance, which was strongest for Antarctica, indicating that the phylogenetic resolution of the rRNA gene may be more suitable to detect geographic isolation at isolated and extreme sites whereas genes or markers with a higher phylogenetic resolution may be required in other regions. Further, ecophysiological microdiversity is much below the resolution of the 18S rRNA gene, indicating that additional marker genes with higher resolution power should generally be included in future studies (FWF project P18767).

We investigated cyst formation and the ecological trigger of cyst formation in several pigmented and unpigmented taxa. The results so far document a species-specific variation in the significance of different triggers. Namely the availability of food bacteria in the heterotrophic and mixotrophic flagellates was responsible for considerable interspecific variation of encystment pattern. Availability of (micro-)nutrients further modified growth and encystment pattern of the pigmented (mixotrophic and phototrophic) strains.

In summary, the investigations highlight the need for combined morphological, ecological and molecular approaches in reconstructing the diversity of protists and the evolutionary scenarios behind the formation of this diversity. The analysis of a very restricted number of laboratory grown strains proved generally difficult or even inadequate for relating ecophysiological laboratory findings to the field situation.

The long-term research on **Algal Ecology** was finished with the retirement of the leading scientist, **Martin Dokulil**, at the end of 2008. Accordingly, the focus of his last year's activity was on summing up the long-tem studies on the River Danube, including the results obtained during the international Joint Danube Survey 2, and on lakes Mondsee and Neusiedl. Research results were presented at three international and one national meetings and summarized in three scholarly articles, including two chapters published in the Encyclopedia of Inland Waters (Dokulil 2009[2], Dokulil & Kaiblinger 2009[3]).

Plankton ecology and ecophysiology

(**Thomas Weisse**, Peter Stadler, Ulrike Scheffel, Michael Moser)

Investigating habitat selection and local adaptation in acid mining lakes

As already outlined in the report of the previous working group, it is currently controversially discussed if the same freshwater microorganisms occur worldwide wherever their required habitats are realized, without any adaptation to local conditions below the species level. Recent studies in our laboratory and elsewhere provided, however, evidence for limited dispersal and local adaptation among wide-spread freshwater ciliates (e.g., Gächter & Weisse 2006, Weisse et al. 2007) and flagellates (Boenigk et al. 2007). Intraspecific, clonal differences in ecophysiological key features such as growth and grazing rates were repeatedly reported for marine and freshwater protists. It now appears that large intraspecific ecophysiological differences, which are less obvious from morphological features and cannot be detected at the level of conserved genes (Weisse et al. 2008, and discussion on previous page), are rather the rule than the exception among free-living aquatic protists (Weisse 2006). The current question is: is this intraspecific variability linked to habitat effects, thus promoting allopatric speciation in freshwater microbes?

Within the FWF project ‘Patterns and processes of adaptation and tolerance to low pH of freshwater plankton’ we investigate the adaptation of planktonic species (flagellates, ciliates, and rotifers) to and their microevolution in acidic mining lakes (AML). AML are extreme aquatic habitats with strongly reduced biodiversity (Gaedke and Kamjunke 2006; Geller et al. 1998). These environments harbor highly specialized microbial biota. Relative to natural ecosystems, all man-made AML are young habitats. The organisms thriving in these lakes may be highly specialized new colonizers (acidophilic species), or they may be generalists (acidotolerant species) taking refuge from otherwise superior competitors or predators that are less tolerant to the harsh environmental conditions. In contrast to most natural lakes, AML are disconnected from other surface water bodies. With respect to biogeography, they may be portrayed as acid island habitats in circumneutral surroundings. Highly acidic AML

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[2] Dokulil, M.T. (2009). Comparative Primary Production. In: Likens, G.E. (ed.), Encyclopedia of Inland Waters. Vol 1, pp. 130-137.

[3] Dokulil, M.T. & Kaiblinger, C. (2009). Phytoplankton Productivity. In: Likens, G.E. (ed.), Encyclopedia of Inland Waters. Vol 1, pp. 210-218.

(pH <3) are fishless and do not harbor waterfowl which are assumed to act as important vectors of microbial dispersal in circumneutral lakes (Weisse 2006). Rates of dispersal are, therefore, most likely reduced in AML relative to natural, circumneutral lakes. Temporal variation, which favors generalist phenotypes and, similar to dispersal, acts agains local adaptation, is also low in AML relative to neutral lakes. Accordingly, AML provide a rare case of suitable ecosystem models to test for the significance of strong habitat selection.

Overall, this research project tests two (null) hypotheses, i.e. that

- acidotolerant species benefit from competitive release under conditions of acid stress
- populations of widely dispersed planktonic microorganisms are virtually identical in similar (acidic) habitats, i.e. the habitat selects and site-specific genetic divergence (microevolution) below the species level is of minor significance

Our three study lakes are located at Langau (Lower Austria) and in Lusatia (Germany). These lakes are remarkably similar in their origin and hydrochemical composition, but the German and Austrian sites are located approximately 325 km apart from each other. All three lakes originated from rising ground water of open-cast pits of lignite mines after the cessation of mining activities and were geogenically acidified (current pH ~2.6).

In pursuit of the first hypothesis, we conducted laboratory experiments with the mixotroph flagellate species *Chlamydomonas acidophila* and *Ochromonas* sp. (three strains), and two ciliate species of the genus *Oxytricha* in 2008. We first assessed the pH reaction norm of each strain/species at the temperature used for maintaining our stock cultures (17.5 °C). In a second step, we exposed the protists to lower (10 °C) and higher (25 °C and, in the case of *Ochromonas* spp., also 35 °C) experimental temperatures to measure the combined effect of pH and temperature stress. Temperature had a significant effect on the pH tolerance of all species tested; high temperature, in particular, narrowed the width of the pH niche of the protist species (Fig. 6). The results shown in Fig. 6 seem to be typical for our study organisms. All species investigated thus far are acidophilic (i.e., with higher fitness at pH <6 than at circumneutral pH) or obligate acidophilic (surviving only at pH <6).

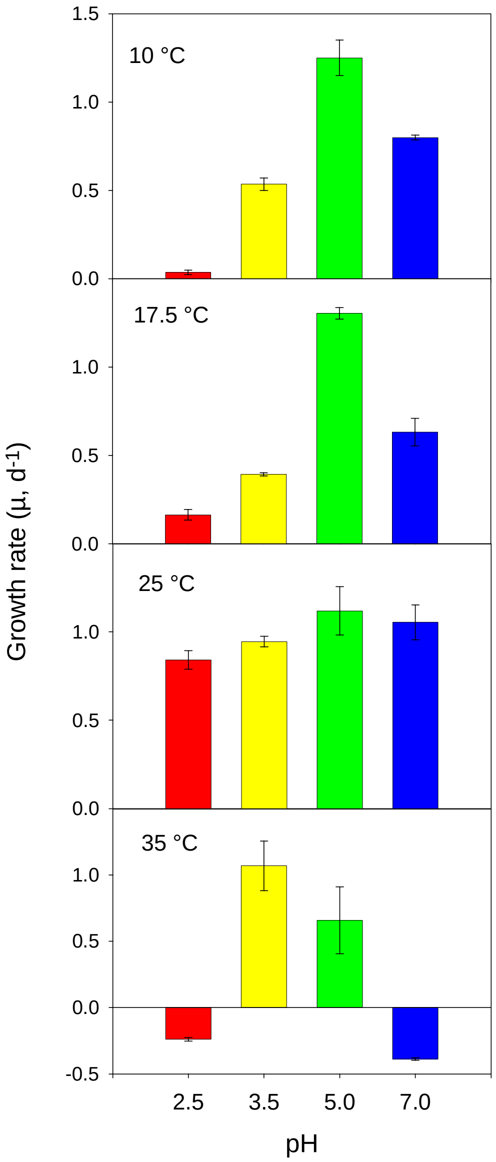

Fig. 6. Population growth rate of the flagellate *Ochromonas* sp. at four different pH and four temperatures. The flagellate was isolated from an acidic mining lake (pH 2.6) at Langau, Lower Austria (Moser & Weisse, in prep.).

To test the second overall hypothesis, we conducted simulated in situ experiments with flagellates and ciliates from the three acidic mining lakes together with our project partners at the University of Potsdam. The experiments were designed to test if there is significant interaction between freshwater protists and the habitats in which they actually or potentially dwell, i.e. to investigate if similar habitats may affect similar organisms differently (Fig. 7).

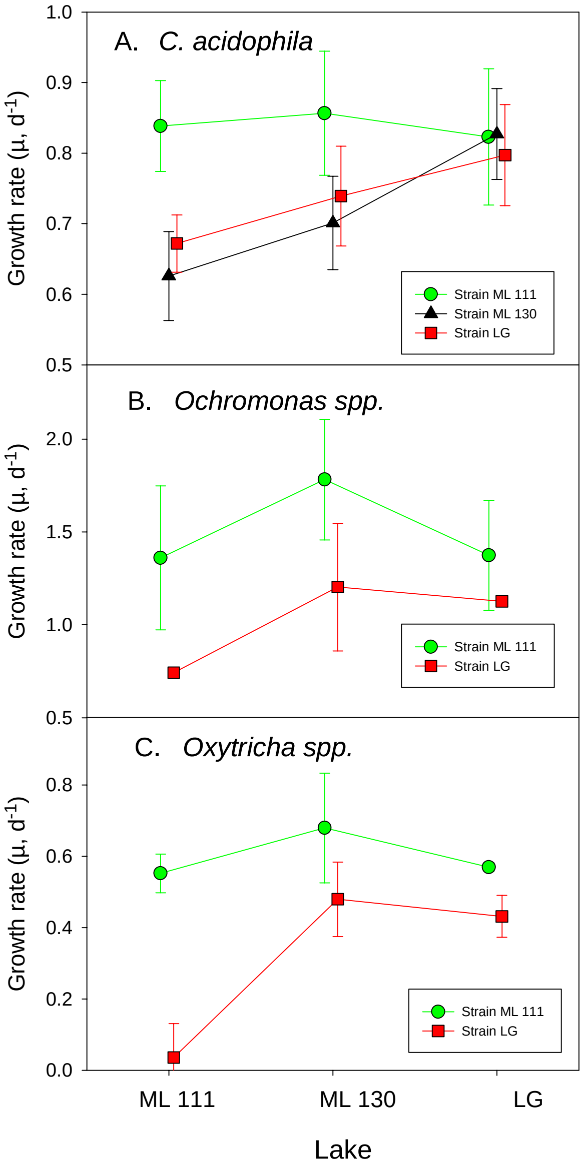

Fig. 7. Mean growth rates of each *C. acidophila* strain in water from all three acid mining lakes (A), mean growth rates of two *Ochromonas* spp. strains isolated from Lake ML 111 and Lake LG in water from all three lakes (B), and mean growth rates of two *Oxytricha* spp. species isolated from Lake ML 111 and Lake LG in water from all three lakes (C). The error bars denote 1 SD (Weisse et al, in prep.).

Three strains of the flagellate species *Chlamydomonas acidophila* were exposed to lake water of their origin and from the two other AML in a cross-factorial design (Fig. 7A). Population growth rates were measured as a proxy for their fitness. Results revealed significant effects of strain, lake (=habitat), and strain x habitat interaction. In a second series of experiments, we measured the growth response of two flagellate strains of the genus *Ochromonas* (Fig. 7B) and two closely related, but different ciliate species of the genus *Oxytricha* (Fig. 7C). Each strain/species was isolated from one of the three study lakes.

Results confirmed the experiments with *C. acidophila*, i.e. we measured significant species x habitat interaction.

In summary, our study demonstrates that the same habitat may affect strains of the same species differently and that similar habitats may harbour different strains of the same or different species. We conclude that strong habitat selection in AML may stimulate local adaptation and allopatric speciation among freshwater microbes. This finding is of general relevance for aquatic microbial ecology because we can reject the earlier assumption that the distribution of aquatic microorganisms can be understood solely in terms of habitat properties (Fenchel, T. 2003: Science 301,925-926).

Predicting the potential impact of short-term temperature fluctuations on carbon flux in small water bodies which may become common in the course of climate change

Small freshwater bodies are abundant and economically and ecologically important on a global scale. Within these, protozoa play an important role in structuring planktonic food webs and sequestering CO_2. We (Montagnes et al. 2008, Global Change Biology 14, 1-16) hypothesized that short-term (~20 days) fluctuations, of 2–10 °C, will significantly alter carbon flux associated with predator–prey interactions within the microbial planktonic food web. We examined the model ciliate, *Urotricha farcta*, which is abundant and common; it was fed the autotrophic flagellate *Cryptomonas* sp., which is also common. Laboratory experiments were conducted over relevant ranges: 8–24 °C; 0–2 $x10^5$ prey mL^{-1}. Mechanistic-phenomenological multiple regressions were developed and fit to the data to obtain relationships for (1) growth rate and volume changes of the flagellate vs. temperature and (2) growth rates, grazing, and cell volume change of the ciliate vs. temperature and prey concentration. Responses revealed interaction between temperature and prey levels on all ciliate parameters, indicating it is inappropriate to apply simple temperature corrections (e.g. Q_{10}) to such functions. The potential impact of such temperature changes on carbon flux was illustrated using a simple ciliate–flagellate predator–prey model, with and without the top grazer, *Daphnia*, added. The model indicated that predator–prey pulses occurred over 20 days, with the ciliate controlling the prey population. For ciliates and prey, carbon production peaked at 20 °C and rapidly decreased above and below this maximum; differences between minimum and maximum were approximately fourfold, for both prey and ciliate, with low levels at 25–30 °C and 10–15 °C. Including literature data to parameterize, the influence of the grazer *Daphnia* did not alter the prediction that the ciliate may control short-term flagellate pulses and temperature will influence these in a nonintuitive fashion.

Palaeolimnology

(**Roland Schmidt**, Kerstin Huber, Kaarina Weckström, Johann Knoll)

Within the project "**Late-Pleniglacial climatic and environmental evolution**" of the Austrian Science Fund (FWF project P18595-B17), Kerstin Huber (Ph.D. thesis) established a diatom-based quantitative inference model for summer epilimnetic water temperature (SEWT) by combining three datasets from the Alps (in total 116 lakes). The application of this transfer function (model prediction: $R^2_{jack} = 0.89$, RMSEP = 1.82 °C) to a sediment core section from Längsee (Carinthia, Austria) enabled for the first time to reconstruct water temperatures in the Alps for the last glacial termination spanning the period from approximately 19,000 to 13,000 years ago (Huber et al., in press). To put the results into the frame of Northern Hemispheric climate change, the inferred temperature curve was correlated with the GRIP oxygen event stratigraphy from Greenland ice-cores, the ice-rafting event of Heinrich 1 (six 'Heinrich events' were named after their discoverer, Heinrich 1988) in the North Atlantic, and glacier stadials in the Alps (Gschnitz, Clavadel/Senders). The presence of a warm period followed by a three-partited colder period related to Heinrich 1 fits well to the GRIP data (Fig. 8). In contrast to the terrestrial vegetation indicated by pollen, diatoms and chrysophytes (cysts) responded much quicker to climate change, thus providing strong climatic indicators. Exemplarily, morphotype differentiation was performed for selected chrysophyte cysts and diatoms (*Cyclotella comensis* complex), which improve the prediction quality of environmental reconstructions. However, non-analog conditions between fossil and recent diatom and chrysophyte cyst assemblages occurred during late glacial. Diatom and chrysophyte cysts were present in Längsee during late glacial, which today are lacking in the calibration training set lakes. Several of the chrysophyte cysts in Längsee were described as new (Huber et al., in press). Regarding the ecological preferences of the chrysophytes represented by cysts, the distribution in the sediment core was compared with the diatom-based SEWT and total phosphorus (TP) reconstructions of Längsee. They indicated 3 groups of cysts focusing to (1) meso/eutrophic conditions, (2) cold waters and/or oligotrophic conditions, and (3) showing no preferences against these variables.

For the validation of the inferred climatic patterns at Längsee and to solve the question how different lake types may respond to climate change, we started within the FWF-project a comparative multi-proxy study at Jeserzer (Saisser) See, Carinthia. This lake, which is ca. 35 km away from Längsee, is shallow. Although species composition differed from Längsee, the inferred SEWT (K. Weckström, postDoc fellowship from the Finnish Academy of Sciences) confirmed the climate reconstruction from Längsee (Schmidt et al., submitted)

The test of a new simultaneous multigroup inference model for the reconstruction of past environmental conditions using our Niedere Tauern lake data set (NT) showed no improvement against the one-organism-group approach (Thompson et al. 2008), which was used, e.g. , for Längsee (diatoms).

Since Längsee indicated rapid response of diatoms and chrysophyte cysts to climate change, we tried to quantify their speed of response to changing environmental conditions during global warming in an Alpine lake (Unterer Wirpitschsee, Niedere Tauern). For this reason we used again the NT dataset established 10 years ago. The investigations indicated slight compositional and environmental changes (mainly pH and alkalinity) during the decade 1998 – 2008. From diatoms, the Fragilariaceae appeared to be particularly sensitive to climate change (Huber et al., submitted).

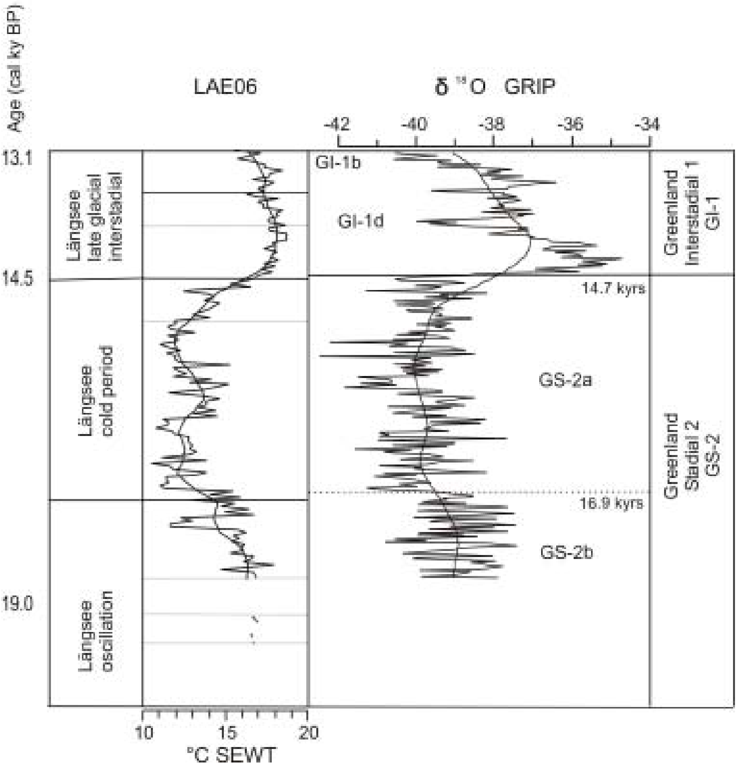

Fig. 8. Diatom-based summer epilimnetic water temperature reconstruction (SEWT) during the last glacial termination in Längsee (Carinthia) compared with the oxygen isotope event stratigraphy of the GRIP ice-core, Greenland (from Huber et al., in press).

During the reporting period we started an initiative called PALDAT (first meeting 10th – 11th March at ILIMNOL) to bring together, for the first time, scientists working on different climate proxies in Austria. A book on “climate change – 20,000 years of climate development in Austria (proxies, data, scenarios)” was published in the meantime[4]. The mentioned FWF

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[4] Schmidt, R., Matulla, C., and Psenner, R. (2009): Klimawandel in Österreich Die letzten 20.000 Jahre ... und ein Blick voraus. Alpine space & environment, Vol. 6, pp 184, innsbruck university press.

project and the project **CLIM-LAND** (**Seasonal climate impact on alpine land-use development**, Austrian Academy of Sciences and the Ministry of Life Sciences) provided major contributions to this book for the understanding of climate development and climate-driven changes and processes in Austria during the time from deglaciation to present. Highlights were disentangling of the complex interactions between climate and human impact in the Alpine lake Oberer Landschitzsee (Schmidt et al. 2008), the novel approach of seasonal climate reconstruction, the reconstruction of warm periods during the Roman and medieval times similar to present, and the close relationship between climate and Alpine land-use, which were reported earlier. Our long-term multi-proxy investigations at Längsee and Oberer Landschitzsee made both lakes to key sites for climate reconstruction in Austria.

Experimental Evolutionary Ecology

(**Claus-Peter Stelzer**, Johanna Schmidt, Anneliese Wiedlroither)

The working group "Experimental Evolutionary Ecology" studies evolutionary processes and their feedback on population dynamics, using freshwater rotifers as model organisms. Within the FWF project P20735 we investigate the causes and consequences of transitions to obligate parthenogenesis in cyclical parthenogenetic rotifers of the genus *Brachionus*. Our research efforts in 2008 were concentrated on two main topics: First, to develop an automated image analysis system as a new tool for the study of experimental rotifer populations and secondly, to quantify the fitness effects of transitions to obligate parthenogenesis in *Brachionus calyciflorus*.

(1) An automated system for sampling and analyzing experimental rotifer populations has been developed and tested extensively (Fig. 9). It relies on image analysis of digital photographs taken from subsamples of the culture. The system works completely autonomously for up to several weeks and it can sample up to 12 cultures at time intervals down to a few hours. It allows quantitative analysis of female population density at a precision equivalent to that of conventional methods (i.e., manual counts of samples fixed in Lugol's solution) and it can also recognize males, which allows detecting temporal variation of sexual reproduction in such cultures. Female body size is another parameter that can be measured automatically with the image analysis system. This feature may be useful for studies onf population productivity and/or in competition experiments with clones of different body size. A manuscript describing this analytical system was recently submitted for publication.

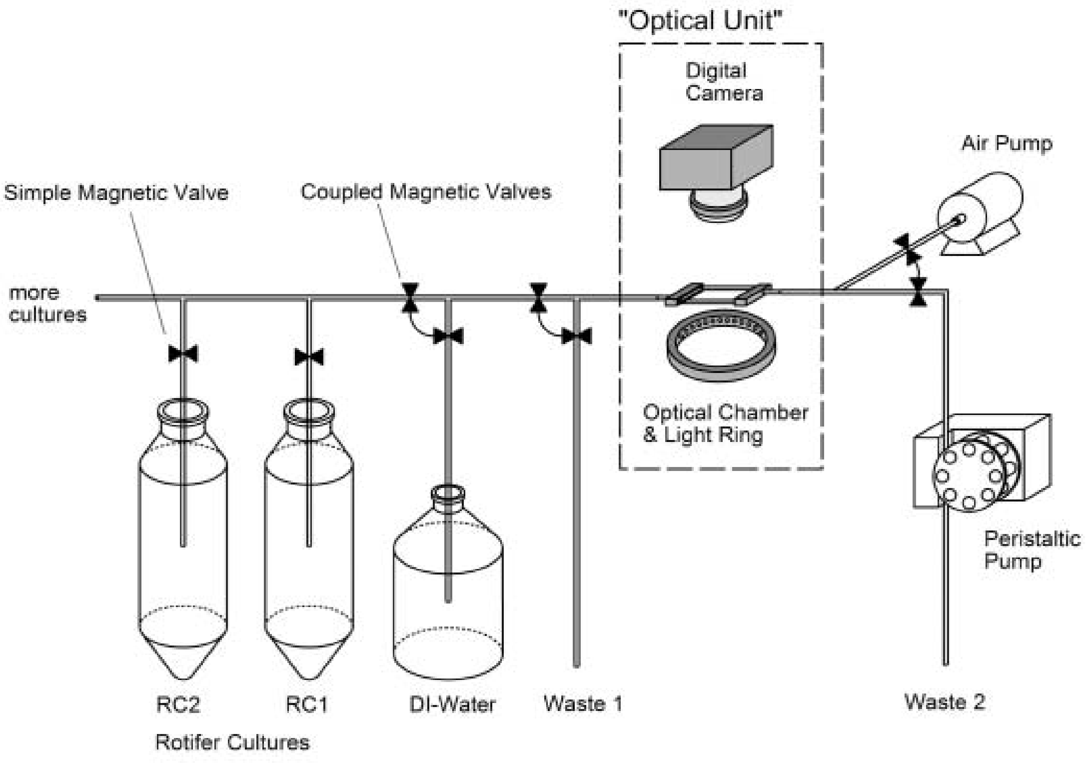

Fig. 9. Schematic drawing of the sampling and image analysis system. For simplicity, only two rotifer cultures (RC) are displayed (the system can handle up to twelve cultures). All electrical parts, i.e. magnetic valves, air pump, peristaltic pump, camera and illumination, can be controlled by a PC (PC and wiring not shown).

(2) Fitness differences between obligate vs. cyclical parthenogenetic strains have been quantified in life history experiments. These experiments suggested that obligate parthenogens should grow faster and enjoy a fitness advantage proportional to the level of sexual reproduction in their competing cyclical parthenogenetic strains. Body size measurements of more than thirty clonal lines have shown that obligate parthenogens are generally smaller compared to cyclical parthenogens (Fig. 10). This was surprising, because both reproductive forms were often highly genetically related and sometimes even derived from the same inbred line.

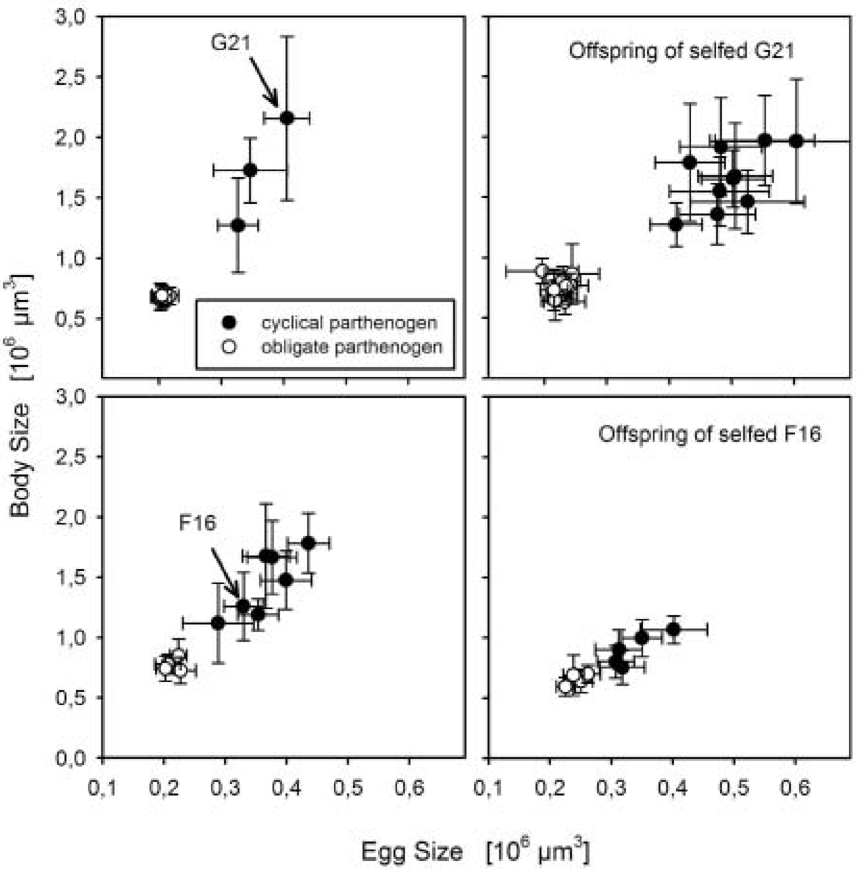

Fig. 10. Body size and egg size in clones of *Brachionus calyciflorus*. Left: First generation of inbred clones; right: second generation of inbred clones (established by self-fertilization of clones G21 and F16, respectively).

Direct competition experiments were used to test if obligate parthenogens are able to displace their cyclical parthenogenetic relatives, which should be the case according to theoretical models. These competition experiments were mainly analyzed using body size estimates by the image analysis system: Since obligate parthenogens were consistently smaller in body size than cyclical parthenogens, changes in mean body size of a population were indicative of shifts in the relative fraction of the reproductive types. In other words, if one competitor replaced the other, the mean body size of the population should shift towards that of the superior competitor in pure culture. We found that obligate parthenogens could indeed displace cyclical parthenogens when starting at equal proportions, but also when invading an already established population of cyclical parthenogens. By contrast, an established population of obligate parthenogens was resistant against invasion of cyclical parthenogens (Fig. 11). These results were based on measurements with the image analysis system and they were subsequently confirmed by direct determination of the reproductive types at the end of the experiments. A manuscript of these results is currently being prepared for publication.

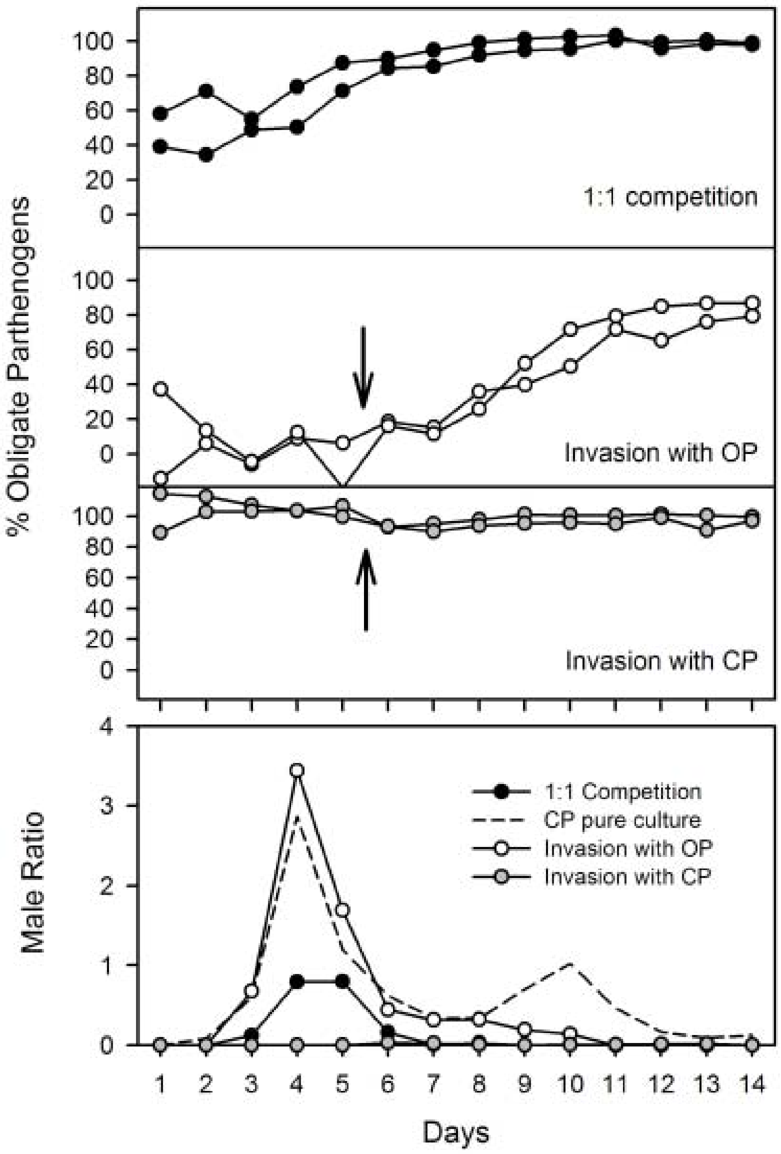

Fig. 11. Competition between cyclical and obligate parthenogens (descendants of clone G21). Estimates for the % obligate parthenogens are based on changes in the mean body size of the population. Arrows show the start of the invasion treatments.

Dissemination of the preliminary results took place mainly in the form of oral presentations. In June 2008, CPS took part in the Evolution 2008 meeting in Minnesota/USA (talk), in September 2008 he attended the DZG meeting in Jena/Germany (poster) and the DGL meeting in Constance/Germany (talk). Results from this project were also presented in two invited departmental seminars: in November 2008 at the University of Zurich/Switzerland and in December 2008 at the University of Göttingen/Germany.

Molecular & Cytogenetic Evolution of Asexual Aquatic Organisms

(**Dunja Lamatsch**, Maria Pichler)

The paradox of sex remains the queen of problems in evolutionary biology. Sexual reproduction is widespread throughout the animal and plant kingdoms, but under certain conditions remains costly compared to asexual reproduction. Sex creates a wide variety of genotypes on which natural selection can act, but it also breaks up favourable gene combinations. Asexuals have the ability to preserve their genome and to propagate genotypes that have a previous history of success. In addition, asexuals can reproduce twice as fast as sexuals, because all, rather than half, of their offspring are themselves capable of reproduction. Although asexual reproduction offers several clear short-term advantages, ruling evolutionary theory dictates that the absence of a mechanism for rapid genetic change will direct clones persisting over long time frames into evolutionary dead ends. Several animal and plant groups nevertheless show a large incidence of asexual reproduction and some lineages might have been fully asexual for many millions of years.

Leaving the University of Sheffield, I (D.L.) started my recent research position on January 15, 2008 at the Institute for Limnology. I am intending to continue my work on asexual ostracods and fishes in cooperation with several European Research institutions.

Sperm-dependent parthenogenetic fishes

The first quarter of the year was occupied with publishing a manuscript about the Amazon molly in BMC Evolutionary Biology (Loewe, Laurence & Lamatsch 2008). This publication raised much attention in the Austrian as well as international press. It also made a contribution in a short TV presentation documenting the activities of the institute (ORF OÖ, 4.7.08), as well as part of "Topic of the month (August): Sex and reproduction" of the ÖAW (http://www.oeaw.ac.at/home/thema/thema_200808_4.html).

In cooperation with Prof. Jukka Jokela (EAWAG, Zürich) and Dr. Jouni Taskinen (Jyväskylä, Finland) I am supervising a student in Finland working on the sperm-dependent parthenogenetic Gibel carp, *Carassius gibelio*, an invasive species (http://www.europe-aliens.org/speciesFactsheet.do?speciesId=53250#) that lately introgressed Finland. During two visits at the EAWAG, Zürich, we analysed the parasite load in relation to paternal introgression and genetic variability in two populations of this asexual fish. The successful cooperation will be continued with funding of EAWAG.

Genetic variability in non-marine ostracods with mixed reproductive modes

In April and May I have been working at the Royal Belgian Institute for Natural Sciences, Brussels, where I analysed data concerning the genetic variability in *Eucypris virens,* and started an ostracod culture at the institution of my collaborators Dr. Isa Schön/Prof. Koen Martens from the EU research training network “Sexasex”. During this time I also completed another manuscript that was accepted by Evolutionary Biology (Lamatsch et al. 2008) and gave an invited talk at the University of Leuven about genetic introgression in asexual fishes as a way to escape the clonal decay.

In August/September I have been working as honorary fellow at the University of Sheffield, UK, to supervise a student of the “Sexasex” RTNetwork and analyse data in cooperation with Prof. Roger Butlin. The analysed data was presented as invited talk and a poster presentation at the Annual Meeting of the genetic Society at Bath, UK. This cooperation, as well as my stay at Brussels, was subsidized by the “Researcher Exchange Program” of the Austrian Academy of Sciences.

Since the commencement of my employment coincided with the start of the institute´s reconstruction the set up of my laboratory facilities, as well as the aquarium for *Poeciliids* was significantly delayed. The interim space needed for the aquarium was generously provided by HR Dr. Albert Jagsch of the Federal Agency for Water Management, Institute for Water Ecology, Fisheries and Lake Resarch, Scharfling (http://www.baw-igf.at/).

Ecology of Freshwater Fish

(**Josef Wanzenböck**, Karl Maier, Maria Pichler, Barbara Pamminger-Lahnsteiner, Patrck Schöttl)

The first months (January to April) of 2008 were completely occupied with work on crossing experiments of Alpine and Baltic whitefish (*Coregonus* sp.). The previous attempts within the research project “Conservation of natural biological resources in Austria: Identification of indigenous whitefish species and extent of potential hybridization with a Baltic species” (Austrian Science Fund, FWF, project L229-B12), to conduct a full diallelic cross of 10 females and 10 males of each group were only partly successful. However, in winter 2007/ 2008 the research group started a third effort to accomplish this task which was successful. To monitor embryonic mortality and dynamics of hatching in 400 families with three replicates (1200 petri dishes with 30 eggs each) was a huge task requiring the full attention of all the personnel available in the research group and additionally the help of up to 12 undergraduate students to manage the enormous work load. Other important activities, normally pursued in parallel, had to be largely reduced which unfortunately is reflected in a low publication record of 2008. The crossing experiments involved the MSc project of Patrick Schöttl which was finished in late 2008 and summarizes the first results of the crossing experiments. Significant differences were found in the embryonic development of the different groups, with pure Alpine whitefish showing the lowest total mortality and pure Baltic whitefish showing the highest vales. Hybrid groups exhibited intermediate trait values (Fig. 12).

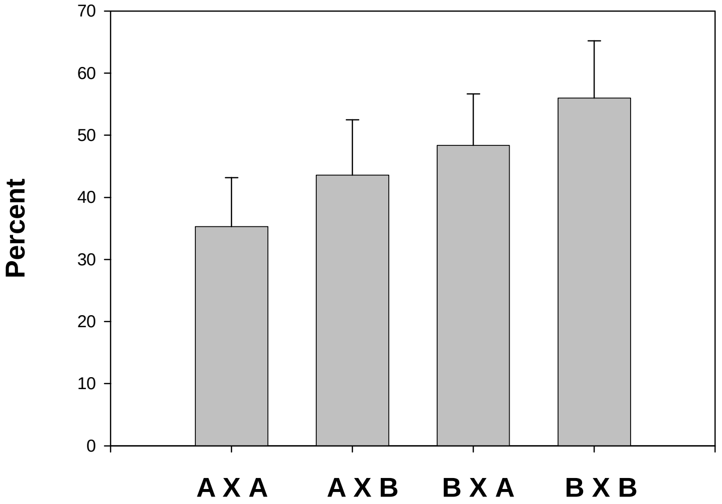

Fig. 12. Total average mortality (% of all eggs fertilized) throughout the embryonic development until hatching for the four crosses (100 families each) between Alpine (A) and Baltic (B) lineages. First letter denotes females, second letter represents males. Means and standard deviations of 100 families are drawn.

Another unexpected, but very promising result, was that the groups also differed with respect to developmental rate, i.e. duration of embryonic development from fertilisation to hatching (Fig. 13).

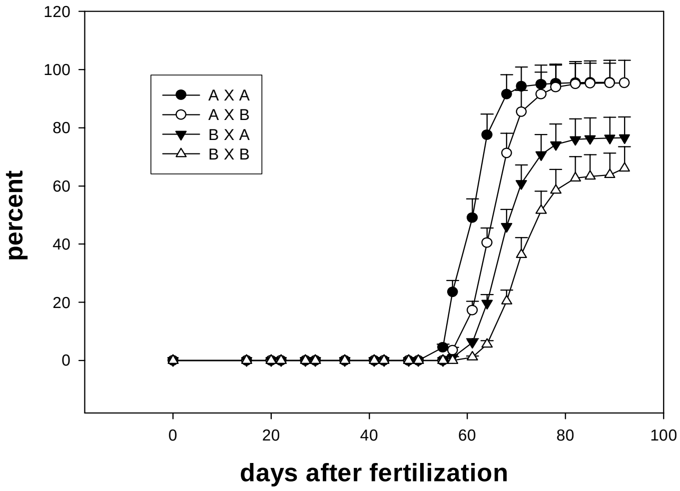

Fig. 13. Time course of hatching (cumulative % of fertilized eggs hatched) during the embryonic development of the four crosses of Alpine (A) and Baltic (B) lineages of whitefish. First letter denotes females, second letter represents males. Means and standard deviations of 100 families are drawn.

After the completion of the crossing experiments work resumed on other aspects which had been previously pursued in the mentioned project on whitefish. Specifically, data sets were analysed on genetic differentiation of native versus introduced whitefish strains in Lake Mondsee. These groups were hypothesised to differ in timing of reproductive activity (introduced fish spawn November/December, native fish mainly in January) which could be verified under experimental conditions and in the field using genetic (microsatellite) markers. These data (in addition to the data of Patrick Schöttl) were presented at the “10th Int. Symp. on the Biology and Management of Coregonids” which took place in August 2008 in Winnipeg, Canada. These data are part of two manuscripts submitted to the conference proceedings (Pamminger-Lahnsteiner et al., submitted, and Wanzenböck et al., submitted).

A symposium called “150 years of Ichthyology in Austria”, celebrating the 150th anniversary of the publication of the famous book by Heckel & Kner “Die Fische der östreichischen Monarchie (Fishes of the Austrian Empire)”, was organized (together with Dr. Sabine Wanzenböck, ILIM, and Dr. Ernst Mikschi, Natural History Museum Vienna) and held at the Museum of Natural History in Vienna, October 23-24, 2008. 15 lectures on the past, present and future activities of all relevant institutions in Austria working in some area of fish biology were presented at the meeting. Most of the lectures will be published in a special issue of the Journal “Österreichs Fischerei (Austrias Fishery)”, scheduled for autumn 2009.

Stream Ecology

(**Maria Leichtfried**, Anneliese Wiedlroither and external cooperation partners)

- The project “Initiative in River Ecology in Sri Lanka: from Science to Application” (IRESA) was continued and its research results were presented at three international meetings (IAD, SEFS, SLAFAR) in 2008. The international workshop “Riverscapes in Sri Lanka: Current knowledge and future challenges“ was co-organized within the framework of 14th Sri Lankan Association for Fisheries and Aquatic Resources Conference (SLAFAR) in Colombo, June 2008. Publication of the conference proceedings as a special volume of the “Int. Review of Hydrobiology” is in preparation. Also in relation to IRESA, a TV movie for high school education was produced in Sri Lanka. The film will be available in three languages (in English, Singhalese and Tamil).
- Within a new PhD study, financed by Austria, ecological experiments around leaf decomposition processes have been carried out on a perennial low order stream (Eswathu Oya) in a wet climatic zone of Sri Lanka. The experiments will continue in 2009.

Comparison of bed sediments in Sri Lankan streams with those in streams previously investigated in Europe and Africa: Bed sediments are important structures in lotic ecosystems. They serve as habitat for many organisms, as food resource, and agents of physical, chemical and biological processes. Bed sediments were studied in temperate and tropical zone streams in Europe, Africa and Asia within various projects during the last years. The investigated European streams are the River Danube (river km 2007) and a low order stream in the Austrian part of the Danube catchment area; the African streams are low order streams in the Rift Valley; the Asian streams are situated on different islands in Indonesia, Philippines and Sri Lanka. The food supply and macrozoobenthic inhabitation in bed sediments of nine streams located in different climatic zones, in different geographical positions, with different anthropogenic pollutions were studied in search for common features (Tab. 1).

In the temperate climate zone, the River Danube was investigated in Lower Austria, at river-km 2007, near Loiben, Wachau. A natural gravel bar is situated on the left streamside, creating a different hydrological situation and new habitats for invertebrate colonization in comparison to the main stream (Fig. 14). The second temperate stream investigated is Oberer Seebach, a 2nd order stream in the Danube catchment, located in a karstic prealpine mountain area, unpolluted, unregulated and in its catchment uninhabited (e.g. Leichtfried 1995). The site was investigated in the framework of long-term project Ritrodat-Lunz (1978 – 2003) of the Biological Station Lunz.

Tab. 1. Basic surface water characteristics of the nine streams investigated; two are located in temperate Europe, four in tropical Asia and three in tropical African zones. Annual means are reported with minimum and maximum values.

		Order	Temperature	Conductivity	pH
Stream	Country		(°C)	(μS_{20}/cm)	
Danube, km 2007	Austria	9	11,3 (0 - 19)	283 - 301	7,5 (7,0 - 7,5)
O. Seebach	Austria	2	6,9 (2 - 12)	212 - 220	7,7 (7,5 - 8,0)
Awu R.	Java, Indonesia	1	17,2 (14 - 22)	85 - 92	7,2 (6,8 - 7,7)
Tubod R.	Leyte, Philippines	1	26,2 (24 - 29)	74 - 112	7,4 (7,2 - 7,6)
Yan Oya	Sri Lanka, dry zone	2	27,0 (26,5 - 27,5)	295 - 594	7,3 (7,1 - 7,5)
Esw. Oya	Sri Lanka, wet zone	2	26,0 (25 - 27)	24 - 38	6,1 (5,2 - 6,9)
Sagana R.	Kenya, Mt. Kenya	1	10,8 (8,0 - 12,4)	30 - 36	6,5 (6,3 - 6,7)
Njoro R.	Kenya, Rift Valley	2	22,9 (18,2 - 29,2)	217 - 335	8,4 (7,4 - 9,9)
Baharini R.	Kenya, Rift Valley	1	28,2 (24,5 - 31,4)	705 - 815	8,6 (8,0 - 9,2)

Awu River is a rain forest stream on the slopes of Mt. Slamet, a 3428 m high volcano in central Java, Indonesia; pristine in the upper, inhabited in the lower reach; investigated within the cooperative Javadat project. Tubod River is a first order rain forest stream in the central mountains on the west reaches of the Leyte Island, Philippines, near the Leyte State University, Baybay, flowing into the sea, also pristine in the upper and slightly inhabited in the lower reach (Leichtfried et al. 2004).

Yan Oya is a seasonal low order stream in the dry climatic zone of Sri Lanka flowing to the Hurulu Wewa Reservoir. Annual floods during the northeast monsoon influence the stream. During the dry period, only the hyporheic zone in the streambed remains inundated, enabling organisms to survive. The level of anthropogenic pollution is moderate (Weliange 2007).

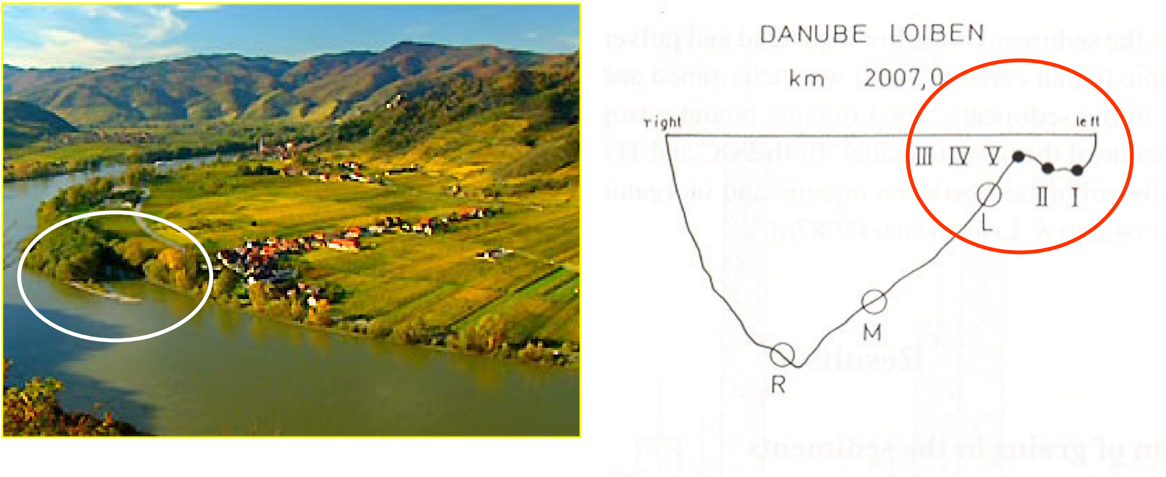

Fig. 14. River Danube near Loiben in Lower Austria, river-km 2007, width 300m, max. depth 8 m

Eswathu Oya is a 2nd order perennial flashy stream, a tributary to the Kelani Ganga (River) with dense riparian vegetation and without any pollution in the upper reach. Lower reach surrounded by rubber tree plantations. Both Sri Lankan streams were investigated within the cooperative project IRESA during 2005 – 2008 (Weliange 2007).

Sagana River is a stream in the catchment of Sana River, Kenya; its headwaters are on the slopes of Mt. Kenya at an elevation of 4000 m, our study site was at 3300 m, unpolluted and uninhabited. This work was part of a PhD study at Nairobi University (Mwangi 2000).

Njoro River is a Kenyan low order stream in the Rift Valley area, passing the Egerton University and campus, largest tributary of the protected soda Lake Nakuru. It is a heavily anthropogenically impacted river and, in its lower part, also industrially polluted. This river was investigated in the framework of a long-term cooperation with Egerton University (e.g. M`Erimba 2004).

Baharini is a short, clean water, grassland stream in the National Park Nakuru (700 – 1000 m), flowing to the famous flamingo Lake Nakuru in the Kenyan Rift Valley. The stream is a very important drinking water source for all animals living around the Lake Nakuru in the National Park. The investigations were done in connection with a PhD study in cooperation with Egerton University in Kenya (Shivoga 1999).

The main energy source for the benthic faunal community of all investigated streams is allochthonous organic matter. Most of it is retained in the bed sediments as biofilm and/or associated organic particles. The unprocessed allochthonous organic matter is only rarely directly available to the animal consumers; it has to be processed by the microorganismic community (mainly fungi and bacteria). According to Marshall (1984), the biofilm is defined by as the biomass of microorganisms and their organic excretions (exocellular polymers) attached to surfaces. Biofilm development in the bed sediments depends on the availability of colonizable surfaces areas, and these are negatively correlated with grain diameter (Leichtfried, 1985). Therefore, up to 88 % of nutrients in bed sediments are stored in grain size classes smaller 1 mm in diameter (Leichtfried, 1988). Sediments in all investigated streams are dominated by grain size classes larger than 10 mm. The processing of the organic matter takes place on the surface of and in the bed sediments.

Fig. 15 shows the nutrient situation in fine bed sediments, where the largest part is stored. A similar pattern emerged both for organic carbon and nitrogen. The concentrations are very low in the three streams on islands Sri Lanka and Leyte, Philippines. There is a trend of higher nutrients in the temperate zone, with the exception of the volcanic Awu River on Java, Indonesia.

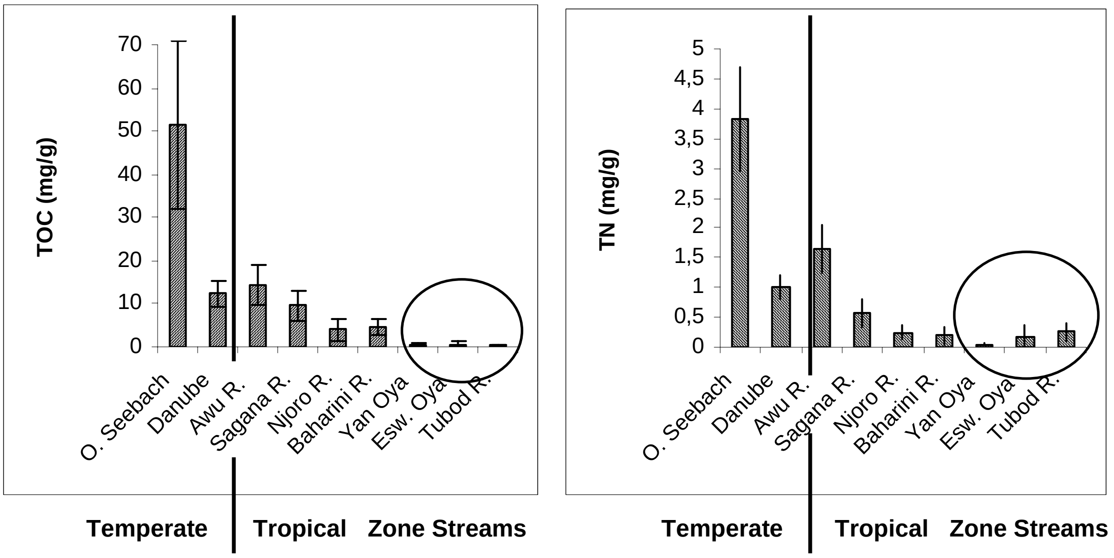

Fig. 15. Annual mean total organic carbon (TOC) and total nitrogen (TN) concentrations in fine bed sediments of the nine streams investigated. Two streams are located in temperate climate (the Austrian Danube and Oberer Seebach), seven in tropical climate.

C/N ratios were calculated as a crude proxy of food quality in the bed sediments. Low C/N ratios, which indicate high food quality for the invertebrate consumer community, were measured in the sediments of all streams investigated on tropical islands (Fig. 16, left). In conclusion, the food level available in the bed sediments of tropical island streams is very low, but the nutritional quality for the consumer community is good. It appears likely that food is a limiting factor for the benthos community, as abundance of macrozoobenthos is low in these island streams (Fig. 16, right).

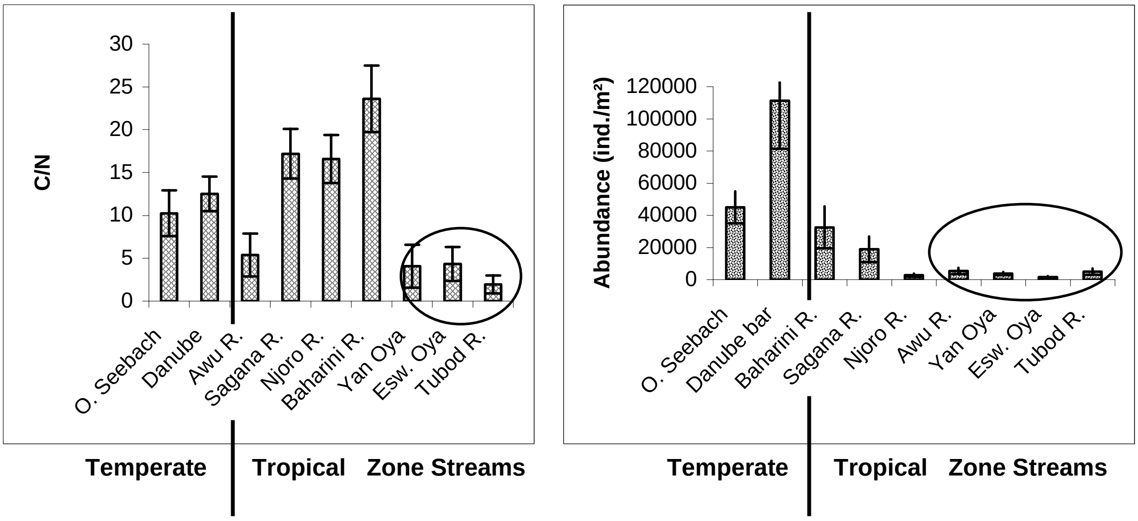

Fig. 16. C/N ratio (left) and abundance of benthic animals in bed sediments (right).

The low macrozoobenthos abundance in the Kenyan Njoro River is caused by heavy anthropogenic and industrial pollution. In contrast, a high invertebrate abundance was found in the Danube, especially in protected shallow areas around the gravel bar. This illustrates the positive effect of such structures for the whole river ecosystem, because the benthic community, together with the microbial biofilm community, plays an important role in the self-purification processes in rivers. Concerning biodiversity, we found higher species richness in mountainous tropical island streams like Eswathu Oya in Sri Lanka or Tubod River, Leyte, Philippines, than in the low land streams, although the abundances in mountain streams are lower (e.g. Weliange et al. 2008).

In summary, bed sediments in continental streams offer higher food supply for the benthos community, but of lower nutritional quality than in tropical island streams. The abundance of the benthic invertebrate community appears to reflect the amount of available food rather than its quality.

International Post-Graduate Training Programmes in Limnology (IPGL)

(**Gerold Winkler**, Regina Brandstätter and many national and international partners)

Since 1975 the Institute manages IPGL as a hub for international post-graduated training, research and networking in the area of limnology and freshwater ecosystem management. IPGL organizes a variety of training programmes and acts as interface platform linking research institutions world-wide. During 2008, 32 experts in aquatic ecology and water resource management attended academic training courses organized and implemented by IPGL. The IPGL participants were drawn from Ethiopia (4), Kenya (9), Poland (1), Saudi Arabia (2), Sudan (1), Tanzania (5), Uganda (8) and Zambia (2). The Austrian Development Cooperation funded 26 participants, 3 participants were sponsored via NFP (The Netherlands Fellowship Programme) and 3 participants by their employing institution. Four M.Sc projects were organized by IPGL and supervised by partner institutions in Austria and Africa (Uganda, Ethiopia, Zambia and Tanzania). All four M.Sc students finished their theses successfully and were awarded the academic title “Master of Science” in April 2008. The results of an IPGL M.Sc. thesis (A. Dagne) were published in a peer reviewed journal.

A fundamental restructuring process of the M.Sc. programme “Environmental Sciences with specialization in Limnology and Wetland Ecosystems” commenced in 2008. IPGL, UNESCO-IHE, Egerton University and the Austrian Development Cooperation agreed to implement the restructured M.Sc. programme in October 2009. The restructured M.Sc.

programme will consist of 4-months taught courses at UNESCO-IHE in The Netherlands, followed by a 4-months period at Egerton University in Kenya and 4-months in Austria organized by IPGL. Subsequently, the M.Sc. research period and thesis writing phase will take place either in the home country of the participants, in Austria, or The Netherlands. The new organizational structure strives for combining the best excellence of all partner institutions. The curriculum development has kicked-off in 2008 following a participatory approach. Academic quality assurance measures have been aligned to both European and East African standards in order to pave the way for an European-African joint degree in 2012.

In June 2008 the exhibition/workshop “Bridging research, technology & development: sustainable water management in Eastern Africa – phase II: initiating interactive stakeholder partnerships for sustainable water resource development” was organized in Kisumu, Kenya (Fig. 17). The workshop involved 140 experts drawn from governmental institutions (national/regional ministries & management bodies), universities, research institutions, civil society, entrepreneurs, industrialists and communities to intensify partnerships for the sustainable development and management of aquatic resources. The exhibition displayed pro-poor innovations and technologies for safe water supply, sanitation, water saving, irrigation, aquaculture, fisheries, fish processing, reduction of water borne & water transmitted diseases and environmental monitoring. National and regional water resource management organizations presented current policies and challenges in its implementation. The Kisumu exhibition/workshop integrated a special workshop on “demand-led research and improving the interaction between researchers and end-users”, which was organized by the EU-funded project “SPLASH”. The workshop participants gave an overview on current research activities on water resource management in Eastern Africa, provided recommendations on research needs in the region and suggested suitable institutional arrangements in North-South collaborations.

Emphasized was the pivotal role of science and technology for providing innovative tools for the sustainable management of aquatic resources, which could act as a milestone for the achievement of the Millennium Development Goals (improvement of water quality & human health, food security via aquaculture & fisheries, and environmental health).

Within the EU-funded research project “BOMOSA” the environmental carrying capacity of small-reservoirs in Eastern Africa and environmental impacts of fish farming were further investigated within the PhD thesis “Effects of cage fish farming systems on phytoplankton and water quality in small ponds of East Africa” carried out by N. Straubinger. Two IPGL organized M.Sc. research projects were carried-out within the BOMOSA framework (F. Degefu, R. Nalwanga). Furthermore, IPGL was also coordinating the dissemination work-package within BOMOSA (update of BOMOSA webpage, development of dissemination material and events, etc.) and the planning of the final BOMOSA conference taking place in September 2009.

Fig. 17. KISUMU WORKSHOP & EXHIBITION, June 2008. Bridging research, technology & development: sustainable water management in Eastern Africa – phase II: initiating interactive stakeholder partnerships for sustainable water resource development.

1.4. Congruence/deviations from medium-term research program 2008-2012

The five years' Mid-term Research Programme (MTRP), originally formulated in German in April 2005, outlined ambitious goals within the Institute's research focus, i.e. investigating the origin and maintenance of (intraspecific) diversity of aquatic organisms and communities. Those goals referred mainly to the establishment of new research fields such as Evolutionary Ecology of Asexual Aquatic Organisms and of new, interdisciplinary cooperations. The former has been achieved (see reports by C.-P. Stelzer and D. Lamatsch in the previous chapter), the latter had to be postponed for the following reasons.

The MTRP lists as prerequisites to reach those goals that the renovation and expansion of the Institute's building at Mondsee will be completed within the next two years (i.e., until mid-2007) and that vacant research positions can be renewed. Last year's Annual Report, however, stated the following under this heading: "It is at present impossible to forecast precisely the extent of impairment of our research by the construction work in the course of the rebuilding and expansion of the laboratory building at Mondsee; this is because a detailed scenario of the reconstruction is pending (state of mid-February, 2008). We currently favour to rent office and laboratory containers at an alternative site to ensure the progress of our research activities in the course of the construction work, which will begin in spring, 2008. Due to the uncertainties resulting from the unknown duration of the reconstruction and the specific expertise of the scientists succeeding U. Humpesch and M. Dokulil in 2009, a more detailed outlook on the Institute's research programme beyond 2010 appears impossible at present." Sixteen months later, at the end of June, 2009, the situation is even worse. The Institute for Limnology was forced to evacuate the main building at the lakeshore at very short notice and moved to various interim facilities in summer, 2008. The Institute is currently spread over five different localities at Mondsee and its surroundings, operating in makeshift laboratories and interim offices. Rebuilding of the Institute's main building was stopped in November, 2008, and an end of the reconstruction period is not in sight. Since over one year, we do not have the means to host guest researchers, and it has become increasingly difficult to attract graduate and undergraduate students within our research projects. The Institute's library is not working, because, due to the limited space, all books, older volumes of journals, and most other items had to be stored in boxes and are thus unavailable. The vacant positions of two scientists could not be filled, because of a general freeze of employment at the Austrian Academy of Sciences effective since the beginning of 2009. The reason is that the AAS and, accordingly, the Institute for Limnology do not have a budget at their disposal in 2009. As a consequence, all research and outreach activities of the Institute had to be cut to the minimum. In short, long and even medium-term planning have been replaced by erratic decision making. In view of this more than unpleasant situation, we refrain from updating and even extending our current research program.

1.5. Current version of the medium-term research program for 2009-2013

Currently not available, see the previous section.

1.6. Publications/speeches/poster presentations 2008

Peer-reviewed journal publications

Auinger, B., Pfandl, K., Boenigk, J. (2008) An improved methodology for the identification of protists and microalgae from Lugol-fixed plankton samples: Combining microscopical analysis with single cell PCR. Appl. Environ. Microbiol. 74:2505-2510

Boenigk, J. (2008) The past and present classification problem in nanoflagellates. Protist 159:319-337.

Christiansen, G., Molitor, C., Philmus, B., **Kurmayer, R.** (2008) Non-Toxic Strains of Cyanobacteria are the Result of Major Gene Deletion Events Induced by a Transposable Element. Mol. Biol. Evol. 25:1695-1704

Christiansen, G., Yoshida, W., Blom, J., Portmann, C., Gademann, K. et al. [..] (2008) Isolation and structure determination of two microcystins and sequence comparisons of McyABC adenylation domains in *Planktothrix* species. J. Nat. Prod. 71 : 1881-1886.

Gächter, E., Weisse, T. (2008) Long-term acclimation of growth rates in the oligotrich freshwater ciliate *Meseres corlissi*. Verh. Internat. Verein. Limnol. 30: 218-222.

Greisberger, S., **Dokulil M.T.** & Teubner, K. (2008) A comparison of phytoplankton size-fractions in Mondsee, an alpine lake in Austria: distribution, pigment composition and primary production rates. Aquat. Ecol. 42: 379-389.

Lamatsch, D.K., Fischer, P., Geiger, M., Lampert, K.P., Schlupp, I., Schartl, M. (2008) Diploids Amazon mollies (*P. formosa*) show a higher fitness than triploids in clonal competition experiments. Evol. Ecol., doi:10.1007/s10682-008-9264-2

Lampert, K.P., **Lamatsch, D. K**, Fischer, Petra, Schartl, Manfred, et al. [..] (2008) A tetraploid Amazon molly, Poecilia formosa. J. Heredity 99: 223-226.

Loewe, L., **Lamatsch, D.K.** (2008) Muller´s ratchet may threaten the Amazon molly and other ancient asexuals. BMC Evol. Biol. 88: 88-108.

Montagnes, D.J.S., Barbossa, A., Boenigk, J., Davidson, K., Jürgens, K., Macek, M., Parry, J., Roberts, E., Šimek, K. (2008) Selective grazing behaviour of freeliving protists: views on and avenues for continued study. Aquat. Microb. Ecol. 53: 83-98.

Montagnes, D.J.S., Morgan, G., Bissinger, J.E., Atkinson, D., **Weisse, T.** (2008) Short-term temperature change may impact freshwater carbon flux: a microbial perspective. Global Change Biol. 14: 2810-2822.

Philmus, B., **Christiansen, G.**, Yoshida, W., Hemscheidt, T., (2008) Posttranslational modification in microviridin biosynthesis. Chem. Biochem. 9: 3066-3073.

Schmidt, R., **Roth, M.**, Tessadri, R., **Weckström, K.** et al. [..] (2008) Disentangling late-Holocene climate and land use impacts on an Austrian alpine lake using seasonal temperature anomalies, ice-cover, sedimentology, and pollen tracers. J. Paleolimnol. 40: 453-469.

Thompson, R., Kamenik, C., **Schmidt, R.**, Pla, S., Rieradevall, M. et al. [..] (2008) Testing a new multigroup inference approach in reconstructing past environmental conditions. J. Limnol. 67: 155-162.

Weisse, T., Strüder-Kypke, M.C., Berger, H., Foissner, W. (2008) Genetic, morphological, and ecological diversity of spatially separated clones of *Meseres corlissi* Petz & Foissner, 1992 (Ciliophora, Spirotrichea). J. Eukar. Microbiol. 55: 257-270.

Weisse, T. (2008) Distribution and diversity of aquatic protists: an evolutionary and ecological perspective. Biodiv. Conserv. 17: 243-259.

Books, Book Sections, Conference Proceedings, Reports, Book Reviews

Anneville, O., Kaiblinger, C., Tadonléké, R.D., Druart, J.-C. & **Dokulil, M.T.** (2008) Contribution of long-term monitoringto the European Water Framework Directive Implementation. In: Proceedings of Taal 2007: The 12th Large Lake Conference, New Delhi, Ministry of Environment & Forests (ed.), p. 1122-1131.

Dokulil, M.T. & Kaiblinger, C. (2008) Assessment of potamoplankton and primary productivity in the River Danube: A review. (CD-ROM).

Dokulil, M.T. & Kaiblinger, C. (2008) Phytoplankton. Full Report + Annexes. (CD-ROM).

Hahn, M.T. and Q. L. Wu (2008) Planktonic bacteria in Chinese and European lakes: Are they different? In: Microbes and the Environment: Perspective and Challenges, S.-J. Liu H. L. Drake (Eds.), Science Press, Beijing, pp. 73-78.

Hahn, M.T. & Q. L. Wu (2008). Planktonic bacteria in Chinese and European lakes: Are they different? In: Microbes and the Environment: Perspective and Challenges, S.-J. Liu H. L. Drake (Eds.), Science Press, Beijing, p. 73-78.

Huber, K., Klee, R., **Schmidt, R.** (2008) Distribution and morphological variability of *Cyclotella*-taxa in the late glacial of Längsee (Austria). Proceedings of the 1st Central European Diatom Meeting 2007, S. 59-62.

Lamatsch, D.K., Stöck, M. (2008) Parthenogenesis and hybridogenesis unisexual fishes., hrsg. v. I.Schoen, K. Martens, P. VanDijk; Berlin.

Wanzenböck, J. (2008) Fish reproduction. In: M. J. Rocha, A. Arukwe and B. G. Kapoor (eds.), 629 pp., Science Publishers, Enfield, NH, U.S.A., 2008. . J. Fish Biol. 73: 1088-1089.

Weisse, T. (2008a) Limnoecology - The Ecology of Lakes and Streams (Book review of Lampert, W. u. Sommer, U., 2nd edition 2007). J Plankton Res. 30: 489-490.

Weisse, T. (2008b) Wilhelm FOISSNER and the German Society for Protozoology. Denisia 23: 9.

Weisse, T. (2008c) The meaning of protist diversity: ecology meets taxonomy. Denisia 23: 297-306.

Wolfram, G. & **Dokulil, M.T.** (2008) Leitfaden zur Erhebung der biologischen Qualitätselemente. Teil B2 – Phytoplankton. (Internet: http://wasser.lebensministerium.at/article/articleview/52972/1/5659).

Diploma Theses

Scheuerl, T. (2008) Metabolic and ecological characterisation of the genome-sequenced bacterium Polynucleobacter necessarius subsp. asymbioticus strain QLW-P1DMWA-1T by ecophysiological investigations. Master Thesis, University of Salzburg.

Lectures and Poster Presentations

Auinger, B. (06.03.2008) Molecular population studies from plankton samples preserved with Lugol's iodine solution – a quantitative approach. Annual Meeting of the Society for Protozoology Rostock/GERMANY.

Bode. S., Adolfsson, S., Bautz, E., Vanderkerkhove, J., Martins, M.J.F., Schmit, O., Mezquita, F., **Ostermaier,** V., **Kurmayer**, R. (17.05.2008) Distribution and abundance of non-toxic mutants in populations of cyanobacteria in the Alps. 1st MySIL Meeting, Lunz am See/AUSTRIA (poster).

Boenigk, J. (01.04.2008) An improved methodology for the identification of protists and microalgae from Lugol-fixed plankton samples: Combining microscopical analysis with single cell PCR. Meeting of the Phycology Section DBG, Wittenberg/GERMANY.

Boenigk, J. (09.04.2008) The past and present dilemma with protist species, diversity and ecophysiological differentiation. Annual meeting of the British Society for Protist Biology Gregynog/UNITED KINGDOM (keynote lecture).

Boenigk, J. (17.09.2008) Quantitative molecular population studies of single-celled eukaryotes reveal heterogenous population structures. Annual Meeting Ecological Society, Leipzig/GERMANY (invited).

Boenigk, J. (28.10.2008) Megasystematics meets microdiversity: Conceptual progress in microeukaryotic diversity research. University Potsdam, Potsdam/GERMANY (invited).

Boenigk, J. (21.09.2008) Population heterogeneity in protists: methodological advances and evolutionary implications. Annual Meeting of the German Zoological Association, Jena/GERMANY.

Boenigk, J. (06.03.2008) Niche separation and coexistence of chrysophytes: The Alpine Lake Gradient Analysis. Annual Meeting of the German Association for Protozoology Warnemünde/GERMANY (Poster).

Hahn, M. (22.02.2008) The Role of Microdiversity in the Ecology of Freshwater Bacterioplankton. Plant Biology Forum, Zurich/ SWITZERLAND (keynote lecture).

Hahn, M. W., Jezberova, J., Jezbera, J., Scheuerl, T. and Brandt, U. (13.11.2008) Biogeography and Ecology of Bacteria: What are Appropriate Taxa for Investigations? Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin/GERMANY (invited)

Durá, L. M., Picazo, A., **Hahn,** M. W., Camacho, y A. (10.09.2008) Distribución y abundancia de bacterias filamentosas (SOL CLUSTER) en LAGUNAS ESPAÑOLAS de distintas caracteristicas determinada mediante hibridación con sondas moleculares fluorescentes (fuish). XIV Congreso de la Asociacion Iberica de Limnologia Huelva/SPAIN.

Jezberova, J., Jezbera, J., **Hahn**, M.W. (09.06.2008) Microdiversity of closely related polynucleobacter genotypes and their co-ocurrence and dynamics in selected freshwater habitats in Central Europe. ASLO Summer Meeting St. John's/CANADA.

Huber, K. (08.09.2008) Diatom-environment relationships in alpine lakes and their application to climate reconstruction. 20th International Diatom Symposium, Dubrovnik/CROATIA.

Huber, K. (10.03.2008) Late-Pleniglacial and Late-Glacial of Längsee (Carinthia): Quantitative climatic and environmental reconstructions of Längsee from siliceous algae. Climate Change in Austria during the past 20,000 years: linking climatic and environmental data from different archives, proxies and time scales (PALDAT), Roland Schmidt, Mondsee/AUSTRIA.

Huber, K. (17.05.2008) Tracking late Pleistocene climatic variations in an alpine lake sediment core. 1st MySIL Meeting, Lunz am See/AUSTRIA (poster).

Jost, S. (06.03.2008) Niche separation and coexistence of chrysophytes: The case study ‘Loibersbacher Teiche’. Annual Meeting of the German Association for Protozoology Warnemünde/GERMANY (poster).

Kurmayer, R. (19.01.2008) Microcystin-producing Cyanobacteria in freshwater environments in Uganda. Hydrobotany Seminar, Institute of Botany, University of Innsbruck, Innsbruck/AUSTRIA (invited).

Kurmayer, R. (13.05.2008) Application and validation of qPCR methods for the monitoring of toxin-producing cyanobacteria in freshwater. Experts Scientic Workshop on

Development, Validation and Implementation of qPCR and PCR Methods for use in Recreational Waters, Maine/UNITED STATES (keynote lecture).

Kurmayer, R. (03.12.2008) Evolution der Toxinsynthese und Populationsstruktur bei toxischen Cyanobakterien. Botanical Colloquium, University of Innsbruck, Innsbruck/AUSTRIA (invited).

Lamatsch, D. K. (18.04.2008) Paradox of Sex. Seminar series, University of Leuven, Institute for Aquatic Ecology, Leuven/BELGIUM (invited).

Lamatsch, D.K. (05.09.2008) Genetic variability in a non-marine ostracod with mixed reproduction. Annual Meeting of the Genetic Society: The evolution of sex and asexual reproduction, Bath/UNITED KINGDOM (invited).

Lamatsch, D.K., Namiotko, T., Rossetti, G., Schön, I., Butlin, R.K. & Martens, K. (05.09.2008) Mitochondrial DNA phylogeny reveals multiple origins of asexuality and cryptic species within a Eucypris virens (Crustacea: Ostracoda) species complex. The evolution of sex and asexual reproduction, Bath/UNITED KINGDOM (poster).

Moser, M., **Scheffel**, U., **Weisse**, T. (06.03.2008) Protists in an acid mining lake - life at the extreme. Annual Meeting of the German Association for Protozoology Warnemünde/GERMANY (poster).

Pamminger-Lahnsteiner, B. (07.11.2008) Verhindern getrennte Laichzeiten von Renken und Maränen im Mondsee deren Hybridisierung? Eine morphologische und genetische Studie. Österreichische Fischereitagung, Mondsee/AUSTRIA.

Pamminger-Lahnsteiner, B. (14.03.2008) Screening lineage specific mtDNA haplo-groups in the larvae of putatively native and introduced stocks of whitefish (Coregonus sp.) in two Austrian lakes. 6th Conference of the German Society of Ichthyology (GFI) Munich/GERMANY.

Pamminger-Lahnsteiner, B. (26.08.2008) Does temporally displaced spawning time prevent the introgression of stocked whitefish species into native species? A morphometric and genetic study in Mondsee (Austria). 10th International Symposium on the Biology and Management of Coregonid Fishes, Winnipeg/CANADA.

Schmidt, R. (11.03.2008) Holocene climatic and environmental changes in an alpine lake (Oberer Landschitzsee, 2076m a.s.l.) tracked by seasonal bioindicator models, pollen and sedimentology. Climate change in Austria during the past 20,000 years, linking climatic and environmental data from different archives, proxies and time scales (PALDAT) (Roland Schmidt), Mondsee/AUSTRIA.

Schmidt, R. (15.06.2008) The Cyclotella comensis complex in alpine and pre-alpine lakes. Central European Diatom Meeting, Trento/ITALY (poster).

Stelzer, C.-P. (22.06.2008) Loss of sex in rotifers: mechanisms and adaptive significance. Evolution 2008, Minneapolis/UNITED STATES.

Stelzer, C.-P. (25.09.2008) Mechanismen und Fitness Konsequenzen von obligater Asexualität bei Rotatorien. DGL Annual Meeting 2008, Konstanz/GERMANY.

Stelzer, C.-P. (19.11.2008) Evolution of obligate parthenogenesis in Rotifera. BEES, Behaviour, Ecology and Evolution Seminar, Zurich/SWITZERLAND (invited).

Stelzer, C.-P. (15.12.2008) Evolution von obligater Parthenogenese bei Rotatorien. Zoological-Anthropological Colloquium, University Göttingen, Göttingen/GERMANY (invited).

Stelzer, C.-P. (19.09.2008) Giving up sex: transitions to obligate parthenogenesis in the rotifer Brachionus calyciflorus. 101. Annual meeting of the German Zoological Society, Jena/GERMANY (poster).

Straubinger, N., Schagerl, M., Kurmayer, R. (31.03.2008) Phytoplanktongemeinschaften und Microcystinproduktion in kenianischen Fischteichen. Annual Meeting of the Section Phycology of the German Botanical Association, Leipzig/ GERMANY (keynote lecture).

Straubinger; N., Schagerl, M., Kurmayer, R. (17.05.2008) Toxin production by cyanobacteria in fish ponds in Kenya. 1st MySIL Meeting, Lunz am See/AUSTRIA (poster).

Wanzenböck, J. (26.08.2008) Experimental evaluation of spawning activity in a native Alpine whitefish population versus an introduced population of whitefish (Coregonus lavaretus complex) in Mondsee, Austria. 10th International Symposium on the Biology and Management of Coregonid Fishes, Winnipeg/CANADA.

Wanzenböck, J. (27.08.2008) Embryonic development and mortality in crossing experiments of a Baltic and Alpine whitefish lineage (Coregonus lavaretus species complex). 10th International Symposium on the Biology and Management of Coregonid Fishes, Winnipeg/CANADA (poster).

Weisse, T. (26.08.2008) Biodiversity and ecology of freshwater ciliates. XXth International Congress of Zoology, Paris/FRANCE (keynote lecture).

Weisse, T., Moser, M., Scheffel, U. Stadler, P. (09.06.2008) Life at pH 2.6 - protists are adapted to extreme conditions. 2008 ASLO Summer Meeting, St. John's/CANADA (invited)

Winkler, G. (21.10.2008) International Post-Graduate Training Programme in Limnology: integrated capacity enhancement programme on freshwater ecosystem research & management in developing countries. UNESCO-IHE meeting, Delft/NETHERLAND (keynote lecture).

1.7. Scientific cooperation 2008

Bigelow Laboratory for Ocean Science, Bigelow, UNITED STATES
(Project: Niche separation and coexistence of chrysophytes in an alpine gradient)
Department of Microbiology and Ecology and Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Valencia, SPAIN
(Ecological and phylogenetic comparison of bacterial populations inhabiting Antarctic and European lakes)
Egerton University, Njoro, KENYA, Typ: Universitäre Lehr- und Forschungseinheit
(International Post-Graduate Training Programmes in Limnology)
Fachbereich Organismische Biologie der Naturwissenschaftlichen Fakultät der Universität Salzburg, Salzburg, AUSTRIA
(Patterns and processes of adaptation and tolerance to low pH of freshwater plankton)
Humboldt Universität Berlin, Institut für Biologie (Genetik), Berlin, GERMANY

Institut für Ökologie, Universität Innsbruck, Innsbruck, AUSTRIA
(Niche separation and coexistence of chrysophytes in an alpine gradient)
Lunds universitet, Lund, SWEDEN
(Niche separation and coexistence of chrysophytes in an alpine gradient)
Nanjing Institute of Geography and Limnology, Nanjing, CHINA
(Habitat specificity of flagellates and ecophysiological significance of microdiversity)
National Fisheries Resources Research Institute (NAFIRRI), Jinja, UGANDA
(Disssertation within IPGL: Molecular and chemical characterisation of toxic cyanobacteria in Ugandan freshwater)
Norwegian Institute for Water Research (NIVA), Oslo, NORWAY
(Disssertation within IPGL: Effects of cage fish farming systems on phytoplankton and water quality in small ponds of East Africa)
Sanofi Aventis, Frankfurt, GERMANY
(Habitat specificity of flagellates and ecophysiological significance of microdiversity)
School of Biological Sciences, Liverpool, UNITED KINGDOM
(Allgemeine Limnologische Grundlagenforschung)
UNESCO-IHE, Institute for Water Education, Delft, NETHERLANDS
(International Post-Graduate Training Programmes in Limnology)
Umweltforschungszentrum Leipzig Halle, Leipzig, GERMANY
(Habitat specificity of flagellates and ecophysiological significance of microdiversity)
University of Hawaii, Honolulu HI, UNITED STATES

Università di Pisa, Pisa, ITALY

(Genetic and ecological diversity of a narrow phylogenetic group of abundant freshwater bacteria)

Universität Leipzig, Leipzig, GERMANY

(Patterns and processes of adaptation and tolerance to low pH of freshwater plankton)

Universität Potsdam, Potsdam, GERMANY

(Patterns and processes of adaptation and tolerance to low pH of freshwater plankton)

(Niche separation and coexistence of chrysophytes in an alpine gradient ())

Universität Stuttgart , Stuttgart, GERMANY

(Habitat specificity of flagellates and ecophysiological significance of microdiversity)

Universität Wien, Fakultät für Lebenswissenschaften, Department für Meeresbiologie, Wien, AUSTRIA

(Disssertation within IPGL: Effects of cage fish farming systems on phytoplankton and water quality in small ponds of East Africa)

Universität Zürich, Zürich, SWITZERLAND

(Niche separation and coexistence of chrysophytes in an alpine gradient)

Veterinärmedizinische Universität Wien, Wien, AUSTRIA

(Niche separation and coexistence of chrysophytes in an alpine gradient)