Direct and indirect effects of fungi and oomycetes on leaf litter degradation by freshwater macroinvertebrates

Christine Aßmann

Konstanzer Online-Publikations-System (KOPS)
URN: http://nbn-resolving.de/urn:nbn:de:bsz:352-opus-122127
URL: http://kops.ub.uni-konstanz.de/volltexte/2010/12212/

Direct and indirect effects of fungi and oomycetes on leaf litter degradation by freshwater macroinvertebrates

Dissertation

zur Erlangung des akademischen Grades
des Doktors der Naturwissenschaften (Dr. rer. nat.)
an der

Universität Konstanz
Mathematisch-Naturwissenschaftliche Sektion
Fachbereich Biologie

vorgelegt von

Christine Aßmann

Konstanz, April 2010

Tag der mündlichen Prüfung: 19.07.2010
Referent: Prof. Dr. Eric von Elert
Referent: Prof. Dr. Karl-Otto Rothhaupt

In der Wissenschaft gleichen wir alle nur den Kindern, die am Rande des Wissens hie und da einen Kiesel aufheben, während sich der weite Ozean des Unbekannten vor unseren Augen erstreckt.

Sir Isaac Newton (1643 - 1727)

Table of Contents

Summary

Detritus may provide a major part of the total organic input into lakes, and littoral communities receive relatively high leaf litter inputs along the shoreline, where it is decomposed and integrated into secondary production. The decomposition processes in lakes are documented in few reports only, and it is therefore important to improve our understanding of the process of leaf conditioning and its effects on the benthic community in lakes. In the present thesis I investigated how the identity of microbial colonisers affects the consumption by macroinvertebrates organisms were examined. For the studies presented here the shredder *Gammarus roeselii* (Amphipoda) and small-particle-feeder *Limnomysis benedeni* (Mysida), a recent invader of Lake Constance were chosen because of their numerical importance in the littoral benthic community of Lake Constance.

During decomposition the physical structure of leaves and their chemical composition changes, and it has been suggested that the increased preference of shredders for conditioned leaves is caused by these changes. Here two experiments were performed, in which alder leaves were exposed in the littoral of Lake Constance. Regular leaf subsamples were analysed for chemical and physical leaf parameters, and the consumption rates of *G. roeselii* were determined in laboratory food choice assays with autoclaved and leached alder leaves as additional food items. In addition to leaf toughness the bulk leaf parameters nitrogen-, carbon-, phosphorus-, protein- and polyphenol content were measured and the ergosterol content was determined by HPLC. Consumption rates of littoral exposed leaves were statistically analysed for effects of leaf parameters using permutation based tests (1st experiment) and a linear model approach (2nd experiment). In both experiments leaf parameters changed and consumption by *G. roeselii* increased significantly with conditioning in the littoral. The negative correlation of polyphenols with shredder feeding corroborated the known repellence by polyphenols. Earlier it was assumed that increasing nitrogen and protein of leaf litter during decomposition lead to enhanced preference by invertebrate shredders. Notwithstanding

studies by others the N- and the protein content decreased over the first time of exposure in the littoral, which suggested that leaf colonising micro-organisms (fungi and oomycetes) could not compensate for leaching of N-containing constituents.

Statistical analyses revealed high co-linearity among leaf parameters, which hampered identification of causal relations between leaf parameters and the shredders' feeding rates. In both exposition experiments increasing ergosterol content over exposition time pointed at increasing metabolically active eumycotic fungal biomass on the littoral-exposed leaves, and therefore aquatic fungi and oomycetes were isolated from leaves that had led to high consumption rates of *G. roeselii*. The fungal and oomycete isolates were cultured and identified based on the sequence of internal transcribed spacer (ITS) regions of rDNA, and the sequences obtained during these studies were deposits into GenBank®.

In freshwaters, fungi are regarded as the most important microbial component on decaying leaves, and aquatic hyphomycetes were considered as the dominant group within the fungal communities on decaying leaves. Surprisingly no aquatic hyphomycetes were found here. However, 9 ascomycete (8 in the class of sordariomycetes) and 4 oomycete strains were isolated from conditioned leaf litter. This suggests, that in lentic freshwaters fungi others than aquatic hyphomycetes play a role in leaf litter degradation. Here, for the first time, oomycetes were demonstrated to affect leaf parameters to a similar extent as other fungal strains and to positively affect consumption by *Gammarus*, which suggest that oomycetes have a greater impact on leaf litter decomposition in freshwaters than hitherto assumed.

In order to experimentally separate effects of leaching and colonisation by fungi or oomycetes, experiments with single isolates growing on autoclaved leaves were performed, in which effects of single strains on leaf parameters and on consumption by *G. roeselii* were assessed. The majority of the different fungi and oomycete isolates on leaves were significantly preferred over controls, and consumption rates by *G. roeselii* proved to be strain-specific. The leaf parameters nitrogen, carbon, phosphorus, protein and polyphenol were affected by colonisation with single isolates, and the magnitude of the effects was

strongly strain-specific. Statistical analysis with a linear model revealed that polyphenol and protein levels were major determinants of the consumption rate of *Gammarus*, suggesting that fungi and oomycetes might indirectly steer consumption by altering the leaf litter content of protein and polyphenols, in particular during later stages of conditioning in the field.

Shredders discriminate between leaves colonised by different fungal and oomycete species, but mechanistically, the mediation of preference by fungi on leaves is not well understood. In order to test the hypothesis, that the strain-specific preference of *G. roeselii* is mediated by attractants or repellents that are constituents of fungi or oomycetes, selected fungal and oomycete strains were grown either in synthetic or leaf extract medium. Mycelia were extracted with solvents methanol or methylene chloride:methanol (2:1, v:v). Leaves covered with these extracts were subjected to choice feeding assays with *G. roeselii*. Methanol extracts proved to be repellent, and lipid extracts had no effect on the preference of G. *roeselii*. These results were contrary to the effects of the single isolates on leaves and suggested that compounds others than lipids or those extracted by methanol mediated the preference of *G. roeselii*. The repellent effect of the extracts of fungi or oomycetes was strongly affected by the carbon source in the growth medium.

The benthic mysid *L. benedeni* has recently invaded Lake Constance. Controlled laboratory experiments revealed that this mysid fed as well on shortly as well as on extensively leached leaf litter of several tree species. The interaction of the measured leaf parameters carbon- and polyphenol content explained 74% of the attractiveness of the leaf litter for the mysid, which suggested that feeding (scraping on the surface) of *L. benedeni* is hindered by the waxes and cutin of the cuticula and by the lignocellulose structure of the leaf. *Fusarium sporotrichoides*, *Microdochium* sp. PVSo2 and Ascomycete sp. PVSo8 growing on leaves elicited an intermediate feeding activity by *L. benedeni*, compared to that of littoral-exposed and autoclaved leaf litter. This suggests that *L. benedeni* feeds unselectively on the different microbial colonisers on decaying leaf litter. For the first time it was demonstrated that *L. benedeni* is a benthic leaf consumer that might potentially facilitate leaf degradation in Lake Constance.

Zusammenfassung

Detritus kann einen wesentlichen Anteil des organischen Eintrags in Seen ausmachen, und litorale Gemeinschaften erhalten relativ hohe Einträge an Laub entlang der Uferlinie, wo dieses zersetzt und in die Sekundärproduktion eingebracht wird. Dekompositionsprozesse sind in Seen nur wenig untersucht. In der vorliegenden Arbeit habe ich untersucht, wie die Identität von mikrobiellen Besiedlern die Konsumption konditionierten Laubs durch Makroinvertebraten beeinflusst. Für die hier gezeigte Untersuchung wurde der Shredder *Gammarus roeselii* (Amphipoda) und der Klein-Partikel-Fresser *Limnomysis benedeni* (Mysida), der erst in jüngster Zeit in den Bodensee eingewandert ist, wegen ihrer zahlenmäßigen Bedeutung in der benthischen Gemeinschaft des Litorals des Bodensees ausgesucht.

Während der Dekomposition verändern sich die physikalische Struktur und die chemische Zusammensetzung der Blätter; und es wird angenommen, dass die gesteigerte Präferenz von Shreddern für konditioniertes Laub durch diese Veränderungen verursacht wird. Hier wurden zwei Experimente durchgeführt, in denen Erlenlaub im Litoral des Bodensees ausgebracht wurde. Regelmäßig wurden von Laubunterproben die chemischen und physikalischen Blattparameter untersucht und in standardisierten Wahlexperimenten die Konsumptionsraten von *G. roeselii* mit den zusätzlichen Futterarten autoklaviertes und ausgewaschenes Erlenlaub bestimmt. Zusätzlich zur Blatthärte wurden die Blattparameter wie der Stickstoff-, Kohlenstoff, Phosphor-, Protein- und Polyphenolgehalt bestimmt und der Ergosterolgehalt mit der HPLC gemessen. Die Effekte der Blattparameter auf die Fraßraten der im Litoral ausgebrachten Blätter wurden mit permutations-basierten Tests (erstes Experiment) und mit einem linearen Modell (zweites Experiment) statistisch ausgewertet. Mit der Konditionierung im Litoral veränderten sich in beiden Experimenten die Blattparameter, und der Fraß von *G. roeselii* stieg signifikant an. Die negative Korrelation zwischen den Polyphenolen und dem Fraß der Shredder bestätigte die schon bekannte abschreckende Wirkung der Polyphenole. Allgemein wird angenommen, dass ein ansteigender Gehalt von

Stickstoff und Protein im Laub während der Dekomposition zu einer gesteigerten Präferenz der invertebraten Shredder führt. Abweichend davon nahm hier der Stickstoff- und Proteingehalt während der ersten Zeit der Exposition im Litoral ab, was darauf hindeutet, dass die Besiedlung mit Mikroorganismen das Auswaschen der stickstoffhaltigen Inhaltsstoffe nicht kompensieren konnte.

Die statistischen Auswertungen ließen ein hohes Maß an Co-Linearität zwischen den Blattparametern erkennen, welches die Identifizierung kausaler Zusammenhänge zwischen den Blattparametern und den Fraßraten der Shredder erschwerte. In beiden Expositionsexperimenten deutete der über die Expositionszeit ansteigende Ergosterolgehalt auf eine ansteigende metabolisch aktive eumycotische Pilzbiomasse auf den im Litoral ausgebrachten Blättern hin. Deshalb wurden von Laub, das zu hohen Fraßraten bei *G. roeselii* führte, aquatische Pilze und Oomyceten isoliert, in Kultur gebracht und, basierend auf der Sequenz der Internal-Transcribed-Spacer (ITS) Regionen der rDNA, identifiziert. Die in den Studien erhaltenen Sequenzen wurden in GenBank® hinterlegt.

In Süßwasser werden Pilze als wichtigste mikrobielle Komponente auf sich zersetzendem Laub angesehen, und aquatische Hyphomyceten werden als die dominante Gruppe innerhalb dieser Pilzgemeinschaften betrachtet. Erstaunlicherweise wurden hier keine aquatischen Hyphomyceten gefunden, wohl aber 9 Ascomyceten (8 aus der Klasse der Sordariomyceten) und 4 Oomyceten. Das deutet darauf hin, dass in stehenden Gewässern nicht aquatische Hyphomyceten sondern andere Pilze eine Rolle in der Laubzersetzung spielen. Zum ersten Mal wurde hier nachgewiesen, dass Oomyceten, ebenso wie andere Pilzarten, die Parameter der Blätter beeinflussten und sich positiv auf den Fraß von *Gammarus* auswirkten. Das deutet darauf hin, dass Oomyceten einen größeren Einfluss auf die Laubdekomposition in Süßgewässern haben als bisher angenommen.

Um die Effekte der Auswaschung die der Besiedlung durch Pilze und Oomyceten experimentell zu trennen, wurden Versuche durchgeführt, in denen die einzelnen Isolate auf autoklaviertem Laub wuchsen und die Effekte der einzelnen Pilze und Oomyceten auf die Blattparameter und den Fraß durch *G.*

roeselii untersucht wurden. Im Vergleich zu den Kontrollen, führte der Großteil der unterschiedlichen Pilze und Oomyceten auf Laub zu erhöhten Fraßraten von *G. roeselii*. Die Blattparameter Stickstoff, Kohlenstoff, Phosphor, Protein und Polyphenol wurden spezifisch durch die einzelnen Isolate beeinflusst. Die statistische Auswertung mit einem linearen Modell machte deutlich, dass die Gehalte an Polyphenol und Protein die bestimmenden Faktoren für die Fraßrate von *Gammarus* waren. Dies deutet darauf hin, dass Pilze und Oomyceten die Konsumption indirekt steuern können, indem sie den Protein- und Polyphenolgehalt des Laubes, im Besonderen während späterer Stadien der Konditionierung im Gewässer, ändern.

Shredder können zwischen Blättern unterscheiden, die mit unterschiedlichen Pilz- und Oomycetenarten besiedelt sind, wobei unklar ist, wie diese Präferenz durch Pilze vermittelt wird. Um die Hypothese zu untersuchen, dass die isolat-spezifische Präferenz von *G. roeselii* durch Attraktantien und Repellentien der Pilze oder Oomyceten hervorgerufen wird, wurden ausgesuchte Pilz und Oomyceten Isolate entweder in synthetischem oder in Blattextraktmedium herangezogen. Die Mycelien wurden mit den Lösungsmitteln Methanol oder Dichlormethan:Methanol (2:1, v:v) extrahiert. In Futterwahlversuchen mit *G. roeselii* und Laub, das mit diesen Extrakten behandelt wurde, erwiesen sich die Methanolextrakte als abschreckend, und die Lipid Extrakte hatten keinen Effekt auf die Präferenz von G. *roeselii*. Diese Ergebnisse stehen im Gegensatz zu den Effekten der einzelnen Isolate auf Laub und deuten darauf hin, dass andere Inhaltstoffe als Lipide oder die durch Methanol extrahierten die Präferenz von *G. roeselii* vermitteln. Der abschreckende Effekt der Extrakte aus Pilzen und Oomyceten wurde stark durch die Art der Kohlenstoffquelle im Wachstums-medium beeinflusst.

Die benthische Myside *L. benedeni* ist erst kürzlich in den Bodensee eingewandert. Hier konnte in kontrollierten Laborexperimenten gezeigt werden, dass diese Myside sowohl kurz als auch intensiv ausgewaschenes Laub von unterschiedlichen Baumarten frisst. Die Interaktion der gemessenen Blattparameter Kohlenstoff- und Polyphenol-Gehalt erklärte 74% der Attraktivität des Laubes für die Myside, was darauf hinweist, dass der Fraß von *L. benedeni* durch Wachse und das Cutin der Cuticula und durch die

Lignocellulosestruktur des Blattes behindert wird. Im Vergleich zu Litoral exponiertem und autoklaviertem Laub, lösten *Fusarium sporotrichoides*, *Microdochium* sp. PVSo2 und Ascomycete sp. PVSo8, die auf dem Laub wuchsen, eine intermediäre Fraßaktivität bei *L. benedeni* aus. Dies lässt vermuten, dass *L. benedeni* die unterschiedlichen mikrobiellen Besiedler des Laubes unselektiv frisst. Hier wurde zum ersten Mal gezeigt, dass *L. benedeni* ein benthischer Laubkonsument ist, der die Laubzersetzung im Bodensee potentiell fördern könnte.

Chapter I

General introduction

General Introduction

One-third of the world's terrestrial area is covered by forest (Otto 1994). This ecosystem plays a significant role in the global carbon-dioxide (CO_2) budget, as it contains one sixth of total earth's atmospheric CO_2 (120 billion tons a^{-1} CO_2 in photosynthetically active plants). Forests are open ecosystems, which exchange material and energy with adjacent forests, open landscapes, the atmosphere and with freshwater lakes and running water systems (Waring and Schlesinger 1985). The forest-derived material comprises dead wood, twigs, branches and leaf litter. In particular the rate of litterfall varies considerably with season, and in the northern hemisphere it shows a maximum in autumn (Waring and Schlesinger 1985; Abelho 2001). Seventy percentage (%) from above ground dead organic material is leaf litter (Waring and Schlesinger 1985), and this constitutes a major energy resource for freshwater and soil ecosystems (Waring and Schlesinger 1985; Abelho 2001).

After the leaf litter is shed from the trees and has fallen to the ground or into water, this non-living particulate organic material is called detritus (Lampert and Sommer 1997), and the process of decomposition begins. Decomposition is defined as a number of interrelated chemical, physical and biological processes, by which organic matter is broken down into smaller particles (fine particulate organic matter, FPOM). During decomposition inorganic nutrients and dissolved organic matter (DOM) are released and then become available for uptake by plants and microorganisms (Waring and Schlesinger 1985; Berg and McClaugherty 2003).

Leaf litter reaches freshwater systems by vertical fall from trees or by lateral movement (e.g. wind; Abelho 2001). In many streams, rivers and lakes, detritus is the dominant energy resource (Webster and Benfield 1986), and the transfer of this allochthonous carbon to aquatic herbivores and detritivores represents a major pathway of energy flow (Webster and Benfield 1986; Reshi and Tyub 2007). Leaves enter the aquatic system as coarse particulate organic material (CPOM) and are subsequently decomposed to DOM and FPOM by several, often simultaneous, processes (reviewed by Abelho 2001). In principle, three

phases of decomposition of dead leaves in freshwaters are observed: a) leaching of soluble organic substances; b) microbial colonisation and degradation, and c) fragmentation by physical abrasion or invertebrate shredding. Microorganisms and invertebrates convert the energy and the nutrients of leaf litter into secondary production (reviewed by Graça 2001).

An early process in decomposition is the leaching of soluble substances from the leaf litter. Leaching begins shortly after leaves have entered the terrestrial or aquatic system, and then the main groups of soluble substances in litter such as sugars, phenolics, hydrocarbons and glycerides (Berg and McClaugherty 2003) are dissolved. In the aquatic environment, leaching mainly takes place within the first 24 to 48 hours, but soluble substances like polyphenols have been shown to continue leaching out for longer periods (Bärlocher 1992b; Abelho 2001).

Simultaneously to leaching, leaves are colonised by microbial decomposers, mainly fungi and bacteria. The enzymatic capabilities of these microbial decomposers enable them to degrade structural leaf components such as cellulose, hemicellulose and lignins (Bärlocher *et al.* 1992; Berg and McClaugherty 2003). The entire process of leaching and microbial colonising of leaf litter is referred to as 'conditioning' (Abelho 2001), and this term describes the changing quality of leaf detritus as food for invertebrates. In general, conditioning is assumed to stimulate leaf decomposition by invertebrates (Suberkropp 1992). With microbial, mainly fungal, colonisation during conditioning, concentrations of nutrients in leaves have been observed to change. Some are leached out whereas others increase. For example it is known that in leaf litter the concentrations of protein, nitrogen and phosphorous increase (Bärlocher 1985; Suberkropp 1992; Graça *et al.* 1993b) and other parameters, e.g. mechanic stability, decrease (Graça and Zimmer 2005). Another aspect of leaf litter decomposition is the fragmentation of the leaf litter into particles of smaller size and an increased surface-area. This fragmentation is partly caused by mechanical forces due to wave action but mainly by invertebrates termed shredders (Webster and Benfield 1986; Suberkropp 1992; Graça 2001).

Often leaves are already colonised by microbes while still attached to the tree (Waring and Schlesinger 1985), and after they enter the water a subsequent colonisation by aquatic fungi and bacteria takes place. It has repeatedly been shown that fungi are the most important microbial component on decaying leaf litter in freshwaters (reviewed by Gessner *et al.* 2007), and fungal biomass associated with decomposing leaves can account for up to 16% of total detrital litter mass (Abelho 2001). Marano *et al.* (2010) reviewed that fungi act as trophic intermediates of energy flow between fallen leaves and higher tropic levels. The colonisation by microbes enhances the decomposition of leaves directly by macerating the leaves and indirectly by increasing the palatability of the leaves for invertebrate leaf degraders, which suggests that fungi play an important role in the decomposition of leaf litter (Otto 1994; Bärlocher 2007). In most decomposition studies from running waters, aquatic hyphomycetes were considered as dominant species of the fungal communities on leaves (Bärlocher *et al.* 1992; Bärlocher 2009), but the presence of fungi of other phyla has also repeatedly been shown (reviewed by Bärlocher 2009).

In the early stages of decomposition, in addition to fungi, oomycetes have been found on decomposing leaf litter (Bärlocher *et al.* 1992). Although oomycetes are well represented in freshwater habitats (Nechwatal and Mendgen 2009), little is known about their ecological function (Zare-Maivan and Shearer 1988; Dix and Webster 1995), although some may play a role in the early breakdown of detritus (Brasier *et al.* 2003).

In freshwaters, leaf litter is consumed primarily by invertebrate shredder organisms like crustaceans (e.g. isopods, amphipods), trichopterans and in some cases freshwater snails (gastropoda; Suberkropp 1992; Graça 2001). Shredders feed on coarse particulate organic material (COPM) thereby reducing it to fine particulate organic material (FPOM), which in turn constitutes a food source for other invertebrates like collectors and gatherers (e.g. chironomids, trichopterans; Graça 1993; Graça *et al.* 2001b) and possible filter-feeders (e.g. bivalves in the littoral through wave caused suspension). It is well established that shredder (aquatic as well terrestrial invertebrates) prefer naturally conditioned over unconditioned leaves (reviewed in Suberkropp 1992

and Maraun *et al.* 2003). Many laboratory experiments have demonstrated that shredders preferred, grew and survived better when they were offered leaf litter colonised with a single fungus (reviewed by Suberkropp 1992 and Graça 2001). This preference for conditioned leaf litter suggests that leaching and microbial colonisation improves the nutritional quality of leaf litter for shredders.

Most studies on leaf litter decomposition and the role of microbial colonisation for feeding by shredders have, however, investigated running water systems. Only a few reports on leaf conditioning (Federle and Vestal 1982; Federle *et al.* 1982) and decomposition in lakes are available (Webster and Benfield 1986; Sabetta *et al.* 2000; van Dokkum *et al.* 2002). In lakes, allochthonous vascular plant material may provide between 10% and 75% of the total organic input (Webster and Benfield 1986), which emphasizes the importance of leaf litter for the littoral of lakes.

Lake Constance is a pre-alpine Lake situated in the south-western part of Germany (47° 39' N, 9° 18' E). With a water surface area of 529 km^2, a maximum depth of 253 and a shore line of 289 km (Ostendorp *et al.* 2004), Lake Constance is the largest German lake. The shore line of Lake Constance shows two different types of habitats: bluffs (e.g. between Meersburg and Überlingen) and plain watersides. Stony habitats have a diverse benthic fauna, in which shredders are abundant (Baumgärtner *et al.* 2008). It is well known that allochthonous input is a major carbon source for lentic food webs (Pace *et al.* 2004), and detritus may provide a major source of the total organic input in lakes (Webster and Benfield 1986). In a large lake like Lake Constance the leaf litter input is of less relative importance than in rivers and streams. In large lakes, leaf litter has a rather punctiform impact and plays a significant role on the local scale in the littoral zone.

Here in the littoral of Lake Constance one of the main shredder organisms is the omnivorous amphipod *Gammarus roeselii* (Fig. I 1A; Mörtl 2004; Baumgärtner *et al.* 2008). It originates from the Balkans and was first described in Paris (Gervais 1835). Before 1974, during the lake eutrophication, *G. roeselii* established itself in Lake Constance and was the dominant gammarid amphipod in Lake Constance until 2002 (Hesselschwerdt *et al.* 2009).

In former days marine, terrestrial and freshwater habitats were undisturbed, had a habitat-fitted diverse flora and fauna and were isolated through natural barriers. The industrialization led to extensive trade and, along with this, to the dispersal of species into nearly all kinds of habitats (Kowarik 2003). These 'biological invasions' are defined as a human-mediated processes, which allow organisms to establish themselves in areas they never would have reached naturally (Kowarik 2003). These anthropogenically introduced invaders (invasion after 1492) are defined as 'neobiota' (Kowarik 2003) and, following this nomenclature, invasive animal species are called 'neozoans' (Boye 2003). Anthropogenically transformed waterways, such as the connections of streams and rivers through canals, (e.g. the Main-Danube Canal as the most important migration route in Europe), accelerate the expansion of invading aquatic species (reviewed by Gergs *et al.* 2008). The main aquatic invasion pathways in Germany are shipping canals, ballast waters, aquaculture and stocking and ornamental trade (Nehring and Klingenstein 2009), which are leading to increasing biological homogenation (Gherardi 2007). Invasive species may either have positive (e.g. by providing abundant food for animal predators) or negative (e.g. replacement of native species by competition) effects on the invaded ecosystems, their members and community structure (Nehring and Klingenstein 2009).

Lake Constance has been invaded by 17 benthic invertebrate species in the last 5 decades (Rey *et al.* 2005; Hanselmann and Gergs 2008). Most of the neozoans appeared in Lake Constance later than in other German freshwaters, because of the natural barrier of the Rheinfall (Schaffhausen) and because of the natural undisturbed habitat structures in the upper river Rhine (Hesselschwerdt *et al.* 2009). The most considerable invaders of the past and recent times in Lake Constance are the zebra mussel *Dreissena polymorpha* (Siessegger 1969), the crayfish *Orconectes limosus* (Hirsch *et al.* 2008), the asian clam *Corbicula fluminea* (Werner and Mörtl 2004), the amphipod *Dikerogammarus villosus* (Rey *et al.* 2005) and the freshwater mysid *Limnomysis benedeni* (Fritz *et al.* 2006).

L. benedeni (Fig. I 1B) is an euryhaline and strong invasive mysid species, which originates from the estuary region of the Ponto-Caspian sea (Grigorovich *et al.* 2002). It migrated fast through the Main-Danube system into the river Rhine system (Bij de Vaate *et al.* 2002) and from there into Lake Constance (Fritz *et al.* 2006; Wittmann 2007). *L. benedeni* was mostly found in the littoral zone of Lake Constance (Weish and Türkay 1975; Wittmann 1995; Gergs *et al.* 2008).

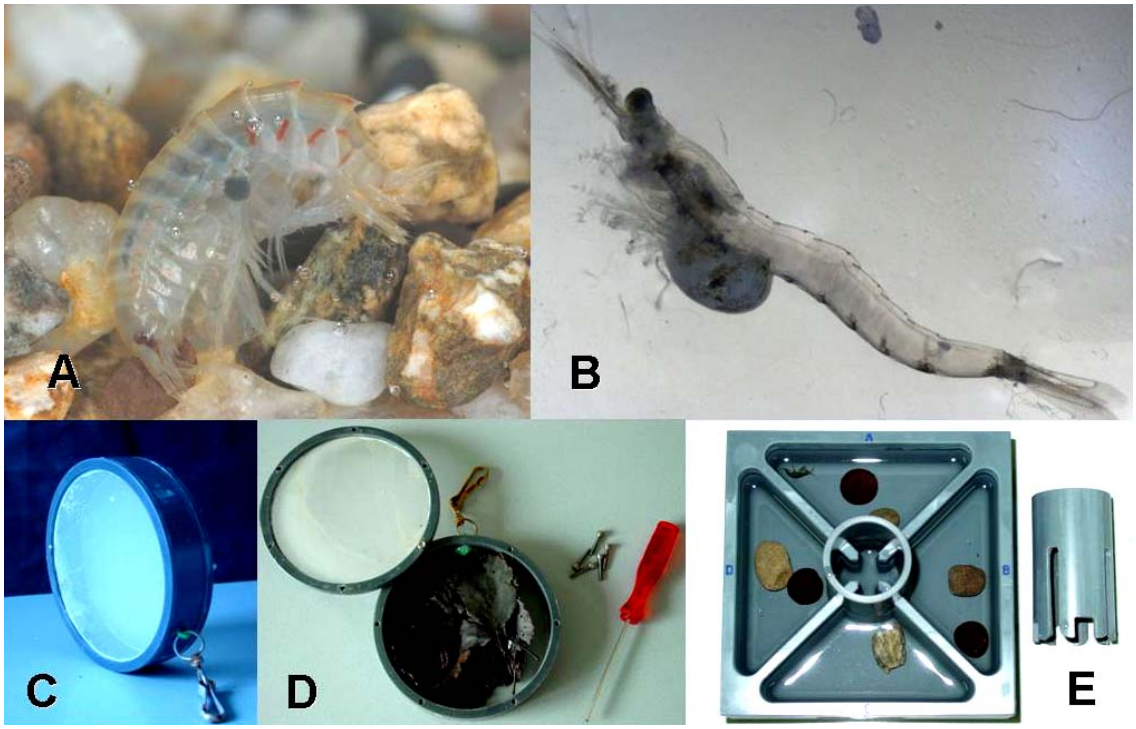

Fig. I 1. Invertebrate decomposers investigated in this study: **A**) the amphipod shredder *Gammarus roeselii* and **B**) the invasive mysid *Limnomysis benedeni*. **C**) and **D**) leaf litter exposition cage for littoral exposure of the leaf litter and **E**) four-chambered polyethylene container used in food choice assays with *G. roeselii*. Photographs: A) courtesy of Dr. M. Mörtl and B) courtesy of A. J. Hanselmann.

In earlier reports *L. benedeni* is described as detritivorous-herbivorous with a preference for smaller food particles (Dediu 1966). However the most recent study of Gergs *et al.* (2008) classified the mysid as an omnivore-herbivore feeder. In this study the mysid fed on algae, epilithon, chironomids and on

conditioned black alder leaves. Therefore the authors assumed that *L. benedeni* used mainly biofilm-associated fungi and bacteria on the leaves as food source. Most leaf litter decomposition studies from streams and lakes focused on trichopterans, amphipods and isopod shredders, which were regarded as shredders with a comparably high feeding activity (e.g. Arsuffi and Suberkropp 1988; Suberkropp 1992; Graça *et al.* 1993b; Graça *et al.* 2001a; van Dokkum *et al.* 2002). It has been documented that in absence of plecoptera and trichoptera other invertebrates, e.g. gastropods, may function as shredders (Graça 2001). In the case of Lake Constance, the densities of the main shredder *G. roeselii* were strongly reduced after the invasion of the ponto-caspian amphipod *Dikerogammerus villosus* (Hesselschwerdt *et al.* 2008). In parallel, *L. benedeni* was able to establish itself in high densities in the benthic community in the littoral of Lake Constance. This led to the hypothesis that this mysid could have an impact on the leaf litter decomposition in the lake and thereby affect the allochthonous energy flow.

In this thesis I investigated the process of leaf litter breakdown by macro-invertebrates from the littoral zone of Lake Constance. I focused on the impact of fungi and oomycetes on the food preference of leaf litter of two benthic crustaceans, *L. benedeni* and, in particular, the amphipod *G. roeselii*.

In the **2nd chapter** I investigated to which extent food preference by *G. roeselii* depends on the microbial colonisation of the detrital material. In a combined field/laboratory approach, leaf litter was exposed (Fig. I 1C and 1D) in the littoral of Lake Constance and the consumption of these conditioned leaves was assessed over time in standardized laboratory assays with *G. roeselii* (Fig. I 1E). After reaching the date of maximum consumption of the exposed leaves, actively growing fungi and oomycetes were isolated from the leaf litter and identified. In order to test for strain-specific effects, these single strains of fungi or oomycetes were assayed for their effects on feeding preferences of *G. roeselii*.

In the **3rd chapter** another conditioning experiment with leaf litter in the littoral was performed, during which leaf litter parameters and the consumption of *G. roeselii* were monitored over time. In order to experimentally separate the effects of leaching and those of fungi or oomycetes on leaf parameters, pure strains of fungi and oomycetes were established as cultures and their effects on leaf parameters and on the consumption of *G. roeselii* were determined. Using these data and those outlined in the 2nd chapter statistical analyses were performed with the aim to identify leaf parameters that determine the consumption of leaf litter by *Gammarus*.

Single strains of fungi or oomycetes can affect the feeding preference of *G. roeselii* indirectly by altering leaf parameters that subsequently affect the preference of the amphipod. Alternatively, the presence of fungi or oomycetes on leaf litter can directly affect the preference due to the presence of attractants or repellents in the microbes. This was tested in **Chapter 4**, in which biomass of selected strains was extracted with organic solvents and these extracts were tested for effects on the food preference of *G. roeselii*.

During my leaf litter decomposition studies with *G. roeselii*, the mysid *L. benedeni* newly invaded Lake Constance and established itself in the littoral benthic community. It is known that the mysid is a detritivorous-herbivorous feeder, and so the question arose if *L. benedeni* feeds on decomposing leaf litter and the leaf associated microbes. In **Chapter 5** I assessed this in two experiments: first by feeding *L. benedeni* with leaf litter of two different physical conditionings, and second by assessing the feeding activity on leaf litter naturally conditioned in the littoral or inoculated with single strains of fungi or oomycetes.

Chapter II

The impact of axenic strains of fungi and oomycetes on the preference of *Gammarus roeselii* for leaf litter

Christine Aßmann, Jan Nechwatal, Karsten Rinke and Eric von Elert

Fundamental and Applied Limnology (In Press)

Abstract

The interaction of microbial colonisation with leaf litter breakdown by the shredder *Gammarus roeselii* was studied in the littoral of large Lake Constance. In a first step we studied selective feeding of gammarids on leaf litter from three different treatments. Littoral exposed, tap water exposed, and autoclaved leaves were offered to *G. roeselii* in standard food-choice assays under laboratory conditions. We found highly selective feeding on littoral exposed leaf litter indicating that microbial conditioning is affecting the shredders feeding behaviour. Neither C, N, and P nor protein nor ergosterol content were positively correlated with the relative consumption rates of littoral exposed leaves. For a more detailed study of the microbial conditioning of the leaves oomycetes and fungi were isolated from the littoral exposed leaves. Based on the sequence of internal transcribed spacer (ITS) regions of rDNA, we identified single strains of *Fusarium sporotrichoides*, *Microdochium* sp., Ascomycete sp., and *Cylindrocladiella parva*; two strains of *Cylindrocarpon* sp.; and three strains of *Pythium* sp. Subsequently food choice assays were conducted using the isolated strains by offering autoclaved leaves and leaves colonised with a single strain. Three strains (*C. parva*, *Cylindrocarpon* sp. 94-2057 and Ascomycete sp.) were preferred by *G. roeselii*, while leaves with *F. sporotrichoides* were avoided. Leaves with each of the three oomycete isolates (*Pythium* sp.) were neither preferred nor rejected by *G. roeselii*. Our results suggest that the selective feeding behaviour of *G. roeselii* is affected by the relative abundance of specific fungal strains. Thus, whether microbial conditioning acts as a repellent or as an attractor for shredders depends on the microbial community on the leaves.

Keywords: Fungi, oomycetes, leaf shredder, food selection, molecular methods, *Gammarus*, amphipod, ergosterol, food preference, leaf litter, conditioning

1. Introduction

Allochthonous organic matter represents an important input into aquatic food webs of lakes (Pace *et al.* 2004) and rivers (Abelho 2001). Among different sources of organic allochthonous input leaves constitute a major allochthonous source in the aquatic food (Abelho 2001). Leaf litter is processed in three overlapping processes: (1) abiotic loss of soluble substances (leaching), (2) microbial colonisation (fungi and bacteria), and (3) invertebrate feeding and physical abrasion (Abelho 2001; Gessner *et al.* 2003). Invertebrate feeding constitutes a key process for the leaf biomass to enter the aquatic food web, and benthic shredders (e.g. amphipods) are often the most important organisms mediating the processing of leaf material (Webster and Benfield 1986; Abelho 2001). During microbial colonisation (conditioning), the total nitrogen, phosphorus, and protein content of leaves increases, and the leaves become softer (Bärlocher 1985; Suberkropp 1992; Graça *et al.* 1993b). At the same time, microbial biomass associated with leaves greatly increases (Suberkropp *et al.* 1983). Various studies (Hieber and Gessner 2002; Gulis and Suberkropp 2003; Gessner 2005) have shown that fungi are the most important microbial component on decaying leaf litter in streams. While hyphomycetes were already extensively studied in respect to their role in leaf conditioning in freshwaters (Bärlocher *et al.* 1992), the role of oomycetes is largely unknown. Oomycetes are known saprophytes or plant parasites in soil and water. They are well represented in freshwater habitats (Nechwatal and Mendgen 2006; Nechwatal *et al.* 2008) and are found on leaf litter in rivers (Bärlocher 1991a; Dix and Webster 1995; Bärlocher *et al.* 1995), but have not been tested in shredder feeding assays until now.

The microbial conditioning of leaf litter increases its palatability for invertebrate shredders (Abelho 2001; Maraun *et al.* 2003). Shredders feed on coarse particulate organic material, reducing it to fine particulate organic material, which in turn constitutes a food source for other invertebrates (Graça 1993). Shredders and grazers from terrestrial and running water systems have a higher preference for leaves colonised by fungi, as demonstrated in many

laboratory experiments (reviewed in Suberkropp 1992 and Maraun *et al.* 2003). These results led to the assumption that microbial colonisation in general improves the nutritional quality of leaf litter for shredders. However, amphipod, isopod or collembolan shredders/grazers clearly prefer certain fungal species or strains colonising leaves over others (Bärlocher and Kendrick 1973; Klironomos *et al.* 1992; Graça *et al.* 1993a; Graça *et al.* 1994a; Maraun *et al.* 2003).

Most of the known mechanisms of leaf litter decomposition are based on studies from terrestrial and running water habitats (e.g. Swift *et al.* 1979; Blair *et al.* 1990; Boulton and Boon 1991; Heneghana *et al.* 1998). Little is known about leaf litter conditioning (Federle and Vestal 1982; Federle *et al.* 1982; Mille-Lindblom *et al.* 2006b) and decomposition (Webster and Benfield 1986; Sabetta *et al.* 2000; van Dokkum *et al.* 2002) in lakes, despite the fact that, Webster and Benfield (1978) indicated that detritus may provide 10 to >75% of the total organic input in lakes, and Pace *et al*. (2004) documented allochthonous inputs into lakes to be a major source for lentic food webs. Moreover, due to the fact that leaf litter enters the lake along the shore, littoral communities receive relatively high allochthonous inputs, and shredders contribute a major proportion to these highly diverse communities (Bohman and Tranvik 2001; Mörtl 2004).

The genus *Gammarus* is regarded as a major shredder, particularly in rivers (Macneil *et al.* 1997). In lakes, the importance of *Gammarus* spp. for leaf litter degradation is less well documented, although *Gammarus* may contribute substantially to the total littoral community biomass (Mörtl 2004; Baumgärtner *et al.* 2008). However, only a few experiments have shown that shredding amphipods were responsible for significant leaf mass loss rates in lakes (Sabetta *et al.* 2000; van Dokkum *et al.* 2002).

We, therefore, aimed in this study to investigate the process of leaf breakdown by shredders from the littoral zone of a large lake and its interaction with microbial conditioning of the leaf material in designed laboratory experiments. For the current study, we assumed that food preference of aquatic shredders depends on the microbial colonisation of the leaves. Thus, microbial processes should mediate leaf litter fragmentation rates and thus should to a large degree

affect the availability of fine particulate organic material for other invertebrates in the food web. We exposed leaf litter in the littoral of Lake Constance and assessed the changing relative consumption over time for these conditioned leaves in standardized laboratory assays. After three weeks, when the point of maximum consumption of the conditioned leaves relative to the tap water conditioned and the autoclaved leaves was reached, we isolated actively growing fungi and oomycetes from the leaf litter and determined their effects on feeding preferences of *Gammarus roeselii* (Gervais). Here the hypothesis was tested, that fungi and oomycetes have a steering role on the preference of the shredder *G. roeselii* in the early stage of leaf litter conditioning.

2. Methods

2.1. Gammarids

Gammarus roeselii (Gervais) was collected with a dip net (mesh size 200 µm) in the littoral of Lake Constance near the Limnological Institute. We chose *G. roeselii*, because it is a common member of the shredder community in the littoral of Lake Constance (Mörtl 2004). For the experiments we used adult individuals of either sex (body lengths 7–12 mm). Animals were starved for 1 day prior to the beginning of each experiment in order to obtain an equal level of starvation of the individuals and the smallest variation in feeding-motivation of *G. roeselii*; which is an approach consistent with many other studies on gammarid feeding (Kli ronomos *et al.* 1992; Gergs and Rothhaupt 2008; Aßmann and von Elert 2009). Gammarid body lengths were measured according to Gergs and Rothhaupt (2008) as the distance between the head anterior and the posterior segment of the pleon using a stereomicroscope (Zeiss Stemi 2000-C, Jena, Germany) with a digital imaging system, which is able to follow the curved shape of the animals. All experiments were run on a 12-h photoperiod at constant temperature (15 °C) in a climate chamber.

2.2. Leaf Litter

Freshly fallen Black Alder leaves (*Alnus glutinosa* (L.) Gaertner) were collected from the ground in autumn 2003 for experiments with leaf conditioning in lake water, tap water and to produce sterile leaves by autoclaving. In experiments using leaf conditioning with single fungal or oomycete species, the leaf material was collected using a nylon net mounted above the ground in autumn 2005. Black Alder leaves were used because of their ubiquitous presence in the riparian vegetation and their comparatively high initial N content of 2.6% nitrogen dry wt. (Schmidt 1996). All collected leaves were air-dried and stored at room temperature in the dark.

2.3. Leaf litter conditioning

We applied three different treatments of leaf conditioning: (i) exposure in the littoral, (ii) incubation in tap water, and (iii) autoclaving. While the littoral exposure should mimic the natural leaching and microbial colonisation processes, the autoclaved leaves served as a control, where no microbial colonisation was allowed. The tap water exposure represents an intermediate treatment, where leaching took place but microbial colonisation was lower.

Starting in July 2005, Black Alder leaves were exposed in the littoral of Upper Lake Constance (N 47° 41.5'; E 9° 12.2') in cages at 0.4 m water depth with contact to the sediment. The cages were constructed of one polyethylene tube (∅ = 125 mm, length 31 mm) and covered with gauze (mesh size 30 µm) on both sides to exclude shredders from the leaf litter. Each cage was filled with eight leaves, equivalent to approximately 2 g dry wt. of pre-soaked alder leaves. In parallel, in the tap water treatment leaves were exposed to tap water at a flow rate of 4320 l day^{-1} (simulating the continuous water exchanges through waves in the littoral) in three 5-l containers (approximately 50 leaves per container). For the experiment, which lasted over 5 weeks, leaves from the littoral and the tap water treatment were harvested in a weekly schedule. In contract to these, the autoclaved treatment did not include any long-term incubation but pre-soaked leaves were autoclaved separately (30 min, 121 °C) for each

experiment, thus providing leaf material that was physically softened and leached but not chemically modified through microbial colonisers.

From leaves from all three treatments equally sized discs (∅ 14 mm) were stamped by a cork borer near the edges of the leaves to avoid leaf veins. The wet weight of the leaf discs was measured (Mettler AE 240) four times to obtain an error below ± 0.1 mg. Prior to each weighting, leaf discs were dipped into deionized water and then dabbed twice with a paper towel to reduce weight fluctuations owing due to excess water on the leaf discs. Although typically dry weight is used in feeding assays (e.g. Graça *et al.* 1993a; Graça *et al.* 1994a; Rong *et al.* 1995), wet weight was used in order to not affect the microbial colonisation of the leaves.

2.4. Food-choice assays with conditioned leaf litter

We performed food choice experiments on a weekly basis over five weeks (i.e. experiments at day 1, 7, 14, 21, 28, and 35). One leaf disc of each of the three leaf disc types was simultaneously offered to one *G*. *roeselii* individual in a transparent polyethylene container (110 × 110 × 37 mm) filled with 250 ml filtered (30 µm) lake water. The food-preference tests were run for 48 h or until 66% of one single leaf disc had been consumed, as estimated visually. The consumption rate on each leaf disc was calculated as the difference between the initial and the remaining wet weights of all three leaf discs divided by the exposition time. Relative consumption (percent) was calculated by dividing the consumption of the respective treatment disc by the total consumption summed over all three treatments in the respective container. We calculated relative consumption in order to compare the outcomes of the experiments independently of the total leaf mass consumed.

2.5. Isolation of fungi and oomycetes

Fungi and oomycetes were isolated from the littoral exposure treatment at day 21. We chose this sampling day, because we noted relatively high relative consumptions for the littoral exposed leaves on that day (after recording elevated relative consumptions for the littoral treatment already at day 7 and

14), which indicated that the microbial colonisation on the leaves was palatable for the gammarids.

The leaves, from which fungi and oomycetes should be isolated, were harvested at day 21 and stored in petri dishes (15°C, 12h photoperiod) in sterile filtered lake water (0.2 µm cellulose acetate filter, FP 30/0.2 CA-S Whatman) until the food choice assay was completed (i.e. on day 23). Pieces of littoral conditioned leaf litter (approx. 2.5 × 2.5 mm) were aseptically cut with a scalpel and transferred to petri dishes containing water agar (2% agar) with antibiotics (90 mg l^{-1} ampicillin, 150 mg l^{-1} streptomycin sulfate). The leaves provided the carbon source; no other carbon sources were added. The petri dishes were incubated at 20 °C with a 12-h photoperiod for 3 days. Actively growing single hyphae extending over the leaf pieces onto the agar were selected and transferred onto malt extract agar (MEA; 1.5% malt extract, 2% agar). Fungal and oomycete cultures were purified from bacteria according to the method described by Abdelzaher *et al*. (1994): Fungal and oomycete hyphae grew vertically through MEA containing antibiotics, and bacteria-free hyphae were scraped off the surface of the MEA. With these hyphae new MEA petri dishes were inoculated establishing our stock cultures. The isolates were examined macroscopically (Stereomicroscope; Stemi 2000-C, Zeiss AG, Germany) and preliminarily grouped according to their macroscopic appearance.

2.6. Identification of fungi and oomycetes

Mycelium from each of 17 fungal and oomycete isolates was scraped off the MEA and homogenized using a pestle in 50 µl sterile water in micro-centrifuge tubes. Chelex 100 resin (10%, Bio-Rad) was added and incubated for 40 min at 65 °C and for 5 min at 90 °C (Wirsel 2002). The homogenate was centrifuged (2300 × g, 15 min), and the supernatant containing DNA was stored at –20 °C. Internal transcribed spacer (ITS) regions 1 and 2 including the 5.8S gene of the ribosomal RNA genes (rDNA) were amplified using the primer pair ITS1/ITS4, as described in White *et al*. (1990) and Gardes and Bruns (1993). PCR products were separated on 1.5% agarose gels (70 × 80 mm; 1×TAE buffer; 45 min, 85 V, 400 mA); bands were visualized with ethidium bromide. When

multiple or weak bands appeared on the gels, the DNA was extracted using a DNeasy Plant Mini Kit (Qiagen, Hilden; Germany) according to the manufacturer's protocol and subsequently amplified. Amplified DNA was digested with restriction endonucleases MspI and AluI (Fermentas) according to the manufacturer's instructions in order to identify groups of isolates with identical restriction fragment length polymorphism (RFLP) banding patterns on 3% agarose gels (70 × 80 mm; 1×TAE buffer; 106 min, 70 V, 400 mA). PCR products of isolates showing unique RFLP patterns were sequenced using the above-mentioned forward and reverse primers by Eurofins MWG Operon (Ebersberg, Germany). BLAST was used to identify the closest related species in GenBank. Fungal and oomycete sequences obtained from GenBank were aligned using BioEdit, version 7.0.5.3 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). RFLP analysis and/or comparison of sequence data were used to for classification of the isolates. The sequences obtained during this study were submitted to GenBank® (accession numbers EU637900 to EU637906, and EU669081 to EU669082).

2.7. Leaf litter inoculated with a fungal or oomycete species

To determine to what extent each of the nine fungal or oomycete species affects the relative consumption of *G. roeselii* for leaf litter, we tested the impact of a single fungal or oomycete species on relative consumption of *G. roeselii* in a separate experiment. Leaves were soaked in tap water and then autoclaved (30 min, 121 °C). Single leaves were placed on a celullose filter (∅= 70 mm) saturated with a mineral solution (0.01 g $MgSO_4$•7 H_2O, 0.01 g $CaCl_2$•2 H_2O, 0.01 g KNO_3, 0.01 g K_2HPO_4, and 0.5 g 2-[*N*-morpholino] ethanesulfonic acid per litre, pH 6.0; Duarte *et al.* 2006) under sterile conditions in a petri dish (∅ = 90 mm). Each leaf was inoculated with the mycelium of an isolate (agar plug placed in the centre of the leaf). Petri dishes were incubated at 20 °C with a 12-h photoperiod. When a fungus or oomycete had fully colonised the surface of the petri dish or had grown through the matrix of the leaf (tips of hyphae grew out of or over the cellulose filter, signifying the "fully conditioned phase"; Bärlocher 1985; determined visually), the leaves were used in the preference

assays. Autoclaved leaves served as the control in the assays. Discs were cut from both types of leaves as described above.

2.8. Food-choice assays with leaves colonised with a single species

Here, the effect of fungal or oomycete mycelium on the leaf litter on the consumption by *G. roeselii* was determined. In two of the four chambers of a grey polyethylene container (108 × 108 × 40 mm), one disc from leaves colonised with a single species and one disc from autoclaved leaves were offered to one *G. roeselii* individual. In another control assay, two autoclaved leaf discs were offered to *G. roeselii*. Each chamber contained a stone shelter of approximately 4 g, and the container was filled with 250 ml filtered (30 µm) lake water. The consumption rate and the relative consumption of *G. roeselii* for either leaves were calculated as described above.

2.9. Leaf parameters

The weekly samples of leaves exposed to the littoral or leaf material colonised with a single fungus or oomycete were pooled, freeze-dried, homogenized with a mortar and pestle, and stored at –80 °C. All leaf litter sample measurements were run in duplicate to exclude systematic errors.

The particulate organic carbon and nitrogen contents were determined using an NCS-2500 analyzer (Carlo Erba Instruments). Prior to determining particulate phosphorus, samples were digested with a 10% potassium peroxidisulfate and 1.5% sodium hydroxide solution at 121 °C for 60 min. Soluble reactive phosphorus in each sample was measured using the molybdate-ascorbic acid method (Greenberg *et al.* 1985) with an autoanalyzer (Technicon). The protein content was measured according to Baerlocher (2005), and polyphenols were measured photometrically as described by Bärlocher and Graça (2005). Ergosterol was extracted in alkaline methanol at 80 °C, followed by a C_{18}-solid-phase extraction (Sep-Pak® VactC18 6cc; Waters) according to Gessner (2005). The extract was quantified by HPLC (LiChrospher® 100 RP-18 column, 5 µm, 250 × 4 mm; Merck Darmstadt; Germany) as described by Gessner (2005), and the ergosterol was then converted to fungal biomass (ergosterol

peak, see Fig. 1 and 2C) using the conversion factor 5.5 mg ergosterol g^{-1} fungal biomass as determined by Gessner and Chauvet (1993).

Leaf toughness was measured with a penetrometer according to Pabst *et al*. (2008); five holes were punched near the edge of one of five leaves. We calculated the average force from the 5 measuring points on each leaf.

2.10. Statistical analyses

Statistical analysis of relative consumption and absolute consumption rates in the three treatments of the food-choice assays with conditioned leaf litter was conducted by applying resampling statistics following the guidelines given in Bärlocher (2005a). The testing strategy in resampling statistics is the same as in classical tests or ANOVA – calculating the likelihood that the values in the different treatments come from the same distribution (Null-hypothesis). In comparison to classical tests and ANOVA, resampling statistics do not make any assumptions about the error distribution, and by that the requirement of normally distributed errors relaxes. Since we repeatedly performed the calculations for each day of the experiment and for all days together, we corrected the significance level by a sequential Bonferroni correction according to Holm (1979). As test statistic for the resampling test we summed the squared differences between within-treatment-means (i.e. for littoral exposed, tap water conditioned and autoclaved leaves) and the grand mean (Bärlocher 2005a). This test statistic was calculated for the measured consumption rates (called the observation vector). In a second step the observation vector was resampled, i.e. an unrestricted permutation of the values was carried out, and the test statistic was calculated for this resampled vector. According to the recommendations by Bärlocher (2005a) we performed 10000 permutations. The p-value is calculated by counting the cases where the test statistic of the resampled vector was equal or higher than the test statistic from the observation value, divided by the number of permutations carried out.

To test for effects of a single fungus or oomycete colonising leaves, relative leaf consumption in the test assays was compared with relative leaf consumption in

the control assays by resampling statistics as described above (in this case by comparing two treatments instead of three). Spearman rank correlations were used to test for significant correlations between the chemical parameters of leaf litter and the relative consumption. In addition, a sequential Bonferroni correction of the Spearman rank correlation was calculated to account for the number of the tests performed (Holm 1979). The calculations for the resampling statistics were performed using R (R Development Core Team, 2006). The module Nonparametric Statistics from STATISTICA 6.0 was used to calculate the Spearman rank correlation coefficients. All levels of significance were set at $\alpha = 0.05$.

Table II 1 Calculated p-values (likelihood for the Null hypothesis saying that consumption in all three treatments come from the same distribution) by resampling statistics according to Bärlocher (2005).

	Absolute consumption rates	**Relative consumption**
Day	**P**	**P**
1	<0.001	<0.001
7	<0.001	<0.001
14	<0.001	<0.001
21	<0.001	<0.001
28	<0.001	<0.001
35	<0.001	<0.001
all	<0.001	<0.001

3. Results

3.1. Relative consumption of in-situ conditioned leaf litter by G. roeselii

Resampling statistics of the consumption rates indicated a highly significant treatment effect (i.e. rejecting the Null hypothesis, see Table II 1). This holds true for the absolute feeding rates as well as for the relative feeding rates and was evident on all sampling days. Even when all sampling days were merged together, the treatment effect was highly significant. The most prominent effect in the temporal development of the relative consumption rates were the increasing relative consumption rates in the littoral exposed treatment starting

around day 14 until the end of the experiment (Fig. II 1). *G. roeselii* preferred leaf litter conditioned in the littoral, and the relative consumption differed depending on the exposure time of leaves. Strongly elevated relative consumption in this treatment was observed at day 21 reaching values surpassing 80%.

Table II 2 Total consumption rate of *G. roeselii* for littoral exposed, tap water conditioned and autoclaved leaf litter from the different feeding assays, n = 14-19.

Exposure day	Total consumption rate [mg wet wt. leaf consumed h^{-1}] ± 2 SE
1	0.34 ± 0.06
7	0.31 ± 0.04
14	0.38 ± 0.08
21	1.06 ± 0.36
28	0.80 ± 0.20
35	0.72 ± 0.18

The relative consumption for littoral-exposed leaves correlated with the total consumption rates of *G. roeselii* (Spearman $R = 0.409$, $p < 0.001$; Spearman rank order correlation, $\alpha < 0.05$), which suggested that the increased attractiveness of littoral-exposed leaves led to a greater overall feeding motivation (appetite) of the gammarids (Table II 2).

Table II 3 Correlation (Spearman rank correlation coefficient R) between relative consumption of *Gammarus roeselii* and several chemical and physical parameters of leaves differing in the incubation period in the littoral of Lake Constance. P-values were adjusted by sequential Bonferroni correction.

Parameter	R	Level of significance
N content	–0.45	$p < 0.001$
P content	–0.30	$p < 0.01$
C content	–0.60	$p < 0.001$
C:N ratio (mol/mol)	–0.16	$p > 0.05$
N:P ratio (mol/mol)	–0.21	$p > 0.05$
C:P ratio (mol/mol)	–0.36	$p < 0.001$
Total phenol content	–0.48	$p < 0.01$
Protein content	–0.56	$p < 0.001$
Ergosterol content	0.21	$p > 0.05$

The relative consumption of littoral-exposed leaves was negatively correlated with all leaf parameters, including the contents of C, P, N, total phenol, and protein (Table II 3, Fig. II 2A). The negative correlation between the N content and preference matches a similarly negative correlation between preference and protein content (Table II 3). The protein content of the leaves declined in general with a minimum at day 14 (Fig. II 2C).

An increase in fungal biomass on leaves was indicated by a six-fold increase in the ergosterol content of leaves during exposure in the littoral (Fig. II 2D). The maximal ergosterol content of 124.4 µg (g dry wt.)$^{-1}$ was found on day 28 (Fig. II 2D). The corresponding fungal biomass equalled 22.6 mg (g dry wt.)$^{-1}$. However, the ergosterol content was not correlated with relative consumption (Table II 3), which suggested that not total fungal biomass, but rather fungal impacts on the leaf structure (leaf matrix) or species-specific effects of the fungal and oomycete colonisers determined the preference of *G. roeselii* for conditioned leaves. We therefore tested the effects of single fungi and oomycete species using strains isolated from leaf litter exposed in the littoral for 21 days, the day of maximal relative consumption for littoral-exposed leaves of *G. roeselii*.

Seventeen fungal or oomycete strains were isolated from actively growing hyphae on leaves. Gel electrophoresis of the amplified ITS1, 5.8S, and ITS2 fragments revealed single bands, indicating pure strains. RFLP analysis of the amplified sequences was used to classify the isolates according to RFLP banding patterns. Eight of the isolates had identical patterns, which suggested a relatively high abundance of this species in the littoral fungal and oomycete community on alder leaves. The patterns of two other isolates were also identical, i.e., in total we identified nine different RFLP types, of which DNA sequences were obtained (Table II 4). Seven of the sequences showed at least 97% similarity to database entries (BLAST) from other studies. Five of the nine RFLP types were assigned to the class Sordariomycetes, one to the Ascomycota, and three to the Oomycetes (Table II 4).

3.2. Relative consumption of leaf litter colonised with a single fungal or oomycete strain by G. roeselii

The relative consumption of leaf litter colonised with *Fusarium sporotrichoides*, *Cylindrocladiella parva*, *Cylindrocarpon* sp. 94-2057 or *Ascomycete* sp. PV So8 differed from that of the control, i.e. autoclaved leaf litter (Fig. II 3, Table II 5).

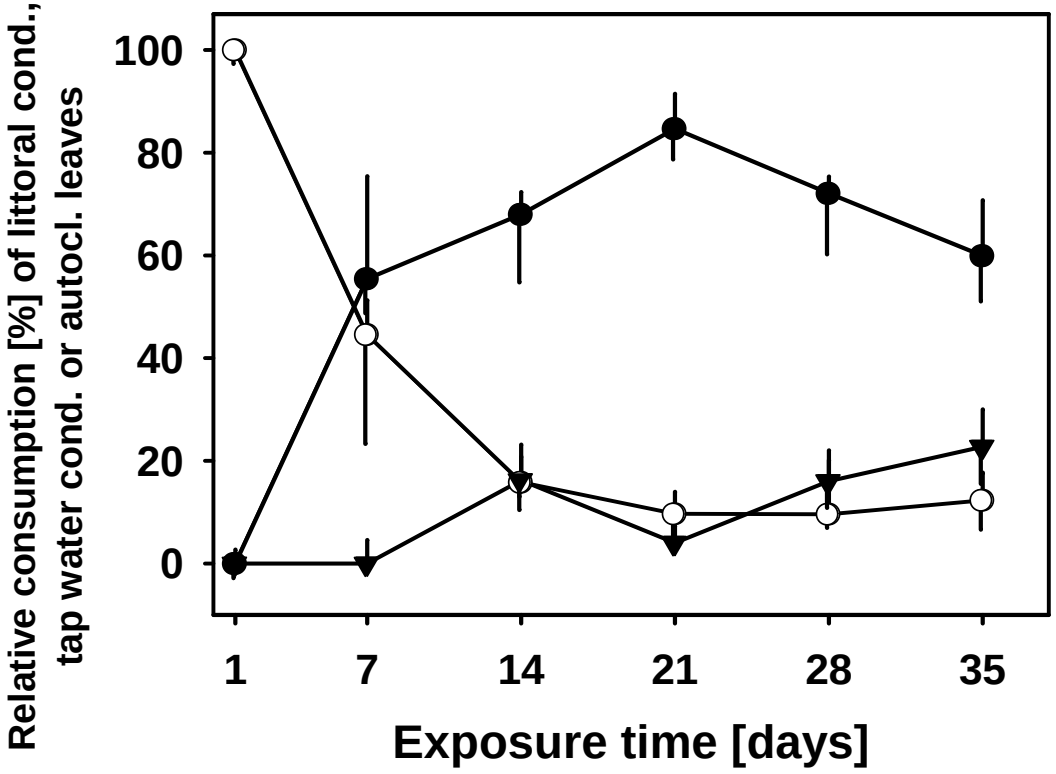

Fig. II 1. Relative consumption on three differently treated food items offered simultaneously to *G. roeselii*. The three treatments consisted of Black Alder leaves (—●—) exposed in the littoral zone of Lake Constance, (—▼—) exposed to tap water, or (—○—) autoclaved. At the time indicated, leaves were sampled and offered to *G. roeselii* in food-choice assays. For each sampling date and treatment the median value and the range including 50% of the data are shown (n = 14–19).

G. roeselii preferred the control over *F. sporotrichoides,* which suggested that *F. sporotrichoides* repelled *G. roeselii*. In contrast, *G. roeselii* preferred leaf litter colonised with *Cylindrocladiella parva*, *Cylindrocarpon* sp. 94-2057 or *Ascomycete* sp. PV So8 over the control, which suggested that these strains

attracted *G. roeselii*. The other species did not significantly affect the relative consumption of *G. roeselii*.

In all cases, colonised leaf material had a higher N content than the control (Table II 6), which corresponded to lower C:N ratios for fungus- or oomycete-colonised leaf litter compared to autoclaved leaf litter. The colonised leaf litter had a higher P content in two out of nine cases and lower C:P ratios in seven out of nine cases (Table II 6). The leaf toughness of the colonised leaves was lower in all nine cases (Table II 6), which indicated that the effects of fungi or oomycetes on the elemental composition of leaves were associated with a reduction in the mechanical stability of leaf litter. The Spearman rank order correlation between leaf parameters and relative consumption revealed that neither the toughness of leaves nor the stoichiometric parameters N, P, and C were significantly correlated with relative consumption. Neither were protein nor polyphenol content correlated with relative consumption.

4. Discussion

Leaf breakdown is generally rapid in habitats with high invertebrate densities. Shredders are responsible for the transformation of coarse material into fine organic matter, and their activity increases the breakdown rate of leaf litter in both streams and lakes (Merritt *et al.* 1984; Kok and van der Velde 1994). However, the importance of the shredder *Gammarus* spp. as consumer of leaf litter in lakes is poorly documented (Sabetta *et al.* 2000; van Dokkum *et al.* 2002).

Gammarids in running waters prefer conditioned over unconditioned leaves (Arsuffi and Suberkropp 1989; Graça *et al.* 1993a; Graça *et al.* 1994a; Graça *et al.* 1994b). Hence, microbial degradation of leaf detritus is considered as one of the major mechanisms determining breakdown rates (Abelho 2001).

Fungal biomass associated with decomposing plant material can exceed 10% of the total litter mass, and fungal biomass can amount to 90 mg g^{-1} organic

Table II 4. Fungi and oomycetes isolated from leaf litter conditioned in lake water for 21 days. Isolates were identified by BLAST analysis of amplified sequences of the internal transcribed spacer regions 1 and 2 (ITS1, ITS2) of the 5.8S rDNA; similarity of the sequences refers to pair-wise alignments with the closest match.

Phylum or class	Species	GenBank accession number	Best BLAST hit; accession no.; similarity [%]
Sordariomycetes	*Cylindrocladiella parva*	EU637905	*Cylindrocladiella parva ASICP1; DQ779786 ; 100%*
Sordariomycetes	*Cylindrocarpon sp. 94-2057*	EU637906	*Cylindrocarpon* sp. 94-2057; AY295305; 100%
Sordariomycetes	*Cylindrocarpon sp. 4/97-1*	EU637900	*Cylindrocarpon* sp. 4/97-1; AJ279490; 100%
Sordariomycetes	*Fusarium sporotrichioides*	EU637901	*Fusarium sporotrichioides* var. *minor* BBA 62425; AF414973; 100%
Sordariomycetes	*Microdochium sp. PV So2*	EU637902	*Microdochium* sp. 4/97-103; AJ279489; 99.6%
Ascomycota	*Ascomycete.sp. PV So8*	EU669082	Leaf litter ascomycete its261; AF502786; 96.8%
Oomycetes	*Pythium litorale*	EU637904	*Pythium litorale* P.03; DQ144637; 100%
Oomycetes	*Pythium sp. JN 1-b*	EU637903	*Pythium* sp. JN-1b; DQ230904; 100%
Oomycetes	*Pythium* sp. PV So7	EU669081	*Pythium* sp. JN-12; DQ237932; 90.3%

mass (dry wt.) associated with alder leaves (reviewed by Gessner *et al.* 2007); therefore, fungi are regarded as key microbial decomposers. In our study, based on the conversion values for ergosterol (Gessner and Chauvet 1993), the fungal biomass on littoral-exposed leaves ranged between 1 and 23 mg (g dry wt.)$^{-1}$ during exposure, which lies in the same range as values for beech and poplar leaf litter in Lake Constance (Pabst *et al.* 2008).

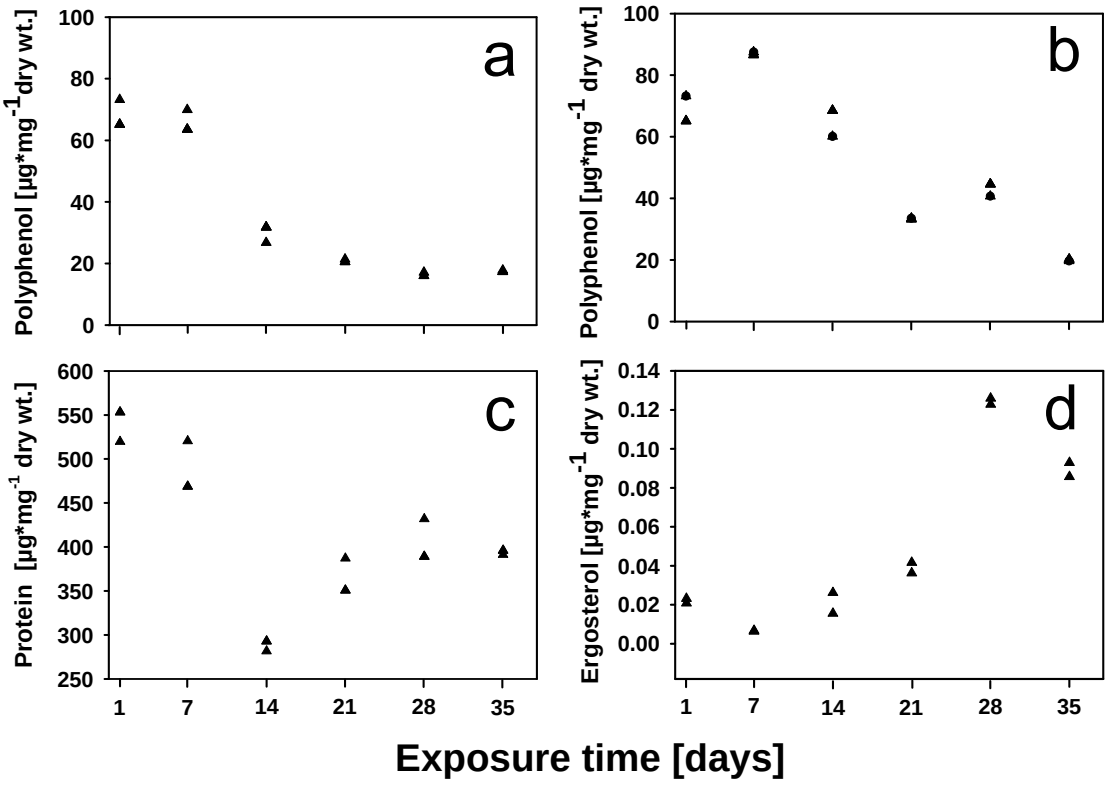

Fig. II 2. (a) Polyphenol, (c) protein, and (d) ergosterol content of Black Alder leaf litter exposed to water in the littoral zone of Lake Constance and (b) polyphenol content of leaves conditioned in running tap water for each day duplicated analyses are displayed.

Here, we report on the interactions between *in situ* conditioning of leaf litter by fungi and oomycetes in a lake littoral and the relative consumption by a benthic invertebrate shredder in standardized behavioural laboratory assays. To our knowledge, this is the first study that investigates the role of oomycete strains in

this microbial conditioning and in food choice assays. We observed that with increased exposure of the leaf litter in the littoral, the relative consumption of *G. roeselii* shifted from autoclaved leaf litter to leaf litter exposed in the littoral; furthermore, also the total amount consumed by *G. roeselii* increased (Fig. II 1, Table II 2).

Table II 5 Calculated p-values of the comparison of relative consumption by *G. roeselii* of un-colonised leaves with leaves colonised with fungal or oomycete species by resampling statistics according to Bärlocher (2005) followed by a sequential Bonferroni correction (Holm, 1979). In the test assays, an un-colonised leaf disc and a leaf disc colonised with fungal or oomycete species were offered. In control assays, two autoclaved (un-colonised) leaf discs were offered. Abbreviations from Figure 3 are given in parenthesis. Significant differences after Bonferroni correction are given in bold.

Fungus or oomycete colonising leaves	**p-value**
Cylindrocladiella parva (Cylin.)	**0.043**
Cylindrocarpon sp. 94-2057 (Cyl. a)	**0.040**
Cylindrocarpon sp. 4/97-1 (Cyl. b)	0.082
Fusarium sporotrichioides (Fus.)	**0.004**
Microdochium sp. PV So2 (Micro.)	0.301
Ascomycete sp. PV So8 (Asc.)	**0.010**
Pythium litorale (Pyth. b)	0.301
Pythium sp. JN 1-b (Pyth. a)	0.301
Pythium sp. PV So7 (Pyth. c)	0.301

Knowing that autoclaving leaf litter has profound impacts on the leaf chemistry, here autoclaved leaves were used as feeding standard in order to justify that any of the leaves will be consumed by *G. roeselii*. In the food choice assays autoclaved leaves served as feeding control in order to provide leaf material with constant stoichiometry and toughness in all food choice assays. It is reasonable to assume that autoclaving led to the initial preference for the autoclaved leaves as it impacts the chemical composition of litter. However, the observed increase over time of the relative consumption of leaf litter conditioned in the littoral relative to these standardized autoclaved leaves clearly reflects changes due to the exposure in the littoral. Our findings are in accordance with

those of Kaushik and Hynes (1971), who showed that detritivores increased their leaf consumption as the leaves became more conditioned.

Leaf litter constitutes a qualitatively poor food source (Bärlocher 1985) for shredders because of its relatively high C:P ratio, which ranges from 215 to 29,900, and the relatively high C:N ratio, which ranges from 11 to 770, compared to the low ratios in the gammarid body (Cross *et al.* 2005; Fink *et al.* 2006). According to Abelho (2001) and Graça and Zimmer (2005) conditioning leads to changes in chemical composition and physical properties of leaves improving its food quality (Suberkropp 1992). In our experiments, neither C, N, and P contents nor the content of ergosterol or protein of the conditioned leaves could explain the observed high relative consumption for littoral exposed leaves of *G. roeselii*. On the other hand, the negative correlation between C:P ratio and relative consumption suggested a stoichiometric effect. However, we believe that stoichiometric ratios are strongly affected by leaching processes that go in parallel with the conditioning and therefore may interfere with each other in a correlation analysis. Moreover, the experiments with single strains do not support the role of C:P ratios for determining food preferences of gammarids but rather suggest that species-specific processes play an important role. In contrast to this, the chemical composition of leaves undergoing conditioning appeared to be important with respect to their polyphenol content as we found a strong negative correlation between polyphenol content and relative consumption.

Polyphenols, known repellents of vertebrate and invertebrate grazers (Rosset *et al.* 1982; Pennings *et al.* 2000; Abelho 2001; Graça and Bärlocher 2005), distinctly declined over exposure time, coupled with a concomitant increase in the preference of *G. roeselii* for Black Alder leaf litter (Table 3, Fig. 2A). Similar declines in tannins and polyphenols have been reported for oak, larch, spruce, and willow leaf litter (Rosset *et al.* 1982; Schofield *et al.* 1998). For alder leaves Bärlocher *et al*. (1995) and Canhoto and Graça (1996) showed a decline of polyphenols (% polyphenol of dry wt.) from 6% (day 1) to 1% (day 28). Our polyphenol values lie in the same range: day 1 with a maximum of 7.3% and

Table II 6. Parameters of autoclaved leaf litter (control) and of leaf litter colonised with single fungal or oomycete strains. Mean values ± 2 SE for the control (n = 4) and mean values for two measurements of colonised leaf material are given. Values that did not fall within the 2 SE of the values obtained for the control are given in bold. N, nitrogen content; P, phosphorus content; C, carbon content.

	Leaf litter constitution (µg mg dry wt.$^{-1}$)						Stoichiometric ratio (mol:mol)		
Leaf litter conditioning	N	P	C	Polyphenol	Protein	Leaf toughness (N)	C:N	N:P	C:P
Autoclaved leaf litter (control)	**25.5 ± 0.4**	**0.2 ± 0.2**	**517.0 ± 4.0**	**36.8 ± 2.8**	**152.4 ± 20.8**	**2.9 ±0.2**	**23.8 ± 0.4**	**252.2 ±26.0**	**6009.3 ± 693.0**
Cylindrocladiella parva	**33.4**	**0.5**	516.9	**20.8**	156.8	**1.0**	**16.2**	**175.1**	**2832.9**
Cylindrocarpon sp. 94-2057	**37.5**	0.2	**503.7**	**27.2**	151.0	**1.7**	**22.3**	**305.1**	**6823.5**
Cylindrocarpon sp. 4/97-1	**31.0**	0.3	**504.8**	**26.4**	169.1	**1.2**	**18.4**	**222.2**	**4090.6**
Fusarium sporotrichoides	**35.8**	**0.5**	**504.7**	**30.4**	**224.4**	**1.4**	**16.5**	**165.6**	**2738.7**
Microdochium sp. PV So2	**36.8**	0.4	519.0	**30.9**	**181.3**	**1.4**	**16.3**	**213.2**	**3470.4**
Ascomycete sp. PV So8	**32.5**	0.3	518.8	**30.2**	**187.5**	**1.8**	**18.5**	227.0	**4207.2**
Pythium litorale	**35.4**	0.2	**504.3**	**42.6**	**192.2**	**2.0**	**19.1**	**286.5**	5469.5
Pythium sp. JN 1-b	**37.4**	0.3	515.3	35.5	**191.5**	**2.1**	**18.1**	234.7	**4247.4**
Pythium sp. PV So7	**34.0**	0.2	**504.3**	**33.3**	**166.0**	**2.0**	**19.9**	**293.4**	5865.4

day 35 with 1.7% polyphenol of dry wt. Our results thus nicely comply with the findings from other studies and accordingly suggest that polyphenols in the leaf litter repel *G. roeselii*. As the polyphenol content decreases during leaf conditioning in the littoral, the repellent effect decreases and the preference of *G. roeselii* for the leaf litter increases. Noteworthy, the polyphenol content of the tap water conditioned leaves decreased during exposition in a similar way, and this was again associated with increasing preference over time (no other leaf parameters were determined; so we could not draw any conclusions about phylloplane fungi which could have possible colonised the leaves). However, in comparison to littoral exposed leaves, the tap-water conditioned leaves were still negatively selected, which indicated that the structure of the microbial communities on the leaves may play a role here.

According to the reviews by Bärlocher (1985) and Suberkropp (1992) increases in the N or protein content of leaf litter lead to an enhanced preference by shredders during leaf decomposition. However, our results of the protein and nitrogen content (negative correlation with the relative consumption) do not corroborate these observations. The protein content of littoral-exposed leaves decreased in general, but the relative consumption of *G. roeselii* for the littoral exposed leaf litter increased .Compared to the study of Gessner (1991), where the protein values ranged from 13% (% protein of dry wt., day 0) to 22% (% protein of dry wt., day 42), our values are almost twice as high with 55 % protein of dry wt. (day 1) and 29 % protein of dry wt. (day 14). Similarly, the nitrogen values reported here (% N of dry wt.) ranged from initially 2.8% to 3.7% at day 35 and lie in the same range as values from Canhoto and Graça (1996) (day 1 = 2.6% and at day 42 = 3.25% % N of dry wt.) for alder leaves.

Our results point to the development of microbial biomass on the littoral-exposed leaves (Baldy *et al.* 1995; Hieber and Gessner 2002) that possibly deterred shredders. These findings suggest that the preference by shredders cannot easily be predicted from bulk parameters like protein content but depends on the fungal species that colonise the material.

In streams, fungi account for 88–99.9% of the microbial biomass on decaying leaves (Kominkova *et al.* 2000), and their biomass can exceed 10% of the total

litter mass (Gessner *et al.* 2007). In a lake, fungal biomass accounted for ≥90% of the total microbial biomass associated with reed (*Phragmites australis*) (Gessner and Newell 1997; Gessner 2005). Ergosterol, a major cell wall constituent of fungi, is widely used as a proxy to determine metabolically active eumycotic fungal biomass (Gessner and Newell 1997; Gessner 2005). In our study, the ergosterol levels increased over exposure time, which suggested an increase in fungal biomass over time. This, however, was not related to the relative consumption of *G. roeselii* for the leaves, which suggested that eumycotic fungal biomass *per se* does not drive *G. roeselii´*s preference. These results should be considered with caution, because ergosterol as a proxy often leads to an overestimation of fungal biomass, since it does not necessarily degrade as rapidly after cell death as has been assumed (Zhao *et al.* 2005). In addition, since ergosterol is lacking in the cell walls of oomycetes (Weete and Gandhi 1996), no conclusions on the effect of leaf colonisation with these water moulds can be drawn. Our results modify earlier reports on the enhanced preference of gammarids for conditioned leaves colonised by fungi (Kostalos and Seymour 1976; Graça 2001). We hypothesized that either strain- or taxon-specific fungal effects or the involvement of oomycetes led to the absence of a correlation between preference and ergosterol. Another possibility is that the leaf structure was modified by the fungi and oomycetes by their enzymatic capabilities (Bärlocher *et al.* 1992), but for the littoral exposed leaves leaf toughness was not measured, so no conclusions could be drawn.

Our results of the chemical parameters of the littoral exposed leaves, which were in most cases negatively correlated with the relative consumption of these leaves (Table 3), are in contrast to other studies, which have shown increased preferences for conditioned leaf litter with increased amounts of nitrogen (reviewed by Bärlocher 1985 and Graça 2001). It has to be mentioned that the bulk of these studies were done in running water systems with the focus on aquatic hyphomycetes as decomposers. Here, in a lentic ecosystem, we found a different species composition of fungi and oomycetes, which may probably have other effects on the leaf chemistry than previously known from aquatic hyphomycetes. Indeed, the occurrence and ecological significance of

oomycetes for litter decomposition has only recently been recognized (Kendrick 2005). We therefore isolated fungi and oomycetes from littoral-exposed leaves at the time of high preference of *G. roeselii* for the leaves. The most common method to establish pure cultures of hyphomycetes from freshwaters is to isolate them from natural foams or conidial suspensions from conditioned leaves (Bärlocher 1991b; Dix and Webster 1995). However, this approach excludes microorganisms that do not form conidia, e.g. non-sporulating fungi and oomycetes. We therefore isolated fungal and oomycete hyphae actively growing on conditioned leaf litter. This innovative approach led to the isolation of six fungal strains and three oomycete strains, which confirms that we did not exclude non-sporulating fungi or oomycetes.

The ITS regions of rDNA have proven to be particularly useful for the separation of fungal taxa at the species or genus level, because the rate of accumulation of mutations in these regions often approximates to the rate of speciation (White *et al.* 1990; Gardes and Bruns 1993). Therefore, we used an ITS-RFLP analysis to classify our isolates into groups of identical banding patterns, with subsequent sequencing of selected members of the groups. Several other studies have shown that RFLP analyses provide a useful tool to distinguish fungal or oomycete isolates from environmental samples (e.g. Brasier *et al.* 2003; Neubert *et al.* 2006; Nechwatal *et al.* 2008). Our isolates thus are likely to represent a significant fraction of the fungal and oomycete taxa actively growing on conditioned leaf litter.

Five of the identified fungal species belong to the class Sordariomycetes, and one to the phylum Ascomycota. Fungi from the class Sordariomycetes grow as decomposers in soil, dung, leaf litter, and decaying wood (Zhang *et al.* 2006). Four of the identified Sordariomycetes (*Fusarium sporotrichioides; Cylindrocladiella parva; Cylindrocarpon* sp. 94-2057, *Cylindrocarpon* sp. 4/97-1) belong to the order of Hypocreales, which includes virulent plant and insect pathogens, as well as mycoparasitic, endophytic, and saprobic species (Bärlocher 1991a; Sabetta *et al.* 2000; Nikolcheva *et al.* 2005). Members of the genus *Fusarium* have frequently been isolated from decaying plant litter in

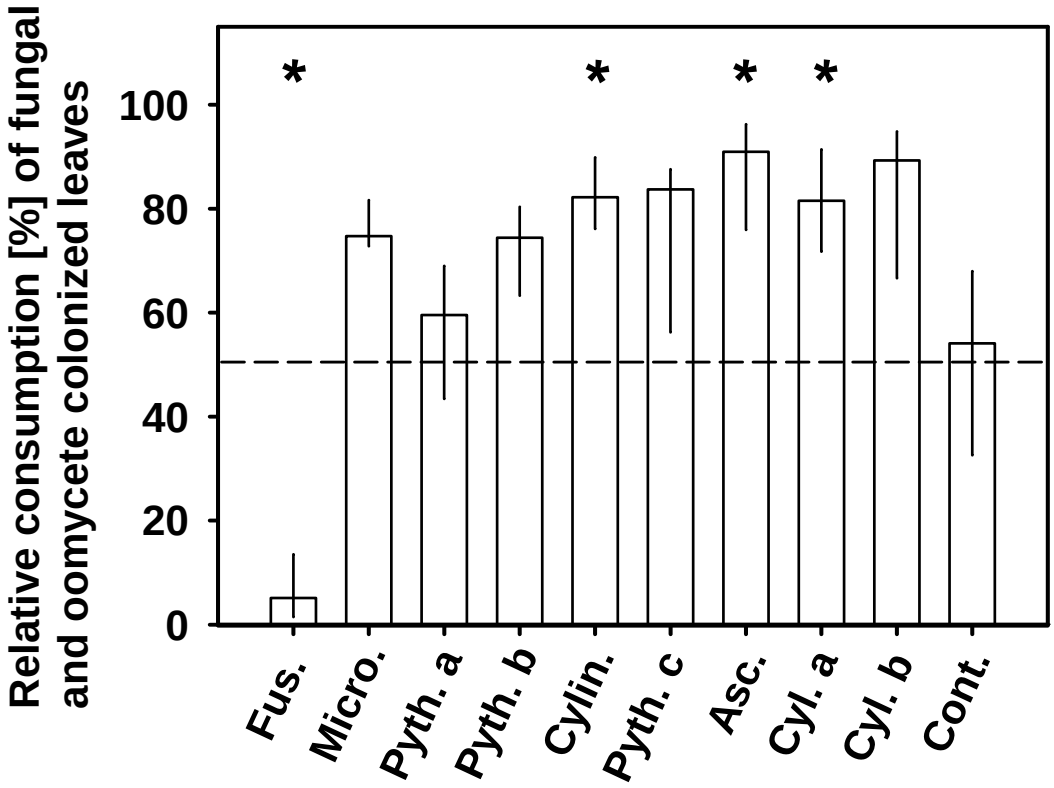

Fig. II 3 Relative consumption of *G. roeselii* (median value and the range including 50% of the data are shown, n = 9–12) for leaf litter colonised with different single fungal or oomycete species over autoclaved leaf litter. Relative consumption of 50 % is indicated by the dashed line. Values above this line indicate that fungal and oomycete species made leaves attractive to *G. roeselii*. Asterisks indicate significant differences to the control after sequential Bonferroni adjustment. Fus., *Fusarium sporotrichioides*; Micro., *Microdochium* sp. PV So2; Pyth. a, *Pythium* sp. JN 1-b; Pyth. b, *Pythium litorale*; Cylin., *Cylindrocladiella parva*; Pyth. c, *Pythium* sp. PV So7; Asc., Ascomycete sp. PV So8; Cyl. a, *Cylindrocarpon* sp. 94-2057; Cyl. b, *Cylindrocarpon* sp. 4/97-1; Cont., control.

freshwater (Bärlocher 1991a; Dix and Webster 1995) and are endophytes on common reed (*Phragmites australis*) in Lake Constance (Wirsel *et al.* 2001). *Cylindrocarpon* sp. is common (Wirsel *et al.* 2001) and *Microdochum* sp. is the most common fungal species found on reed tissue from Lake Constance (Neubert *et al.* 2006). We also isolated three oomycetes from the order Peronosporales (*Pythium* sp. JN 1-b, *Pythium litorale*, *Pythium* sp. PV So7), which are known saprophytes or plant parasites in soils and water. *Pythium* spp. are well represented in freshwater habitats (Nechwatal and Mendgen

2006; Nechwatal *et al.* 2008) and are found on leaf litter in rivers (Bärlocher 1991a; Dix and Webster 1995). In Lake Constance, several *Pythium* species have been reported on common reed (Nechwatal and Mendgen 2006; Nechwatal *et al.* 2008).

In our study the effect of an individual fungus or oomycete on the relative consumption of *G. roeselii* for conditioned leaf litter was tested. Colonisation of the leaf litter by a single strain affected between five and nine different leaf parameters, and these effects were strain-specific. Leaves colonised with *C. parva, Cylindrocarpon* sp. 94-2057 and Ascomycete sp. PV So8 were significantly preferred over control leaves. Since the preference of *G. roeselii* for colonised leaf litter was not correlated with the putative food quality indicators N, P, and total protein content, the effects are strain specific. Preference for leaf litter colonised with a single fungal strain has been reported earlier (Arsuffi and Suberkropp 1989), but those results were obtained in multiple-choice experiments in which leaf litter colonised by different fungi was offered simultaneously to *Gammarus* spp.: The observed preferences of *Gammarus* spp. are the difference of attraction to one fungus and repellence by another strain and hence cannot be attributed to a single strain. Such an experimental setup might correspond to leaf litter in a late phase of conditioning, when all leaf litter has been colonised by various fungi. In contrast, we investigated the role of fungi during the early stages of conditioning, and only two choices were offered: leaves with one strain of fungus or oomycete and leaves free of fungi and oomycetes. Our results indicate that certain fungi colonising leaf litter attract *G. roeselii* and lead to enhanced rates of shredding, while other fungi repel the grazer.

Three of the six isolated fungi and all three of the isolated oomycetes, however, had no effect on the relative consumption of *G. roeselii*. Arsuffi and Suberkropp (1989) reported that 2 of the eight tested fungi had no effect on the preference of *Gammarus* sp., and it is tempting to speculate that the high percentage of strains that had no effect in that study is due to the inclusion of oomycetes. Little is known about the ecological function of oomycetes (Zare-Maivan and Shearer 1988; Bärlocher 1991a; Dix and Webster 1995). Some may play a significant

role in the early breakdown of plant litter and detritus (Brasier *et al.* 2003) and might be a food source for shredders (Fano *et al.* 1982), but the preference of shredders for oomycetes was not determined.

Only one of our species, the fungus *F. sporotrichoides,* repelled *G. roeselii*, whereas most of the fungal strains (five of eight) tested by Arsuffi and Suberkropp (1989) were repellent, and, in contrast, Bärlocher and Kendrick (1973) reported an increased preference for *Fusarium* sp. These differences again point towards strain- or species-specific effects of fungi on the preference of *Gammarus* sp., as has been repeatedly shown (Graça *et al.* 1993a; Graça *et al.* 1994a; Rong *et al.* 1995).

Our study focused on the leaf litter decomposition in a lentic ecosystem. In lotic and lentic studies, generally, comparisons with terrestrial habitats are missing. Edwards (1974) described that the decomposition sequence of leaf litter on soil in a similar way as the one given in Albelho (2001) and Gessner *et al*. (2003) for aquatic habitats. From earthworms it is known that they prefer conditioned over unconditioned leaves (reviewed by Maraun *et al.* 2003), which confirms our and other results for shredders from aquatic habitats. Similarly, collembolans and earthworms have been shown not only to prefer leaves colonised with fungi over un-colonised leaves but as well to discriminate between different fungal species (reviewed by Maraun *et al.* 2003). This suggests that the process of leaf litter decomposition in terrestrial and aquatic ecosystems is not as different as assumed.

In running waters gammarids play an important role in leaf litter fragmentation (Macneil *et al.* 1997); note, however, that gammarids are absent in soft-water, acidic streams). Running freshwaters, like small rivers and streams, are commonly canopy shaded and have large leaf litter inputs. In lakes detritus may provide 10 to >75% of the total organic input (Webster and Benfield 1986). But large lakes, like Lake Constance, have relatively smaller leaf litter inputs compared to rivers and streams, and in precipitous littoral regions leaves are probably drifting into deeper regions. Although leaf litter input may not represent an important process on the ecosystem scale in large lakes, leaves play a significant role on the local scale in the littoral zone of large lakes. Littoral

communities contain a significant proportion of shredders (Mörtl 2004) like gammarids, and in the littoral of Lake Constance patches of leaf litter are found, where the litter is processed by invertebrates e.g. gammarids.

In conclusion, we have shown that even on highly preferred conditioned leaf litter, fungi and oomycetes can have either neutral, positive, or negative effects on the food preference of gammarids for the leaves. This implies that not fungal biomass in general but the relative abundance of such strains may determine the relative consumption of conditioned leaves by *G. roeselii* and thus the rate of leaf litter decomposition in the littoral.

5. Acknowledgements

We are grateful to Prof. Dr. M. O. Gessner for his substantial support in the analysis of ergosterol. We thank C. Gebauer und P. Merkel for excellent technical assistance with the leaf litter analyses. We thank C. Geiss and S. Pitlik for assistance with the food-choice assays and molecular analyses, and acknowledge Dr. W. Nagl, P. Fink and R. Gergs for their help with statistics. We acknowledge M. Wolf for manufacturing the cages and four-chambered containers. We thank S. Raub for comments on, and improving the English of an earlier version of this manuscript. We acknowledge K. A. Brune for editing the English language of the manuscript. This study was supported by a grant to E. v. E. from the German Research Foundation (DFG) within the Collaborative Research Centre SFB 454 *Littoral Zone of Lake Constance*.

Chapter III

Consequences of the colonisation of leaves by fungi and oomycetes on leaf consumption by a gammarid shredder

Christine Aßmann, Karsten Rinke, Jan Nechwatal and Eric von Elert

Freshwater Biology (In Revision)

Abstract

1. Black alder (*Alnus glutinosa*) leaf litter was exposed in the littoral zone of Lake Constance in autumn. Subsamples were analysed for leaf parameters and consumption by *Gammarus roeselii* under standard conditions at regular intervals (exposition experiment).

2. Six of eight measured leaf parameters showed significant effects on the consumption rates of *G. roeselii*. Statistical analyses revealed high co-linearity among leaf parameters. This co-linearity hampered identification of causal relations between leaf parameters and the shredder's feeding behaviour.

3. On dates with a high consumption rate of the exposed leaves, single strains of fungi and oomycetes were isolated and grown on sterile leaf litter. Leaf parameters displayed high variability between the different strains, suggesting pronounced strain-specific effects on leaf conditioning.

4. In a second experiment we measured consumption rates of *G. roeselii* that were offered leaves colonised by single strains of fungi and oomycetes (single isolates experiment). Autoclaved leaves served as a model substrate where leaching had already taken place. The consumption rates were significantly different between the single isolates. Oomycetes proved to be attractive for *G. roeselii* and thus are potentially important mediators of the shredding activity of this amphipod shredder.

5. Whereas the high co-linearity in the exposition experiment obscured the identification of key leaf parameters for shredder feeding behaviour, the single isolates experiment provided a clearer picture, since leaching processes were excluded from this experiment. We identified protein and polyphenol content as major leaf parameters determining the feeding behaviour of *Gammarus*.

6. Single isolates of fungi and oomycegtes growing on leaves had greatly varying effects on leaf parameters and on the consumption by *G. roeselii*, which indicates strain-specific effects on leaf-litter consumption by shredders and suggests that the overall effect on naturally conditioned leaves depends on the relative abundance of individual strains on decomposing leaves.

Keywords: *Gammarus*, oomycete, fungi, leaf litter, food preference

1. Introduction

A large fraction of allochthonous organic matter in freshwaters is provided by the litterfall from the riparian vegetation (Abelho 2001), and the transfer of this allochthonous carbon to herbivores and detritivores represents a major pathway in the energy flow (Reshi and Tyub 2007).

Leaves that have entered freshwaters lose soluble inorganic and organic substances during the so-called 'leaching'. They are also colonised by micro-organisms, and it has been shown that the biomass of the leaf-associated micro-organisms greatly increases over time (Suberkropp *et al.* 1983; Gessner *et al.* 2007). The microbial community associated with decomposing plant material is dominated by fungi, which are regarded as the main microbial decomposers of leaf litter. Leaf-associated fungal biomass may constitute up to 16% of total detrital mass in freshwaters (Abelho 2001; Gessner *et al.* 2007). These simultaneous processes of leaching and colonisation are referred to as "conditioning" of leaf litter (Golladay *et al.* 1983; Abelho 2001).

Leaf conditioning affects secondary production in aquatic ecosystems because the palatability of leaf litter to invertebrate shredders increases through the conditioning (Abelho 2001); particularly shredders have been shown to feed preferentially on conditioned leaves (Chergui and Pattee 1993; Graça *et al.* 1994b; Kiran 1996). It has been recognized that in this respect leaching is the dominant process during the first 24-48 hours (Bärlocher 2005b) after leaf material has entered the freshwater system and that microbial colonisation becomes increasingly important afterwards. However, up until now it has been less clear how leaching and colonisation by microbes interact with regard to the feeding preferences of shredders.

The following changes have frequently been postulated to occur during the process of leaf conditioning: Secondary compounds such as polyphenols are lost (Bärlocher and Graça 2005), nitrogen, protein and phosphorus content increase, (Bärlocher 1985; Suberkropp 1992; Graça *et al.* 1993b), leaf toughness is reduced (Graça and Zimmer 2005), and leaves become more palatable to shredders. According to Graça (2001), the shredders' preference for leaves is determined by three factors: leaf toughness, nutritional value, and

presence of secondary compounds (e.g. polyphenols). Decreasing leaf toughness and increasing nutritional value may be attributed to microbial colonisation, whereas the loss of secondary compounds is also associated with leaching. In summary, it is empirically well established that leaf conditioning enhances the utilisation of the leaf material by shredders, but the underlying mechanisms are less clear.

The picture is further complicated by (i) the fact that shredders' preferences for individual fungi is strain-specific (Graça *et al.* 1993a; Graça *et al.* 1994b) and that there have even been observations of repellent fungal strains (Aßmann *et al.* in press). Furthermore, since leaching and colonisation act simultaneously (Abelho 2001; Graça 2001), the effects appear to be correlated with each other in the field, so that disentangling the effects of leaching and colonisation is not feasible by statistical approaches alone.

Most knowledge about the mechanisms behind leaf litter decomposition in freshwaters is derived from running water systems. In contrast, there have only been a few reports on leaf litter conditioning (Federle and Vestal 1982; Federle *et al.* 1982) and degradation (Webster and Benfield 1986; Sabetta *et al.* 2000; van Dokkum *et al.* 2002) in lakes. However, allochthonous vascular plant material may provide between 10% and 75% of the total organic input in lakes (Webster and Benfield 1986) and are a major source for lentic food webs (Pace *et al.* 2004). It is therefore important to better understand leaf conditioning and its effects on the benthic community in lakes. Furthermore, aquatic fungi, especially aquatic hyphomycetes, have already been studied extensively with respect to their role in leaf conditioning in freshwaters (Bärlocher *et al.* 1992), but the function of oomycetes is largely unknown. Oomycetes are saprophytes or plant parasites in soil and water and are well represented in freshwater habitats (Nechwatal and Mendgen 2006; Nechwatal *et al.* 2008). They have been found on leaves in running waters (Bärlocher 1985; Bärlocher 1991a; Dix and Webster 1995; Gessner *et al.* 2007) and were until now assessed only in a few shredder/grazer feeding assays (Aßmann *et al.* 2009; Aßmann and von Elert 2009; Aßmann *et al.* in press). Here, in addition to single fungal isolates, we therefore as well included strains of oomycetes.

The aims of this study are twofold. In a first experiment we investigated leaf conditioning in the field (exposition experiment) and its effect on consumption by *Gammarus roeselii* (GERVAIS), an important shredder in the littoral zone of lakes (Mörtl 2004; Baumgärtner *et al.* 2008). We monitored key parameters of the leaves (toughness, nutritional value, polyphenol content) over the whole exposition period. This enabled us to (i) quantify the correlation between these parameters with each other and over time and (ii) determine the effects of the changing parameters on the consumption of *Gammarus*. Note that in the first experiment leaching and colonisation (also called conditioning) act simultaneously. In a second experiment (single isolates experiment) we inoculated non-conditioned leaves with single strains of fungi and oomycetes isolated from leaf litter that had been exposed in the littoral zone. However, through autoclaving these leaves were intensely leached in the absence of colonisation. Earlier studies have suggested that autoclaved leaves constitute an appropriate model system for leached leaves (Aßmann *et al.* 2009; Aßmann *et al.* in press). These autoclaved leaves were inoculated with single strains of fungi or oomycetes. We again measured leaf parameters and consumption by *Gammarus* of the colonised (single isolates) leaves and of autoclaved leaves as a control. This allowed us to (i) study the exclusive effects of microbial colonisation excluding the simultaneous effects of leaching, (ii) investigate the strain-specific effects on the feeding behaviour *of Gammarus* (FABRICIUS), and (iii) quantify how much of the observed variability in feeding of *Gammarus* among different strains of fungi and oomycetes can be explained by the leaf parameters.

2. Methods

2.1. Gammarids

Specimens of *G. roeselii*, a common shredder in Lake Constance (Mörtl 2004), were collected with a dip net (mesh size 200 µm) in the littoral zone near the Limnological Institute of the University of Konstanz. Using a stereomicroscope (Zeiss Stemi 2000-C, Jena, Germany) connected to a digital imaging system

body length was measured according to Gergs and Rothhaupt (2008). Only adults of both sexes (body lengths 7 – 12 mm) were used; they were starved for one day prior to the experiments. The animals were reared and the experiments were run in a climate-chamber at a constant temperature (15 °C) with a photoperiod of twelve hours.

2.2. Leaf Litter

Freshly fallen black alder leaves (*Alnus glutinosa* [L.] GAERTNER) were collected from the ground in autumn 2003 and used for the exposition experiment. For the single isolates experiment, black alder leaves were collected with a nylon net mounted above the ground in autumn 2005. All collected leaves were air-dried and stored at room temperature in the dark until needed for the experiments.

2.3. Exposition experiment

We applied three different treatments of leaf conditioning in the exposition experiment (as described in Aßmann *et al.* in press): (i) exposition in the littoral, (ii) incubation in running tap water, and (iii) autoclaving. We applied two exposition regimes (littoral and tap-water) for two reasons: (i) another conditioning treatment adds another contrast to our statistical analysis, and (ii) to provide a more protected and controlled experimental treatment in case of major disturbances in the field. Starting in October 2005, black alder leaves were exposed in the littoral of upper Lake Constance (N 47° 41.5'; E 9° 12.2') in cages (excluding shredders; for details see Aßmann *et al.* in press) at 0.4 m water depth with contact to the sediment. Each cage was filled with eight pre-soaked alder leaves (approx. 2 g dry wt. each). In parallel, 5 l containers containing approx. 50 leaves each were exposed to tap water at a flow rate of 4300 l day^{-1}, simulating the continuous water exchange in the littoral. For the experiment, which lasted for more than six weeks, leaves from the littoral and the tap water treatment were harvested weekly. In contrast, the autoclaved treatment did not include any long-term incubation, but pre-soaked leaves were autoclaved (30 min, 121 °C) before each experiment, thus providing leaf

material that was physically softened and leached but not chemically modified through microbial colonisers.

2.4. Food assays with leaves from the exposition experiment

From leaves originated from all three treatments in the exposition experiment, leaf discs (∅ 14 mm) were stamped using a cork borer near the edges of the leaves (to avoid larger leaf veins). The wet weight of the leaf discs was measured (Mettler AE 240) four times to obtain an error of less than ± 0.1 mg. Prior to weighing, the discs were dipped into deionized water and then dabbed with a paper towel to reduce weight fluctuations owing to excess water on the leaf discs. Although dry weight is usually measured in feeding assays (e.g. Graça *et al.* 1993a; Graça *et al.* 1994a; Rong *et al.* 1995), we used wet weight in order to not affect the microbial colonisation of the leaves.

Single discs from each of the three treatments were simultaneously offered to a single *G. roeselii* in a four-chambered polyethylene container (108 × 108 × 40 mm). Every chamber contained a shelter (a stone of approx. 4 g). A single leaf disc from each of the three different treatments was placed in each of three chambers of the container. The container was filled with 250 mL filtered (30 µm) lake water, and a single *G. roeselii* was added. The feeding assays ran for 48 h or until less than a third of the leaf disc remained (visual inspection). For each feeding assay, the absolute consumption rate for each leaf material was calculated as the difference between initial and residual wet weights of each leaf disc divided by the duration of the experiment, expressed in mg leaf consumed per individual and hour (mg * Ind.$^{-1}$ * h^{-1}). The assays with conditioned leaf material were replicated using different individuals of *G. roeselii* (n = 14 to 19).

2.5. Single isolates experiment

After the exposition experiment (with littoral or tap water exposure) in which leaching and microbial conditioning acted together on the leaves, we separated the effects of conditioning and leaching from each other in a second experiment. Here, we used leaves for which the leaching process was almost

completed (by autoclaving) in order determine the effects of microbial conditioning on leaf parameters. Autoclaving reduces leaf toughness and dissolves soluble organic and inorganic substances out of the leaf matrix (Aßmann *et al.* in press) and additionally leaf endophyte fungi were defeated.
In order to study the effects of microbial conditioning in a controlled way, we inoculated autoclaved leaves with single isolates of oomycetes or fungi isolated from leaves exposed in the littoral zone of Lake Constance (see below). We were interested in the emerging variability of leaf parameters among leaves colonised by different strains of fungi and oomycetes. We decided to work with single isolates/strains instead of the natural microbial community because we had hypothesized that different fungal and oomycete strains affect leaf parameters differently and thus modify the palatability of leaves for shredders in a strain-specific way.

2.6. Isolation and identification of fungi and oomycetes

Fungi and oomycetes were isolated from the littoral exposure treatment on days 22 and 36. We chose these sampling days because we had noted relatively high absolute consumptions for the littoral-exposed leaves on these days (after having recorded elevated absolute consumptions for the littoral treatment already on days 8 and 15). This suggested that the microbial leaf community was palatable for the gammarids. Fungi and oomycetes were isolated from the littoral-exposed leaves, and isolates were purified from bacteria (according to (Aßmann *et al.* in press) in order to establish stock cultures. The isolates were examined macroscopically (Stereomicroscope; Stemi 2000-C, Zeiss AG, Germany) and preliminarily grouped according to their macroscopic appearance.
Mycelium from pure cultures of the thirteen fungi and oomycetes isolates was used for taxonomical classification. DNA extraction, amplification of the ITS (internal transcribed spacer) regions 1 and 2 including the 5.8S gene of the ribosomal RNA (rDNA) genes, RFLP analyses and sequence analyses were carried out as described in Aßmann et al. (in press). RFLP analyses and comparison of sequence data was used for identification of the isolates. The

four different sequences obtained during this study have been submitted to GenBank® (see Table 1 for accession numbers). Due to the unexpectedly high abundance of identical oomycete isolates, we selected two independent isolates with identical RFLP patterns and sequences (36e and 36c; Table III 1).

2.7. Food-choice assays with leaves colonised by single isolates

A total of 14 different strains of fungi and oomycetes were used for the single isolates experiment. Nine of these isolates were obtained from leaves exposed in the littoral zone of Lake Constance in July/August 2005 within the study of Aßmann et al. (in press). In order to increase the number of identified isolates in this study, we repeated the procedure of isolating strains in October/November 2005 during our exposition experiment. This second isolation of fungi/oomycete strains resulted in another five strains (see above).

We determined how each of the 14 fungal or oomycete isolates affected (i) leaf parameters and (ii) the consumption by *G. roeselii*. The inoculation with fungi and oomycetes and the incubation to the 'fully conditioned phase' (Bärlocher 1985) of the leaves were conducted as described in Aßmann et al. (in press). Autoclaved leaves (control) and the colonised leaves were offered in food assay experiments. Leaf discs were cut from leaves of the two different treatments (i.e. colonised or autoclaved) as described above (food assay with leaves from the exposition experiment). One disc from the conditioned leaves and one from the control leaves were offered together to a single *G. roeselii* in the four-chambered polyethylene container as described above. In a control assay, two autoclaved leaf discs were offered to *G. roeselii*. Absolute consumption rates were calculated as above. We replicated the food assays for each isolate and the control with at least nine individuals (n = 9 to 12).

2.8. Leaf parameters

The weekly samples of the littoral-exposed and tap-water-conditioned leaves were freeze-dried and homogenized with mortar and pestle and stored at -80 °C. Samples from autoclaved leaves and from leaves inoculated with a single fungus or oomycete were treated alike. All measurements of leaf litter

samples were performed in duplicate to reduce the chance of systematic errors. Particulate organic carbon and nitrogen content was determined with an NCS-2500 analyzer (Carlo Erba Instruments), and particulate phosphorus was determined according to Aßmann *et al.* (in press). Protein content was measured as according to Baerlocher (2005); total polyphenol content was determined photometrically as described by Bärlocher and Graça (2005).

We measured the ergosterol content (as according to Gessner 2005); for details of the C18-solid-phase extraction and HPLC column see Aßmann *et al.* (in press) of the littoral-exposed leaves in order to monitor fungal development (Gessner *et al.* 2007). Note that oomycetes do not contain ergosterol (Weete and Gandhi 1996); the ergosterol content thus provides no information about oomycete colonisation. A penetrometer (Pabst *et al.* 2008) was used to determine the leaf toughness (N) by puncturing five points of the edge region of five leaves from every treatment.

2.9. Statistical analyses

Statistical analysis of the absolute consumption rates in the exposition experiment with littoral-exposed and tap-exposed leaf litter was conducted using a linear model approach. First we averaged the measured consumption rates and leaf parameters in each treatment (littoral-exposed, tap-exposed and autoclaved) and on each sampling day (days 1, 8, 15, 22, 29, 36, and 43). Since all the single leaf discs from each treatment were offered together, the absolute consumption on one leaf disc depended not only on the preference of the gammarid for that leaf disc, but also on its preference for the other discs. The autoclaved leaves served as a control, because the quality of this food source remained constant over the whole experiment. For this reason, we then adjusted the consumption rates on littoral-exposed and tap-exposed leaves based on the average consumption on the control leaves, i.e. the mean consumption rate of autoclaved leaves was subtracted from the consumption

Table III 1. **1** Fungi and oomycetes isolated from leaf litter conditioned in lake water in summer (**§**) (Aßmann et al., in press) and autumn 2005 (exposition experiment) (†). Isolates were identified by BLAST analysis of amplified sequences of the internal transcribed spacer regions 1 and 2 (ITS1, ITS2) and the 5.8S rDNA; similarity of the sequences refers to pair-wise alignments with the closest match.

Phylum or class	Species	GenBank accession number	Best BLAST hit; accession no.; similarity [%]
Ascomycota	*Epicoccum* sp. PV Wi 22e †	EU740394	*Epicoccum nigrum* (LINK); AY787697. 100.0%
Ascomycota	*Epicoccum* sp. PV Wi 36a †	EU740397	*Epicoccum nigrum* isolate H2F1 (LINK); EU529998. 100.0%
Ascomycota	*Cylindrocarpon* sp. PV Wi 22k †	EU740396	*Cylindrocarpon* sp. EXP0565F (WOLLENWEBER); DQ914670. 98.9%
Ascomycota	*Cylindrocladiella parva* §	EU637905	*Cylindrocladiella parva* ASICP1(ANDERSON); DQ779786 ; 100%
Ascomycota	*Cylindrocarpon* sp. 94-2057 §	EU637906	*Cylindrocarpon* sp. 94-2057 (WOLLENWEBER); AY295305; 100%
Ascomycota	*Cylindrocarpon* sp. 4/97-1 §	EU637900	*Cylindrocarpon* sp. 4/97-1 (WOLLENWEBER); AJ279490; 100%
Ascomycota	*Fusarium sporotrichioides* §	EU637901	*Fusarium sporotrichioides* var. *minor* BBA 62425 (SHERBAKOFF); AF414973; 100%
Ascomycota	*Microdochium* sp. PV So2 §	EU637902	*Microdochium* sp. 4/97-103 (SPRAGUE); AJ279489; 99.6%
Ascomycota	Ascomycete *sp*. PV So8 §	EU669082	Leaf litter ascomycete its261(GILBERT); AF502786; 96.8%
Oomycetes	*Pythium litorale* §	EU637904	*Pythium litorale* P.03 (NECHWATAL); DQ144637; 100%
Oomycetes	*Pythium* sp. JN 1-b §	EU637903	*Pythium* sp. JN-1b (NECHWATAL); DQ230904; 100%
Oomycetes	*Pythium* sp. PV So7 §	EU669081	*Pythium* sp. JN-12 (NECHWATAL); DQ237932; 90.3%
Oomycetes	*Pythium* sp. PV Wi 36c †	FJ882625	*Pythium litorale* (NECHWATAL); EU637904. 96.3%
Oomycetes	*Pythium* sp. PV Wi 36e †	EU740398	*Pythium litorale* (NECHWATAL); EU637904. 96.3%

rates of littoral-exposed and tap-exposed leaves. We did the same adjustment based on the control treatment for all leaf parameters (leaf toughness, protein content, etc). Finally, we calculated linear models between the adjusted consumption rates as a dependent variable and the adjusted leaf parameters as independent variables.

Linear models were also used for analysing the absolute consumption rates in the single isolates experiment. Again, we first calculated the average consumption rates and leaf parameters for each isolate and for the control. Since we wanted to find out which isolates were significantly preferred over the control treatment, we then defined a linear model in which the intercept is equal to the average consumption rate in the control treatment. We achieved this by subtracting this average consumption rate in the control treatment (0.065 mg wet weight*h^{-1} * $ind.^{-1}$) from the consumption rates of all isolates (i.e. adjusted based on the control treatment). This means that a consumption rate of zero in the transformed data of a treatment corresponds to the consumption rate in the control treatment. We then calculated a linear model with an intercept of zero, with the consumption rates as a dependent variable and the isolate as the independent variable. Since the controls by definition had a mean of zero, we removed them from the analysis. All statistical analyses were performed using R (R Development Core Team, 2006), and the significance level of the analyses was $p = 0.05$.

3. Results

3.1. Absolute consumption and leaf parameters in the exposition experiment

The absolute consumption rate for black alder leaf litter of all three treatments (littoral-exposed, tap-water-conditioned and autoclaved) changed over time during the experiment. While at the beginning almost exclusively autoclaved leaves were consumed at a very high level, littoral exposed leaves were increasingly preferred later on (Fig III 1a). From day 22 onwards, the consumption rate of littoral-exposed leaves was about 0.2 mg*$ind.^{-1}$*h^{-1}. Leaves

incubated in tap water were hardly eaten in the first weeks, but consumption of these leaves increased steadily over the course of the experiment. Nevertheless, consumption of tap-water-conditioned leaves always remained below 0.15 mg*ind.$^{-1}$*h^{-1}. On days 8 and 15 we noted a similar consumption rate in littoral-exposed and the autoclaved leaves, but afterwards the consumption of autoclaved leaves remained at a very low level (below 0.05 mg*ind.$^{-1}$*h^{-1}).

The ergosterol content of littoral-exposed leaves increased during exposure, indicating intensive colonisation by fungi on the leaves starting between day 15and 22; a maximum of 35 µg*g^{-1} dwt was reached on day 36 (Fig. III 1h).

The other measured leaf parameters (littoral exposed leaves) changed considerably during the experimental time (Fig III 1b – 1g): While leaf toughness as well as polyphenol and nitrogen content decreased strongly, protein and phosphorus content showed only minor changes.

There is considerable co-linearity between the leaf parameters in the littoral and tap-water exposed leaves, i.e. the different leaf parameters were correlated to each other and developed similarly over time. We quantitatively explored these correlations by calculating a matrix of Pearson product-moment correlation coefficients between all leaf parameters (including N:C and P:C ratios) and exposition time. Fourteen of 36 combinations were significant (Table III 2). In fact, many parameter combinations showed significant correlations because of their similar development over exposition time. For example, the strong decrease in leaf toughness is accompanied by a corresponding decrease in polyphenol content over exposition time.

The statistical analysis of the absolute consumption rate of the littoral-exposed leaves (exposition experiment) as a dependent variable and leaf parameters as independent variables showed that polyphenol content and leaf toughness of the leaves explain a relatively high degree of variability (r^2 above 0.6; Table III 3, Littoral) of the absolute leaf consumption. In addition, correlations of the protein and P content and the P:C ratio with absolute consumption were highly significant (r^2 above 0.5; Table III 3, Littoral). However, since most of these parameters were simultaneously correlated with each other as indicated above,

we cannot distinguish potential causal relationships from pseudo-correlation due to co-linearity.

3.2. Absolute consumption and leaf parameters in the single isolates experiment

Absolute consumption varied between leaves inoculated with different fungal and oomycete isolates compared to the control (autoclaved leaves). Nine of fourteen isolates (6 ascomycetes and 3 oomycetes) were significantly preferred by *G. roeselii* over the control (Fig. III 2a and Table III 4).

Table III 2 Pearson product-moment correlation coefficients (Pearson's r) between experimental day and leaf parameters in the exposition experiment. Since the development of leaf parameters over time were qualitatively similar in the littoral exposed and tap-water experiments, results from both treatments were merged. Bold numbers and asterisks indicate significant differences: $p < 0.001$ '***', $p < 0.01$ '**', $p < 0.05$ '*'.

Variable	Leaf toughness	Polyphenol	Protein	P	N	C	N:C	P:C
Day	**-0.758 ****	**-0.932 *****	-0.010	**0.547 ***	-0.167	-0.286	0.091	0.422
Leaf toughness		**0.647 ***	-0.044	**-0.773 ****	-0.041	0.358	-0.428	**-0.563 ***
Polyphenol			0.060	-0.425	0.342	0.334	0.081	-0.408
Protein				-0.401	0.348	**0.714 ****	-0.270	**-0.655 ***
P					-0.034	**-0.665 ***	**0.645 ***	**0.875 *****
N						**0.637 ***	**0.623 ***	-0.407
C							-0.205	**-0.938 *****
N:C								0.442

Regarding the parameters of the single isolates experiment, the leaf toughness of fungal and oomycete colonised leaves was always lower than in the control (Fig. III 2b). In the case of protein and N content, the fungal and oomycete colonised leaves had higher values than the control (autoclaved leaves, Fig. III 2c and 2d) did. In most cases the P content of the fungal and oomycete

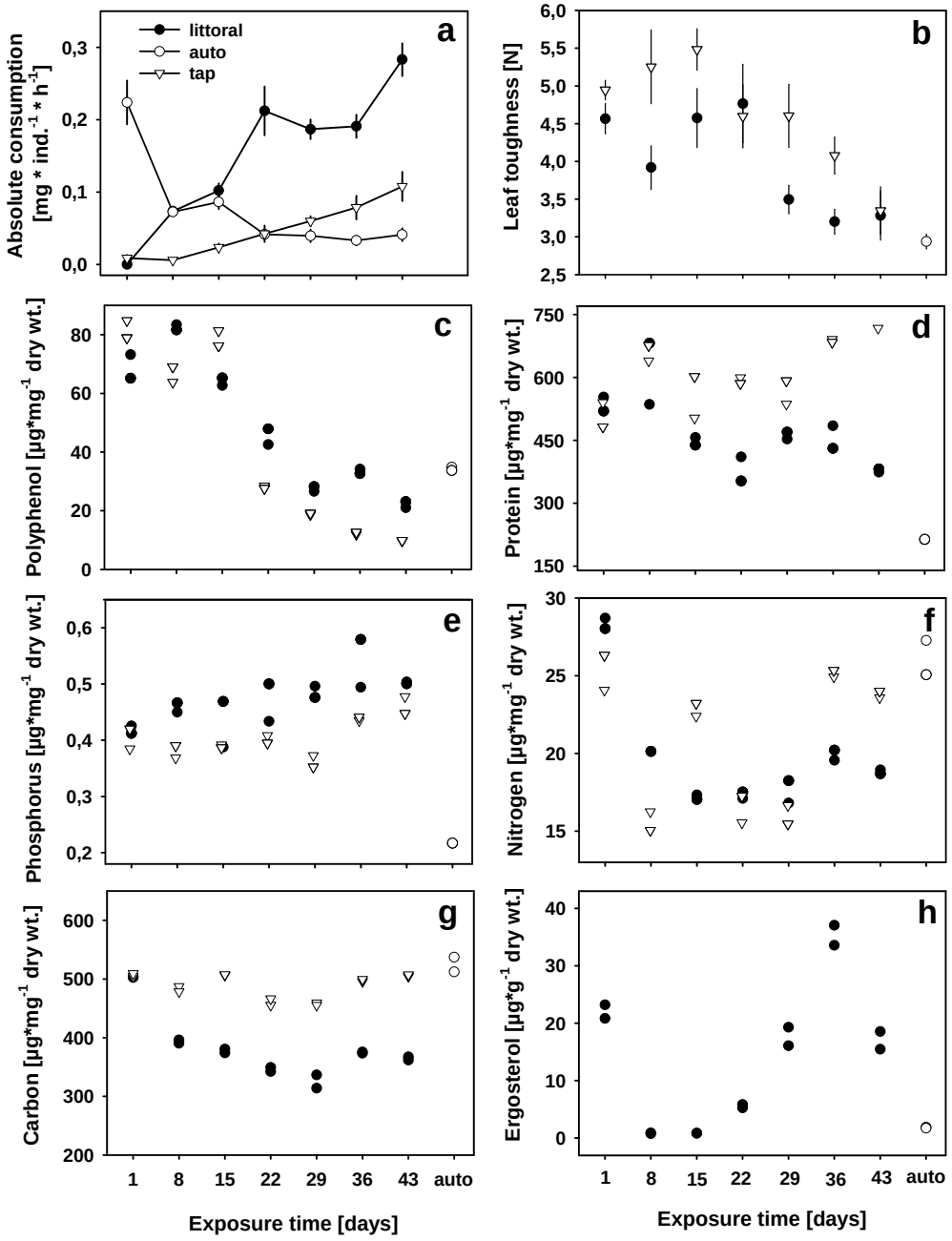

Fig. III 1. Results from the exposition experiment: (a) Absolute consumption rates (mean value ± SE, n = 14–19) of three differently treated food items offered simultaneously to *G. roeselii*. The three treatments consisted of black alder leaves exposed in the littoral zone of Lake Constance (filled circles), exposed to tap water (open triangles), or autoclaved (open circles). Leaf parameters for each sampling date and treatment are shown as follows: (b) Leaf toughness, (c) polyphenol, (d) protein, (e) phosphorus, (f) nitrogen, (g) carbon and (h) ergosterol content of leaves in the littoral and tap water treatment and for autoclaved leaves (auto) are displayed. Duplicated analyses are shown for polyphenol, protein, nitrogen, phosphorous, carbon and ergosterol; for leaf toughness the mean value ± SE from five leaves with five measurements per leaf is shown.

colonised leaves was higher than that of the control (Fig. III 2e). However, the different fungal and oomycete isolates have very different effects on the measured parameters and as well on the absolute consumption by *G. roeselii*. For example, leaf colonisation by *Fusarium sporotrichoides* had no effect on the consumption by *G. roeselii*, but the colonisation with this fungus made the leaves softer (low leaf toughness) and increased the protein, N and P content compared to control (Fig. III 2). These effects are in contrast to the widespread view that fungal colonisation generally increases leaf palatability for shredding invertebrates (Graça 2001).

The statistical analysis of the absolute consumption rate of the fungal- and oomycete-colonised leaves as a dependent variable and their leaf parameters as independent variables showed that polyphenol content and protein content of the leaves explain a large amount of variability (r^2 above 0.4; Table III 3, Isolates) of the absolute consumption of the leaves.

Comparing the statistical results of the littoral and the single isolates experiment (Table III 3), two of eight parameters (the polyphenol and the protein contents) were significantly correlated with absolute consumption in the single isolates experiment, whereas in the exposition experiment six of eight parameters (polyphenol, protein and P content, N:C and P:C ratios) were significantly correlated with the absolute consumption.

4. Discussion

We conducted two experiments to study the effects of fungi and oomycetes on chemical and physical leaf parameters and on the feeding activity of an invertebrate shredder. In a first experiment (exposition experiment), we measured the dynamic development of leaf parameters during conditioning and its effects on the consumption by *G. roeselii*. In a second experiment (single isolates experiment), the influence of single fungal and oomycete isolates on leaf parameters and consumption by the gammarid were studied.

Interpretation of statistical analyses

A large part of our findings is based on extensive statistical analyses of the data sets by using linear models. This was necessary because we aimed at quantifying the degree of covariation among leaf parameters over the course of leaf conditioning. In fact, we found strong correlations between numerous leaf parameters. For example, out of 36 correlations analysed in Table III 2 we found 14 to be significant (about 40%). The high co-linearity among leaf parameters obscured the identification of key leaf parameters for shredder feeding behaviour, because it creates a high number of significant relationships which may be mostly pseudo-correlations. This is impressively demonstrated by the analysis of the eight leaf parameters in Table III 3, of which six showed a significant effect on the consumption rates of *G. roeselii*. At this stage it is still unclear which of these six parameters have a real mechanistic effect on the consumption rate and which are only the consequence of pseudo-correlation. Repeating the feeding assays in the single isolates experiment with autoclaved, i.e. extensively leached, leaves helped to reduce the number of significant parameters: Only two leaf parameters, polyphenol and protein content, were of significance. These results provide evidence that polyphenol and protein contents are the most important leaf parameters mediating the shredders' preference through colonisation by fungi and oomycetes.

Note that we did not correct the p-values in our statistical analyses for repeated testing (e.g. Bonferroni correction); we may thus have accepted the risk of spurious correlations, particularly in the analysis presented in Table III 2. However, we interpreted the results in Table III 2 in a rather explorative way, and the incidence of a few spurious correlations (the expectation value would be 2) does not harm our interpretations.

Leaf toughness and polyphenol content were important leaf parameters in the exposition experiment (Table III 3). This finding is in agreement with the statement of Graça (2001) that leaf toughness and content of secondary compounds are main factors determining shredder preference. Although leaf toughness may represent an important parameter in the field, it was not significant in our single isolates experiment, for which polyphenol and protein

content were considerable parameters. These results indicate that the effect of microbial colonisers on leaf toughness is not as important as the effect on polyphenol or protein content. It is substantial to note that this finding does not necessarily imply a minor relevance of leaf toughness for shredder preference in the field – it merely indicates that microbial colonisers affect shredder activity by changing other leaf parameters in addition to leaf toughness. Note that the leaf toughness of the autoclaved leaves in the single isolates experiment was in approximately the same range as that of the littoral-exposed leaves in the last stage (days 29 to 43) of the exposition experiment (Fig. III 1b).

Table III 3 Effects of adjusted leaf parameters (see Methods) on adjusted consumption rates in the exposition experiment (exposure in the littoral) and in the single isolates experiment (leaves colonised with single fungus or oomycete). Pearson product moment correlation coefficients (*r*), coefficients of determination (r^2) and p-values are given (*p*). Bold letters indicate significant p-values.

Experiment		Leaf toughness	Polyphenol	Protein	P	N	C	N:C	P:C
Exposition	*r*	-0.806	-0.811	-0.754	0.753	0.279	-0. 485	0.576	0.759
	r^2	0.650	0.657	0.568	0.567	0.078	0.236	0.332	0.576
	p	**0.000**	**0.000**	**0.002**	**0.002**	0.335	0.079	**0.031**	**0.002**
Single isolates	*r*	-0.478	-0.678	-0.658	0.031	-0.012	0.358	-0.077	0.008
	r^2	0.228	0.460	0.433	0.001	0.000	0.128	0.006	0.000
	p	0.084	**0.008**	**0.010**	0.917	0.969	0.209	0.793	0.979

Dynamics of consumption and leaf parameters

In the exposition experiment, the preference by *G. roeselii* shifted from autoclaved leaf litter to littoral-exposed leaves with increasing incubation time (Fig. III 1a). As has already been shown for shredder organisms (Kaushik and

Hynes 1971), here consumption by *G. roeselii* increased with ongoing conditioning time of leaf litter.

In earlier studies it has been suggested that the increased preference of shredders for conditioned leaves is caused by changes in the chemical composition and the physical properties of leaves during conditioning (Abelho 2001; Graça and Zimmer 2005). Accordingly in our exposition experiment, the chemical, biological and physical parameters of tap water- and littoral-exposed leaves changed markedly during exposition. Secondary leaf compounds such as polyphenols are known repellents of vertebrate and invertebrate grazers (Rosset *et al.* 1982; Pennings *et al.* 2000; Abelho 2001; Graça and Bärlocher 2005). The polyphenol content of the alder leaves (tap-water- and littoral-exposed) decreased during exposition, a result which is in accordance with earlier studies (Bärlocher *et al.* 1995; Canhoto and Graça 1996). In parallel to the polyphenol content, leaf toughness also decreased strongly during exposition (tap- and littoral-exposed, Fig. III 1b).

It has been shown that the leaf litter contents of nitrogen and protein increase during decomposition, leading to enhanced preference by shredders (Bärlocher 1985; Suberkropp 1992). However, the results of our exposition experiment do not support this notion. The N content as well as the protein content of the littoral-exposed leaves decreased over the first three weeks of exposure (Fig. III 1f). We hypothesise that proteins and N-containing substances such as amino acids and other N-rich compounds were leached out of the leaves, and that simultaneously growing microbes (fungi and oomycetes) could not compensate for this loss. Only after three weeks the protein and nitrogen levels began to increase slightly, indicating that microbial growth became more important than loss processes. In contrast, the effect of microbial colonisation on the protein and nitrogen contents of the leaf litter was clearly visible in the single isolates experiment (Fig. III 2), in which leaves had been leached extensively prior to microbial conditioning. These two observations indicate that leaching constitutes a major loss of nitrogen and protein, which is significant over several weeks in the field. Other studies of leaf conditioning observed increasing nitrogen and protein contents during microbial colonisation coinciding with

increasing shredder consumption (Suberkropp 1992). Although we also found in the single isolates experiment that increasing protein contents go along with microbial colonisation the link with shredder consumption appeared to be less clear. In fact, the protein content of the colonised leaves correlated negatively with consumption. That points strain specific effects of fungi and oomycetes on shredder preference. Most likely, other leaf characteristics than bulk protein or nitrogen content determine the attractiveness of leaf material, and the production of these substances differs between strains of fungi and oomycetes.

Compared to the initial parameters of the autoclaved leaves (and control), all leaf parameters were affected by colonisation with single isolates (Fig. III 2b – 2e). However, the magnitude of the effects showed considerable variation depending on the type of isolate, demonstrating that the effect of microbial colonisers is strongly strain-specific. Bärlocher and Kendrick (1973) showed that protein content of leaves inoculated with single fungi increased compared to the initial content and varied depending on the species. Corroborating these findings, the protein content of our single isolates on leaves showed an inhomogeneous picture as well: The values were highly variable, and almost all values were higher than the control. Other leaf parameters also showed considerable variation within the single-strain experiment; for example, the phosphorus content varied by a factor of about four (Fig. III 2e).

In addition to the leaf parameters, the consumption rates by *G. roeselii* also varied significantly between the different fungal and oomycete colonised leaves (Table III 4, Fig. III 2a). Nine out of fourteen isolates (fungus or oomycete) on leaves were preferred over control leaves (Fig. III 2a), and none was rejected. However, the consumption rate varied considerably between the different strains, e.g. the consumption rate of leaves colonised by *Fusarium sporotrichoides* was approximately ten times lower than that of leaf litter colonised by *Cylindrocladiella parva.* This confirms the results of earlier studies in which the preferences of amphipod shredders were shown to vary depending on the individual fungi species (Bärlocher and Kendrick 1973; Graça *et al.* 1993b; Graça *et al.* 1994b). Other studies even found deterring effects of fungal strains on *G. roeselii* (Aßmann *et al.* in press).

Table III 4: Results of the statistical analysis of absolute consumption rates, of *G. roeselii* for fungal and oomycete colonised leaves (single isolates experiment), by a linear model. Negative estimates for the regression coefficient indicate lower consumption rates than in the control treatment, positive values indicate higher consumption rates. ANOVA of the model indicated high significance ($F_{14,117}$ = 11.53, $p < 0.001$). Asterisks indicate regression coefficients being significantly different from zero: $p < 0.001$ '***', $p < 0.01$ '**', $p < 0.05$ '*'.

Leaf conditioning	**Abbreviation**	**Estimate for regression coefficient**	**SE**	**t-value**	**p-value**	
Fusarium sporotrichioides	Fus.	-0.04205	0.05583	-0.753	0.45286	
Microdochium sp. PV So2	Micro.	0.15625	0.05583	2.799	0.00600	**
Pythium sp. JN 1-b	Pyth.a	0.09836	0.05885	1.671	0.09732	
Pythium litorale	Pyth.b	0.08125	0.05583	1.455	0.14826	
Cylindrocladiella parva	Cylin.	0.38803	0.05885	6.593	1.30e-09	***
Pythium sp. PV So7	Pyth.c	0.15465	0.05583	2.770	0.00652	**
Ascomycete sp. PV So8	Asc.	0.25414	0.05885	4.318	3.31e-05	***
Cylindrocarpon sp. 94-2057	Cyl.a	0.27435	0.05583	4.914	2.93e-06	***
Cylindrocarpon sp. 4/97-1	Cyl.b	0.32336	0.05885	5.495	2.32e-07	***
Epicoccum sp. PV Wi 22e	Epi. a	0.06012	0.06242	0.963	0.33742	
Cylindrocarpon sp. PV Wi 22k	Cyl.c	0.15705	0.05583	2.813	0.00576	**
Epicoccum sp. PV Wi 36a	Epi. b	0.11175	0.06242	1.790	0.07599	
Pythium sp. PV Wi 36c	Pyth.d	0.14375	0.05583	2.575	0.01128	*
Pythium sp. PV Wi *36e*	Pyth.e	0.13525	0.05885	2.298	0.02333	*

Statistical analysis of the single isolates experiment revealed polyphenol and protein levels to be of particular importance for determining the consumption rate of *Gammarus*, which suggests that fungi and oomycetes might indirectly steer consumption by altering the leaf litter content of protein and polyphenols. Polyphenol can be actively degraded by fungi and bacteria (Bhat *et al.* 1998). However, in our exposition experiment the polyphenol content did not correlate with the ergosterol content (Pearson product-moment correlation: polyphenol content - ergosterol content; $r = -0.34$, $p = 0.24$) of the exposed leaves that was used as a proxy for fungal biomass (Gessner and Newell 1997; Gessner 2005). In fact, the loss of polyphenol from leaf litter in the exposition experiment occurred before an increase of ergosterol was detectable. It is reasonable to assume that the active reduction of polyphenols by fungi and oomycetes (additionally as early colonisers which are not detectable via ergosterol) was obscured by leaching of the polyphenols in the early decomposition phase. In fact, the loss of polyphenol from leaf litter in the exposition experiment occurred before an increase of ergosterol was detectable. It is reasonable to assume that the active reduction of polyphenols by fungi and oomycetes (additionally as early colonisers which are not detectable via ergosterol) was obscured by leaching of the polyphenols in the early decomposition phase.

Rong et al. (1995) assumed that food selection of shredders is not exclusively determined by macronutrients but also by specific fungal secondary substances that mediate attraction or repellence. Although Rong *et al*. (1995) found that nonpolar extracts of fungi mediated attraction and repellence of fungi, this observation could not be reproduced for some of the isolates investigated here: Aßmann and von Elert (2009) found that the type of carbon source that fungi and oomycetes were grown on affected the impact of fungal secondary products on the preference of gammarids. Therefore, it seems that the mechanisms behind the mediation of preference by fungi are not well

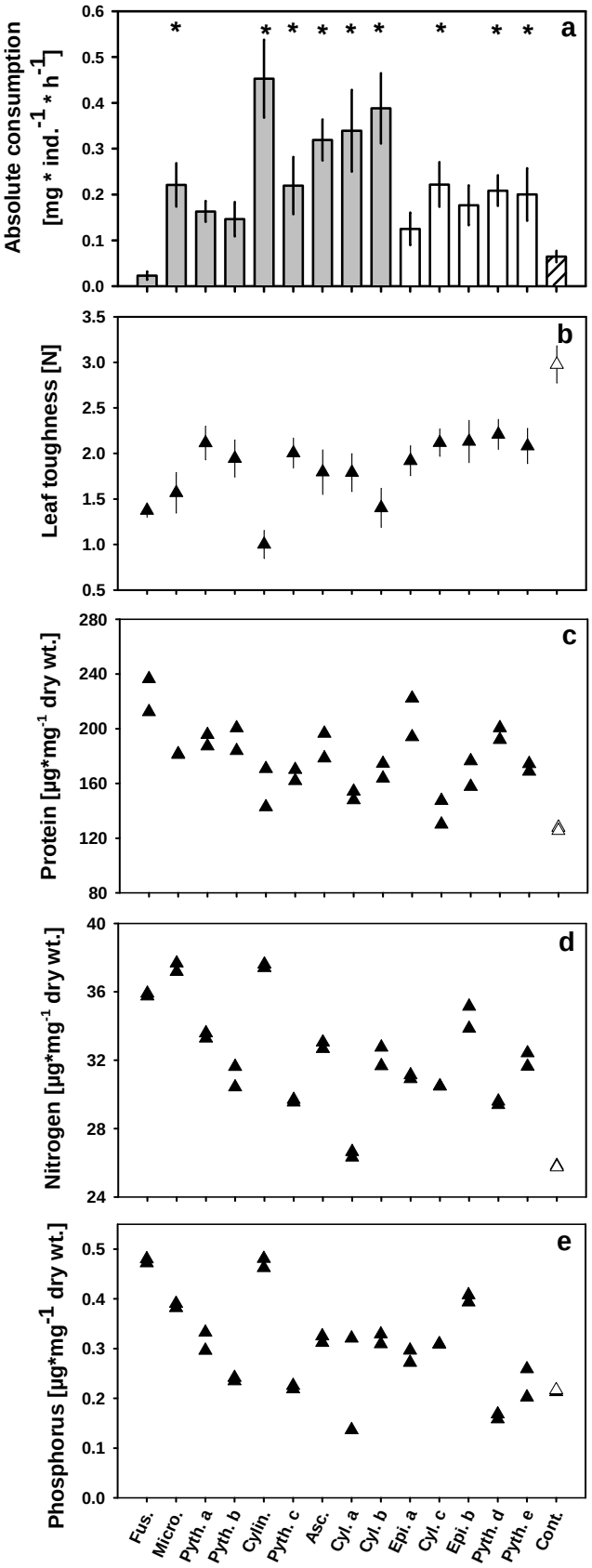

Fig. III 2. Results from the single isolates experiment: (a) Absolute consumption of *G. roeselii* (mean value ± SE are shown, n = 9–12) for leaf litter colonised with different single fungal or oomycete isolates. Grey-coloured columns are fungal and oomycete isolates on leaves from the summer-experiment; non-coloured columns are from the autumn-experiment and striped column is the control. In the following panels (b) leaf toughness, (c) protein, (d) nitrogen and (e) phosphorus content of the black alder leaves colonised with the respective isolates are displayed. Duplicated analyses are given for protein, nitrogen and phosphorous, while for leaf toughness the mean value ± SE from five leaves with five measurements per leaf is shown. Asterisks on the top panel indicate significantly different consumption rates between control and the respective strain. Fus., *Fusarium sporotrichioides*; Micro., *Microdochium* sp. PV So2; Pyth. a, *Pythium* sp. JN 1-b; Pyth. b, *Pythium litorale*; Cylin., *Cylindrocladiella parva*; Pyth. c, *Pythium* sp. PV So7; Asc., Ascomycete sp. PV So8; Cyl. a, *Cylindrocarpon* sp. 94-2057; Cyl. b, *Cylindrocarpon* sp. 4/97-1; Epi. a, *Epicoccum* sp. PV Wi 22e; Cyl. c, *Cylindrocarpon* sp. PV Wi 22k; Epi. b, *Epicoccum* sp. PV Wi 36a; Pyth. d, *Pythium* sp. PV Wi 36c; Pyth. e, *Pythium* sp. PV Wi 36e; Cont., control.

understood (Graça 2001). Here we found that the natural microbial community was highly attractive for *G. roeselii* (exposition experiment). However, further behavioural assays revealed strain-specific effects on consumption by *G. roeselii* (single isolates experiment), which strongly suggests that increased consumption of leaf litter is caused by the relative composition of attractive and neutral strains of fungi and oomycetes rather than by the microbial community on leaf litter in general.

Role of oomycetes in leaf conditioning

Fungi account for 88-99.9% of the microbial biomass on decaying leaves (Baldy and Gessner 1997; Hieber and Gessner 2002), and fungal biomass associated with decomposing plant material can exceed 10% of total litter mass (Gessner *et al.* 2007). In our exposition experiment the ergosterol content of the littoral exposed leaves increased during exposure until day 36, indicating an increasing fungal biomass on leaf litter. Some studies assume fungi to be the key microbial decomposers on leaves (reviewed by Abelho 2001). Ergosterol, a major cell membrane component (membrane lipid) of fungi, is often used to determine metabolically active eumycotic fungal biomass (Gessner and Newell 1997; Gessner 2005). At the same time it is well known that ergosterol is absent from the cell membranes of oomycetes (Weete and Gandhi 1996)and that oomycete species are well represented in the early stages of decomposition of leaf litter (Bärlocher 1992b; Wielgoss *et al.* 2009). Due to the absence of an easily accessible parameter for the determination of abundance, the role of oomycetes in leaf conditioning is not well understood, which is why we included oomycetes in this study. We were able to isolate five strains of oomycetes that all belong to *Pythium* (PRINGSHEIM), a genus which is well represented in freshwater habitats (Dix and Webster 1995) and has been regularly found on conditioned leaf litter in streams and lakes (Chamier *et al.* 1984; Bärlocher 1991a; Dix and Webster 1995). For example, *Pythium litorale* (NECHWATAL) has been described as an abundant leaf litter saprophyte in Lake Constance (Nechwatal and Mendgen 2006; Nechwatal *et al.* 2008; Wielgoss *et al.* 2009).

This study is the first report in which three out of five oomycete isolates were found to positively affect *Gammarus*' consumption. The effects of oomycetes on leaf litter parameters were similar to those of other fungal strains. Due to the presence of oomycetes in the early stages of conditioning (Bärlocher 1992b; Wielgoss *et al.* 2009), these findings strongly suggest that oomycetes have a greater impact on the early leaf litter decomposition in freshwaters than hitherto assumed.

5. Acknowledgements

We are grateful to M.O. Gessner, Dübendorf, Switzerland for his substantial help in establishing the analysis of ergosterol. The authors thank C. Gebauer und P. Merkel for their excellent technical assistance with the leaf litter analyses. In addition, we thank C. Geiss for her assistance with the food choice assays. We also appreciate M. Wolf's help in manufacturing the experimental exposure cages and the four-chambered containers. We acknowledge F. Bartlett for editing the English language of the manuscript. This study was supported by a grant to E. v. E. from the German Research Foundation (DFG) within the Collaborative Research Centre SFB 454 *Littoral Zone of Lake Constance*.

Chapter IV

The impact of fungal extracts on leaf litter on the food preference of *Gammarus roeselii*

Christine Aßmann and Eric von Elert

International Review of Hydrobiology 94 (4): 484-496

Abstract

We investigated the effect of methanol and methanol/methylene chloride extracts of the oomycete *Pythium* sp. JN 1-b and of the fungi *Ascomycete* sp. PVSo8, *Fusarium sporotrichoides,* and *Cylindrocarpon* sp. 94-2057 on the food preference of *Gammarus roeselii*. The preference for leaf discs coated with these extracts compared to uncoated leaf discs was tested in food-choice assays. Methanol extracts
of all strains repelled *G. roeselii*, and the effect of the extract concentration on relative consumption was strain specific. The repellent effect of these extracts, especially of extracts of *Cylindrocarpon* sp., decreased when the fungi were grown on leaf extract medium as opposed to synthetic medium containing sucrose. None of the methanol/methylene chloride extracts affected the food preference of the gammarid. We conclude that biologically active compounds were extracted from fungi and an oomycete were soluble in methanol but not in methanol/methylene chloride. Only repellent activity was observed with the extracts, and relative ratios of repellents and attractants might determine the consumption of fungi by *G. roeselii*.

Keywords: Fungi, *Pythium* sp., leaf shredder, food selection, organic solvent

1. Introduction

Numerous aquatic shredders, such as gammarids, prefer microbially conditioned leaf litter over unconditioned leaves (Arsuffi and Suberkropp 1989; Graça *et al.* 1993b; Graça *et al.* 1994a; Graça *et al.* 1994b). The main microbial decomposers of leaf litter in freshwaters are fungi (Abelho 2001), and their biomass can exceed 10% of the total litter mass in streams (Gessner *et al.* 2007). Detritivores increase their consumption of leaves as the leaves are conditioned and become softer and more palatable, and nutrients become available (Kaushik and Hynes 1971). Nitrogen, phosphorous and protein contents also increase during leaf decomposition (Bärlocher 1985; Rossi 1985; Suberkropp 1992; Graça *et al.* 1993b; Abelho 2001; Graça and Zimmer 2005). Not only leaf conditioning but also the colonisation of the leaves by various fungal species lead to preferential consumption by shredders (Graça 2001). Indeed, many laboratory experiments have shown that shredders discriminate among leaves colonised by different species of fungi (for reviews, see (Suberkropp 1992; Graça 2001).

A variety of different fungi and oomycetes have been found on decaying leaf litter (Nikolcheva and Bärlocher 2004; Shearer *et al.* 2007). Although terrestrial fungi are present when leaves enter streams, they are later replaced by aquatic hyphomycetes, which produce enzymes that degrade the major leaf polysaccharides (reviewed by Bärlocher 1992a). Rong *et al.* (1995) reported that *Gammarus tigrinus* fed with leaves conditioned by a single aquatic hyphomycete prefered leaves with a high content of easily extractable proteins and carbohydrates, and this was interpreted as a shredder preference for conditioned leaves with a high nutritional value. However, total consumption was not correlated to the lipid, protein, polysaccharide, or phenol content of the leaves or the fungal biomass. From this and other studies (Bärlocher and Kendrick 1973; Suberkropp *et al.* 1983) with leaves inoculated with a single fungal strain, it has been concluded that food selection is not determined exclusively by the macronutrient concentrations but also by species-specific fungal attractants and repellents. Here we investigated whether chemical

substances from three fungi and one oomycete involved in the conditioning of leaf litter in the littoral of Lake Constance affect the food preference of the amphipod shredder *Gammarus roeselii* (GERVAIS). In laboratory experiments, we examined the effects of organic extracts of these strains on the food preference of *G. roeselii*. To differentiate between chemically and structurally mediated preferences, we also tested the effect of fungal mycelia.

2. Methods

2.1. Gammarids

G. roeselii was collected using a dip net (mesh size 200 µm) in the littoral zone of Lake Constance. Body lengths were measured according to Gergs and Rothhaupt (2008) using a stereomicroscope (Zeiss Stemi 2000-C, Jena, Germany) connected to a digital imaging system. Only adults of both sexes with body lengths between 7.0 and 12 mm were used; animals were starved for one day prior to the beginning of each experiment. All experiments were run in chambers at 15 °C and a 12 : 12-h dark : light photoperiod.

2.2. Leaf Litter

Black alder leaves (*Alnus glutinosa* (L.) GAERTNER) were collected with a nylon net mounted above ground in autumn 2005. Leaves were air-dried and stored at room temperature in the dark until used.

2.3. Fungi and Oomycetes

The following pure strains of fungi and oomycetes were isolated from littoral conditioned leaf litter in July 2005 and tested: the fungi *Fusarium sporotrichoides* (GenBank accession number EU637901), *Ascomycete* sp. PV So8 (EU669082), and *Cylindrocarpon* sp. 94-2057 (EU637906), and the oomycete *Pythium* sp. JN 1-b (EU637903). Fungi were grown either in sterile Czapek's solution medium (3.0 g $NaNO_3$, 1.0 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7\ H_2O$, 0.5 g KCl, 0.01 g $FeSO_4 \cdot 7\ H_2O$, 30.0 g sucrose per litre; Tuite 1969) or in

sterile leaf extract medium to provide a more natural carbon source with a lower carbohydrate content. Sterile leaf extract was made by autoclaving six dried black alder leaves in 250 mL water and then straining the leaves; 15 mL of sterile leaf extract was added to 150 mL sterile H_2O to obtain the medium. Erlenmeyer flasks with approximately 150 mL medium were inoculated with a small piece of mycelium grown on malt extract agar; cultures were incubated at 20 °C and gently shaken in dim light. After 7–21 days, the developed mycelium (Ø = 5–7 mm) was washed in sterile water, frozen at –80 °C, subsequently freeze-dried, reduced to small pieces using tweezers, and stored at –80 °C.

2.4. Fungal Extracts and Food-Choice Tests

Extracts of fungal or oomycete were coated on leaf discs at a 10 : 1, 2 : 1 or 1 : 1 ratio of leaf biomass to extracted fungal or oomycete biomass (Table 1) according to Rong *et al.* (1995). The fungi and oomycete (30, 150, or 300 mg mycelium dry wt. for the 10 : 1, 2 : 1, and 1 : 1 ratios, respectively) were extracted with 4.5 mL methanol at 4 °C for 150 min; the extracts were centrifuged at 3500 × g for 10 min, and the pellet was discarded. The solvent control was 4.5 mL methanol. In addition, an aliquot of the methanol extract of *Cylindrocarpon* sp. 94–2057 was evaporated to dryness (rotary evaporator, 30 °C) and re-dissolved in the same volume of absolute ethanol; as a control, an aliquot of pure methanol was treated similarly. Fungal or oomycete lipids were extracted according to von Elert and Stampfel (2000). Dried mycelium was divided into six portions of approximately 5 mg dry wt. (10 : 1) or 25 mg dry wt. (2 : 1). Each portion of mycelium was extracted once with 8 mL methanol : methylene chloride (1 : 2, v : v), and then twice with 5 mL. Particles were removed by centrifugation (3500 × g, 10 min), and supernatants of all six fungal biomass extractions were pooled and evaporated to dryness (rotary evaporator, 30 °C). Dried samples were re-dissolved in 4.5 mL absolute ethanol. As a control, 108 mL methylene chloride:methanol (2 : 1, v : v) was treated similarly. All extracts and controls were stored at –20 °C until used. Leaves were incubated for 2 days in tap water and then autoclaved (30 min, 121 °C) prior to each experiment. Leaf discs were cut from the edges of these leaves using a

cork borer (Ø = 14 mm); care was taken to avoid leaf veins. Each leaf disc was weighed four times to obtain a wet weight with an error ≤ 0.1 mg. Prior to each weighing, the leaf discs were dipped in de-ionized water and then dabbed dry to reduce weight fluctuations. After weighing, the leaf discs were dabbed dry again and coated with 75 µL of a methanol or ethanol fungal extract, or with pure solvent (solvent control); aliquots of 25 µL were applied to each leaf disc and allowed to evaporate until dry before the next aliquot was applied. With the 10 : 1 leaf biomass:fungus/oomycete biomass ratio, 75 µl fungal or oomycete extract corresponded to 0.5 mg dry wt. of fungal/oomycete mycelium per disc. The 2 : 1 and 1 : 1 ratios corresponded to 2.5 and 5.0 mg mycelium dry wt., respectively, per disc. Leaf discs without applied solvent served as feeding controls in the assays. Food-choice assays were carried out in a grey polyethylene container (108 × 108 × 40 mm) filled with 250 mL filtered (30 µm) lake water and divided into four chambers. Each chamber contained a stone shelter of approximately 4 g. Two control chambers contained only one leaf disc. The two test chambers contained one leaf disc coated with extract and one leaf disc without extract. In solvent control assays, leaf discs coated with solvent were used instead of discs coated with extract. One *G. roeselii* individual was introduced into each container and allowed to feed for max. 48 hours. Each assay and the corresponding controls were replicated 6–11 times using different *G. roeselii* individuals. The total leaf consumption within a test container was the sum of the consumption of the two leaf discs. The consumption of the leaf disc coated with extract was normalized to the total consumption and expressed in relative values (percent). Here percent values were used, because total leaf consumption differed in each replicate, and otherwise comparison with the other experiments would not have been possible. The effect of an extract on consumption was determined by comparing the consumption in the test assays with the consumption in the control assays using t-tests. To compare different extracts, the net consumption of each extract was calculated by subtracting the mean consumption obtained in the control assays for each extract from each of the consumption values obtained in the test assays, expressed in percent. Lower values of relative consumption as for the control indicated that the extract

had a repellent effect. The net consumptions of the various extracts were compared by one-way ANOVA and Tukey HSD post-hoc test.

2.5. Repellence-Attraction Assays with Extracted Fungal Mycelia

Fungi grown in sterile leaf extract medium was washed with sterile water, extracted with methanol or methanol/methylene chloride (2 : 1, v : v), and centrifuged at 3500 × g for 10 min. The supernatant was discarded. The solvents remaining with the mycelium were evaporated using a gentle flow of N2; the mycelium was then washed with sterile water and stored in sterile water at –20 °C. Assays were carried out as described above with the following modifications. Two opposing chambers in the polyethylene container were sealed off with plastic foil. The remaining two chambers each contained a stone shelter of approximately 4 g. One *G. roeselii* individual was introduced into the container and allowed to acclimate for 45 min prior to the test. A piece (approximately 12 × 5 mm) of extracted mycelium was added to one of the chambers; a piece of mycelium that had not been extracted was placed in the other chamber. After a 5 min acclimation period, the presence of the *G. roeselii* individual in either of the chambers was recorded at 5 min intervals for a period of 60 min. The assays were repeated eight times.

2.6. Statistical Analyses

To account for the inherent high variability of behavioural responses, the level of significance was set to P = 0.1. To test for effects of a single fungal extract, leaf consumption in the test assays was compared pair-wise with leaf consumption in the control assays in t-tests. When data of a control assay were used in more than one t-test, the level of significance was adjusted sequentially according to Bonferroni (Rice 1988). The net leaf consumptions of different concentrations of extracts were compared by one-way ANOVA. Post-hoc Tukey HSD was calculated for subsequent pair-wise comparison of the tests. The data obtained with the methanol extract at a 2 : 1 leaf biomass : fungal biomass ratio and with the methylene chloride : methanol extract did not show homogeneity of

Table IV 1. Summary table of experiments assessing the effect of fungal or oomycete extracts on the consumption of leaves by *G. roeselii*. Leaf discs were coated with various concentrations of extracts. C, Czapek's solution medium; LEM, leaf extract medium.

Extract concentration (leaf biomass:fungal/ oomycete biomass)	Fungus or oomycete	Culture medium	Extraction solvent	Solvent used for application to leaf disc	Number of replicates
10:1	*Fusarium sporotrichoides* *Pythium* sp. JN 1-b *Ascomycete* sp. PV So8 *Cylindrocarpon* sp. 94-2057	C	Methanol	Methanol	6–8
2:1	*Fusarium sporotrichoides* *Pythium* sp. JN 1-b *Ascomycete* sp. PV So8 *Cylindrocarpon* sp. 94-2057	C	Methanol	Methanol	7–9
1:1	*Fusarium sporotrichoides*	C	Methanol	Methanol	7–9
10:1	*Cylindrocarpon* sp. 94-2057	C	Methanol	Ethanol	7–11
10:1	*Fusarium sporotrichoides* *Pythium* sp. JN 1-b *Ascomycete* sp. PV So8 *Cylindrocarpon* sp. 94-2057	C	Methanol:methylene chloride	Ethanol	7–9
2:1	*Fusarium sporotrichoides* *Pythium* sp. JN 1-b *Ascomycete* sp. PV So8 *Cylindrocarpon* sp. 94-2057	C	Methanol:methylene chloride	Ethanol	7–11
10:1	*Fusarium sporotrichoides* *Pythium* sp. JN 1-b *Ascomycete* sp. PV So8 *Cylindrocarpon* sp. 94-2057	LEM	Methanol	Ethanol	7-10

variances after arcsine-square-root transformation; however, when large, balanced assays with many replicates are used, a violation of the assumptions of the analyses of variance (ANOVA) may only cause minor errors in designs (Underwood 1997). All other data were used without transformation because the net consumptions showed homogeneity of variances (Underwood 1997). All ANOVAs were performed using the GLM module Basic; t-tests were carried out with the statistics and tables module of the statistical package STATISTICA 6.0 (StatSoft, Inc. 2004; Tulsa, Okla., USA).

3. Results

3.1. Food-Choice Experiments with Fungal Extracts

To determine how each of the fungal or oomycete extracts affected *G. roeselii's* consumption of leaf litter, discs of sterile black alder leaves were supplemented with extracts from a single fungus or oomycete and used in the food-choice assays (Table IV 1). A lower relative consumption than for the control was observed for the extracts of the oomycete *Pythium* sp. JN 1-b and the fungi *Ascomycete* sp. PV So8 and *Cylindrocarpon* sp. 94-2057 at a ratio of leaf biomass : fungal/oomycete biomass of 10 : 1 (Fig. IV 1a, Table IV 2), which indicated that these extracts had a repellent effect. In contrast, the consumption of the leaf disc coated with the methanol extract of *Fusarium sporotrichoides* of the same concentration did not differ from the consumption of the control leaf disc (Fig. IV 1a; Table IV 2). *F. sporotrichoides* and *Ascomycete* sp. PV So8 extracts with five-fold higher concentrations (2 : 1 leaf biomass : fungal biomass) elicited a higher repelling than the control (Fig. IV 1b, Table IV 2). However, the same concentration of the extract of the oomycete *Pythium* sp. JN 1-b and the fungus *Cylindrocarpon* sp. 94-2057 did not lead to a difference in relative consumption as compared to the control (Fig. IV 1b, Table IV 2). *F. sporotrichoides* extract with ten-fold higher concentrations (1 : 1 leaf biomass:fungal biomass) also led to a significant repellence of *G. roeselii* (Fig. IV 2a, Table IV 2), and this repellence did not differ from that of the extract with

five-fold higher concentration (one-way ANOVA; Figs. IV 1a and IV 2a, Table IV 3). We concluded that the effect of the extract concentration on consumption was strain specific. The *Cylindrocarpon* sp. 94-2057 methanol extract had a higher repellent effect on *G. roeselii* than the same extract dried and re-dissolved in ethanol (one-way ANOVA; Fig. IV 1a and Fig. IV 2b, Table IV 3), and this re-dissolved extract in ethanol was not preferred over the ethanol control (Fig. IV 2b, Table IV 2). Coating of leaf discs with methanol : methylene chloride (lipid) extracts of *F. sporotrichoides*, *Pythium* sp. JN 1-b, *Ascomycete* sp. PV So8, or *Cylindrocarpon* sp. 94-2057 at a 10 : 1 or 2 : 1 leaf biomass:fungal/oomycete biomass did not lead to differences in relative consumption by *G. roeselii* compared to the controls (Fig. IV 1c, d, Table IV 2). The methanol extracts of the fungi and the oomycete grown in leaf extract medium did not affect the relative consumption by *G. roeselii* compared to the controls (Fig. IV 1e, Table IV 2). The net consumption of leaf discs coated with methanol extracts differed from the net consumption of leaf discs coated with the corresponding methanol : methylene chloride extracts (Fig. IV 1a and Fig. IV 1c, Table IV 3). However, the net consumption of leaf discs coated with methanol extracts of *F. sporotrichoides*, *Pythium* sp. JN 1-b, *Ascomycete* sp. PV So8, and *Cylindrocarpon* sp. 94-2057 did not differ, and the net consumption of leaf discs coated with the methanol : methylene chloride extracts of these strains did not differ (Fig. IV 1a and Fig. IV 1c, Table IV 3). The net consumption of leaf discs coated with methanol differed from methanol : methylene chloride extracts at a leaf biomass:fungus/oomycete biomass of 2 : 1 (Fig. IV 1b and Fig. IV 1d, Table IV 3). The net consumption of leaf discs coated with methanol extracts of *F. sporotrichoides* at a leaf biomass : fungus/oomycete biomass of 2 : 1 was lower than that of leaf discs coated methanol : methylene chloride extracts at the same concentration. However, the net consumption of leaf discs coated with methanol extracts of *Pythium* sp. JN 1-b, *Ascomycete* sp. PV So8, or *Cylindrocarpon* sp. 94-2057 at a leaf biomass : fungus/oomycete biomass of 2 : 1 did not differ (Fig. IV 1b and Fig. IV 1d, Table IV 3).

The net consumption of leaf discs coated with methanol extracts of *Cylindrocarpon* sp. 94-2057 grown in leaf extract medium repelled much less than that grown on sucrose in Czapek's medium (Fig. IV 1a and Fig. IV 1e, Table IV 3). The net consumption of leaf discs coated with methanol extracts of the other three strains grown on leaf extract medium also repelled less than that of leaf discs coated with methanol extracts of these strains grown in Czapek's medium, but the effect of the carbon source was not as significant (Fig. IV 1a and Fig. IV 1e; Table IV 3).

3.2. Repellence-Attraction Assays with Extracted Fungal Mycelia

In arenas containing *F. sporotrichoides* mycelia, either extracted with methanol : methylene chloride or unextracted, *G. roeselii* preferentially visited the arena containing the extracted mycelium (Fig. IV 3a, Table IV 2). In all other cases, no preferences were observed between extracted and unextracted mycelia (Fig. IV 3, Table IV 2).

4. Discussion

It has repeatedly been shown that colonisation of leaf litter by fungi goes hand in hand with enhanced leaf litter consumption by shredders (reviewed by Suberkropp 1992). Shredders have furthermore been shown to differentially feed on leaves colonised by different fungal species (reviewed by Graça 2001), which suggests that fungi have a steering role in the rate of leaf litter fragmentation by shredders. However, the mechanisms behind the mediation of preference by fungi are not well understood (Graça 2001). Here we hypothesized that the mediation of preference of *G. roeselii* is due to attractants or repellents that are constituents of fungi or oomycetes.

Bärlocher and Kendrick (1973) have reported an increased consumption by *Gammarus pseudolimnaeus* of leaves covered with *Fusarium* sp. In contrast, *G. roeselii* was deterred in our study from alder leaves covered with *Fusarium*

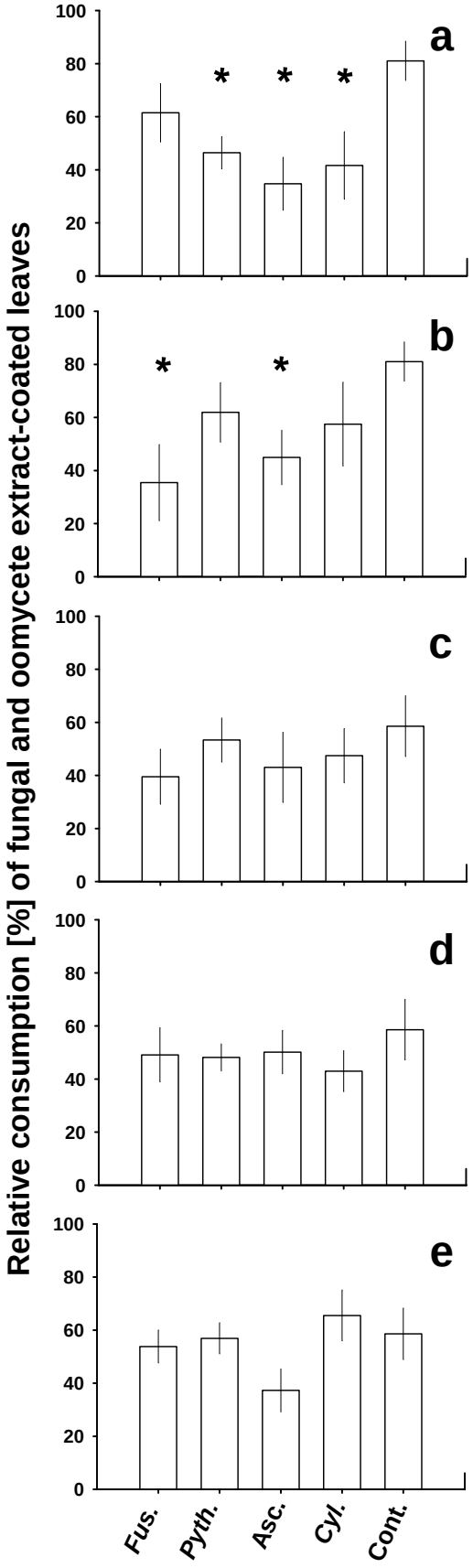

Figure IV 1. Relative consumption of leaf discs by *G. roeselii* in food-choice assays with leaf discs coated with fungal or oomycete extract and uncoated leaf disc. In control assays, one leaf disc coated with solvent and one uncoated leaf disc were offered. Consumption that was lower than in the control indicates a repellent effect. Leaf discs coated with a methanol extract at a leaf biomass: fungal/ oomycete biomass of (a) 10 : 1, and (b) 2 : 1. Leaf discs coated with a methanol : methylene chloride extract at a leaf biomass : fungal /oomycete biomass of (c) 10 : 1, and (d) 2 : 1. (e) Leaf discs coated with a methanol extract of a single fungus or oomycete grown on leaf extract medium. Values are given as mean ± *SE*.

sporotrichoides ratios of 2 : 1 and 1 : 1. This suggests a strain-specific preference or repellence for leaves covered with fungi. But *G. roeselii* was not affected by leaves coated with *F. sporotrichoides* at a leaf biomass : fungal biomass ratio of 10 : 1. A 10 : 1 ratio was also used by Rong *et al.* (1995) for other fungi, but this amount of extracted *F. sporotrichoides* biomass was apparently inadequate for an effect in our assays, although this amount of extract of the oomycete *Pythium* sp. and the fungi *Ascomycete* sp. and *Cylindrocarpon* sp. did suffice.

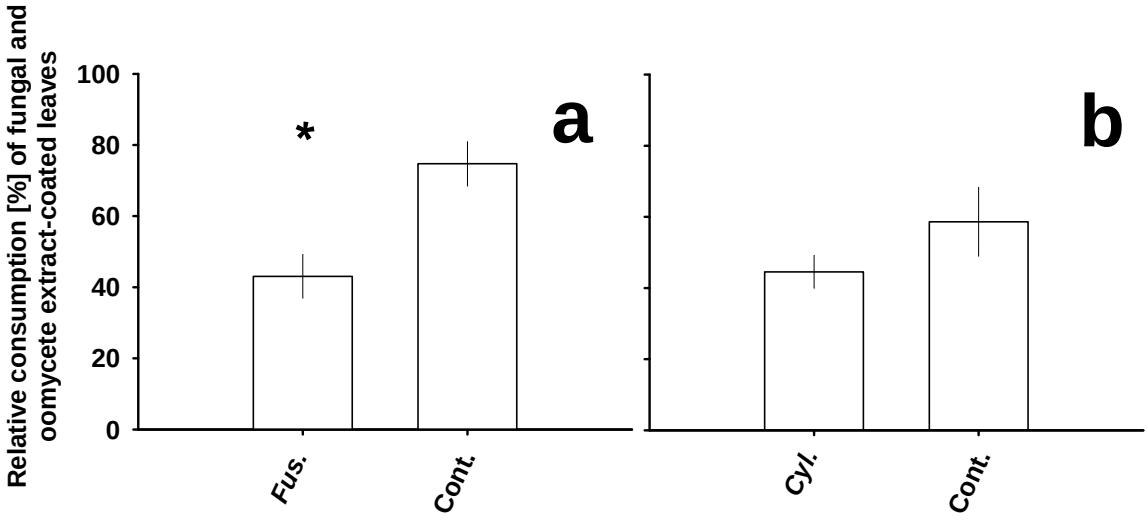

Figure IV 2. Relative consumption of *G. roeselii* in food-choice assays of leaf discs coated with (a) a methanol extract of the fungus *F. sporotrichoides* at a leaf biomass:fungal biomass ratio of 1 : 1 or (b) a methanol extract of the fungus *Cylindrocarpon* sp. at a leaf biomass : fungal biomass ratio of 2 : 1 or,dried and re-dissolved in ethanol. For details, see legend of Figure 1. Values are given as mean ±*SE* of *n* = 7–11; asterisks indicate significant differences. *Fus., Fusarium sporotrichoides*; *Cyl., Cylindrocarpon* sp. 94-2057; Cont., control.

Gessner *et al.* (2007) have reported that fungal biomass associated with decomposing plant material ranged from 1.8% to 20% of the total litter mass. Hence, a fungal extract at a leaf biomass : fungal biomass ratio of 10:1 is in the range of the natural biomass of fungi on decaying leaves. At least with *F. sporotrichoides,* the repellence of *G. roeselii* clearly was concentration dependent, which shows that a certain threshold concentration is required to reproduce the same repellence observed by fungal mycelium on leaves.

Table IV 2. Results of pair-wise comparisons (*t*-test) of consumption by *G. roeselii* of leaf discs uncoated or coated with various concentrations of fungal or oomycete extracts. A 10 : 1 ratio of leaf biomass to fungal or oomycete biomass corresponds to an extract of 0.5 mg fungal or oomycete dry wt. In the test assays, an uncoated leaf disc and a leaf disc coated with extract were offered. In control assays, an uncoated leaf disc and a leaf disc coated with solvent were offered. When a control assay was used for multiple comparisons, this was taken into account by sequential Bonferroni correction (RICE, 1989). Significant differences after Bonferroni correction are given in bold.

Solvent and extract concentration (leaf biomass:fungal/oomycete biomass)	**Fungus or oomycete**	**t-test (P-value)**	**Figure**
Methanol 10:1	*Fusarium sporotrichoides*	0.281	1a
	Pythium sp. JN 1-b	**0.011**	
	Ascomycete sp. PV So8	**0.005**	
	Cylindrocarpon sp. 94-2057	**0.036**	
Methanol 2:1	*Fusarium sporotrichoides*	**0.018**	1b
	Pythium sp. JN 1-b	0.293	
	Ascomycete sp. PV So8	**0.024**	
	Cylindrocarpon sp. 94-2057	0.202	
Methanol 1:1	*Fusarium sporotrichoides*	**0.005**	2a
Methanol 10:1, re-dissolved in ethanol	*Cylindrocarpon* sp. 94-2057	0.529	2b
Methanol:methylene chloride 10:1	*Fusarium sporotrichoides*	0.201	1c
	Pythium sp. JN 1-b	0.693	
	Ascomycete sp. PV So8	0.047	
	Cylindrocarpon sp. 94-2057	0.354	
Methanol:methylene chloride 2:1	*Fusarium sporotrichoides*	0.478	1d
	Pythium sp. JN 1-b	0.326	
	Ascomycete sp. PV So8	0.468	
	Cylindrocarpon sp. 94-2057	0.178	
Methanol 10:1 (grown in leaf extract medium)	*Fusarium sporotrichoides*	0.688	1e
	Pythium sp. JN 1-b	0.889	
	Ascomycete sp. PV So8	0.135	
	Cylindrocarpon sp. 94-2057	0.649	
Methanol:methylene chloride extract vs. uncoated leaf disc	*Fusarium sporotrichoides*	**0.051**	3a
Methanol extract vs. uncoated leaf disc	*Fusarium sporotrichoides*	0.907	3b
Methanol:methylene chloride extract vs. uncoated leaf disc	*Ascomycete* sp. PV So8	0.957	3c
Methanol extract vs. uncoated leaf disc	*Ascomycete* sp. PV So8	0.278	3d

Indeed, it remains to be determined how changes in the relative content of such repellents caused by, *e.g.,* environmental factors, might affect the food preference of *G. roeselii* such that the preference is no longer a fixed characteristic for fungal strains, but rather the result of their growing conditions.
It has previously been shown that amphipods prefer food covered with a single fungus (Graça *et al.* 1993b; Rong *et al.* 1995). Our observation that methanol extracts of the fungi *Cylindrocarpon* sp. 94-2057 and *Ascomycete* sp. PV So8 did not attract *G. roeselii* but rather repelled the gammarid, leads us to conclude that compounds other than those extracted by methanol mediate the preference. The food selection of aquatic insects is influenced by their nutritional needs because their food often contains low amounts of available

Table IV 3. Results of pair-wise comparisons of net consumption by *G. roeselii* of leaf discs coated with fungal or oomycete extracts. The two types of extracts obtained from the three fungi and one oomycete were analyzed by one-way ANOVA with subsequent Tukey HSD test. Significant effects are given in bold.

Compared extracts	Fungus or oomycete	One-way ANOVA	Tukey HSD (*P*–value)
10:1 in methanol:methylene chloride vs. 10:1 in methanol	*Fusarium sporotrichoides*	$F_{1;60}$ = 2.34	0.848
	Pythium sp. JN 1-b	P = **0.021**	0.834
	Ascomycete sp. PV So8		0.505
	Cylindrocarpon sp. 94-2057		0.934
2:1 in methanol:methylene chloride vs. 2:1 in methanol	*Fusarium sporotrichoides*	$F_{1;60}$ = 2.34	**0.018**
	Pythium sp. JN 1-b	P = **0.017**	0.947
	Ascomycete sp. PV So8		0.793
	Cylindrocarpon sp. 94-2057		0.999
10:1 in methanol vs. 10:1 in methanol (grown in LEM)	*Fusarium sporotrichoides*	$F_{1;68}$ = 3.21	0.997
	Pythium sp. JN 1-b	P = **0.003**	0.533
	Ascomycete sp. PV So8		0.854
	Cylindrocarpon sp. 94-2057		**0.054**
2:1 in methanol vs. 1:1 in methanol	*Fusarium sporotrichoides*	$F_{1;16}$ = 0.48	–
		P = 0.499	
10:1 in methanol vs. 10:1 in methanol (dried and re-dissolved in ethanol)	*Cylindrocarpon* sp. 94-2057	$F_{1;15}$ = 3.36	**0.087**
		P = **0.087**	

proteins, carbohydrates, and lipids (Cargill *et al.* 1985a). Cargill *et al.* (1985a) showed that five *Trichoptera* species preferred leaf discs coated with pure unsaturated 18- and 20-carbon fatty acids, which suggests that lipids from aquatic hyphomycetes are important intermediates in the energy transfer from detritus to detritivores. Lipid extracts of three aquatic hyphomycetes led to the same patterns of leaf consumption by *Gammarus tigrinus* as leaf litter inoculated with those fungi (Rong *et al.* 1995). We therefore tested whether

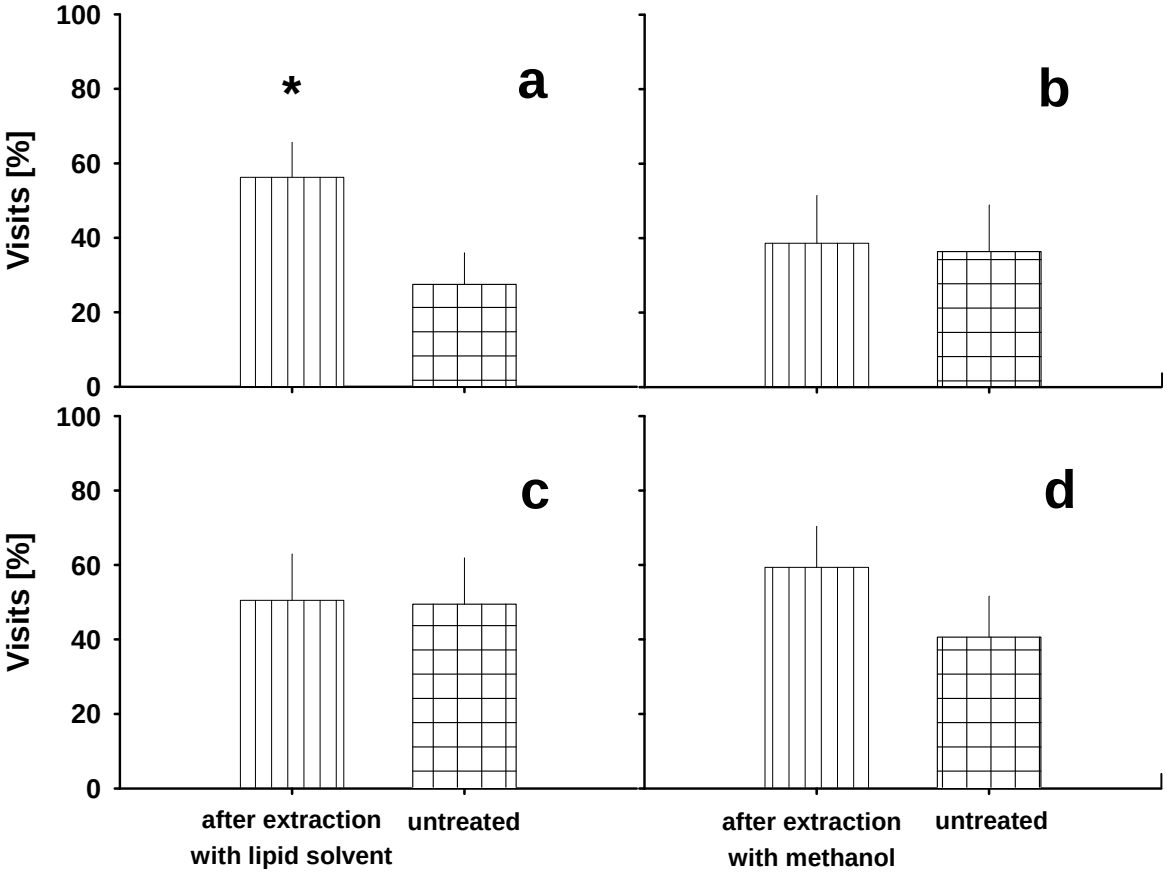

Figure IV 3. Relative frequency of visits by *G. roeselii* to untreated fungal mycelium versus fungal mycelium extracted with methanol or methanol : methylene chloride prior to the experiment. The frequency of visits was determined by behavioural choice assays. a) and b): Relative frequency of visits to mycelia of *Fusarium sporotrichoides*; and (c and d) relative frequency of visits to mycelia of *Ascomycete* sp. PV So8. Mean ±*SE* (*n* = 8). Percent values of visits to solvent-extracted mycelia were subjected to pair-wise comparison with visits to unextracted mycelia. Asterisks indicate significant differences after sequential Bonferroni adjustment. CH2Cl2 : CH3OH, methanol : methylene chloride.

G. roeselii preferred leaf discs coated with methanol : methylene chloride-extracted lipids from our three fungi and one oomycete. However, in our experiments, none of the lipid-containing extracts attracted *G. roeselii*, which suggests that lipids from these organisms were not responsible for the preference of these food organisms by *G. roeselii*.

Although oomycetes have been reported to be present on conditioned leaf litter in streams (Bärlocher 1991a; Dix and Webster 1995), their consumption by amphipods has only been investigated in one study (Fano *et al.* 1982). Here, we investigated the effect of oomycetes on the consumption of leaf litter by *G. roeselii*. The methanol extract of the oomycete *Pythium* sp. JN 1-b coated on leaf discs clearly repelled *G. roeselii*. The presence of compounds that reduce the consumption of *Pythium* sp. JN 1-b points toward possible negative interactions between oomycetes and shredders. In our experiments, we assumed that the amount of oomycete biomass, which was in an early decomposition phase (Bärlocher 1992a), is comparable to the amount of fungal biomass on decaying leaves, *i.e.,* in the range of 1.8% to 20% of the total litter mass (Gessner *et al.* 2007); however, the oomycete biomass on conditioned leaf litter has never been determined. In future experiments, this could be determined by real-time PCR, as demonstrated by Kernaghan *et al.* (2008) for a *Pythium* species in soil.

In all of our experiments, we allowed the organic solvents in the extracts to completely evaporate prior to testing in our assays. However, whereas the methanol extract of *Cylindrocarpon* sp. 94-2057 coated on leaf discs repelled *G. roeselii*, the same extract dried and redissolved in ethanol and applied to leaf discs did not. We therefore recommend using methanol rather than ethanol in such experiments to obtain the maximum response of *G. roeselii*.

As in earlier studies (Cargill *et al.* 1985a; Cargill *et al.* 1985b; Rong *et al.* 1995), we cultured the oomycete and the fungi in a synthetic medium with sucrose prior to extraction. The finding that none of the extracts were attractive to *G. roeselii* led us to postulate that growth on sucrose in a synthetic medium might result in the absence of attractants. We therefore grew the fungi and oomycete in leaf extract medium, which contains a more natural carbon source. Extracts

of *Cylindrocarpon* sp. 94-2057 and *Ascomycete* sp. PV So8 grown on leaf extract medium did not repel *G. roeselii,* which indicated that the type of carbon source affects the biological activity of the repellent compounds in these two fungi.

Clearly a laboratory approach has its limitations, when extrapolating the results to natural conditions. Here we determined food preference in multiple choice experiments, and this artificial situation probably does not tell the whole story about the effect of food types on consumers' life history traits in the field. Preference is not necessarily correlated with fitness of the consumer, and therefore our experiments show only a snapshot of the possible scenario in nature. So far, only non-selective organic solvents have been applied, and the chemical nature of the biologically active compounds has not been elucidated, so that the dynamics of repellence in a fluctuating environment remain to be investigated.

In conclusion, biologically active compounds that affected the consumption of leaf litter by *G. roeselii* were extracted from fungi and an oomycete by methanol, but not by methanol : methylene chloride, which extracts lipids. This could indicate that polar rather than nonpolar chemical cues mediate food consumption by *G. roeselii*. Since growth of the fungi and oomycete on leaf extract medium instead of sucrose-containing synthetic medium reduced the repellent effects of the extracts, non-natural carbon sources probably affect the feeding of shredders in ways that do not necessarily reflect the natural situation. The presence of fungal compounds that also repell *G. roeselii* suggests that the relative ratios of repellents and attractants might determine consumption of fungi by *G. roeselii*. Since changes in environmental conditions could lead to varying ratios of repellents and attractants, the food preference might not be a fixed characteristic of fungal strains, but might rather be modulated by changing environmental conditions.

5. Acknowledgements

We thank Patrick Fink, René Gergs and Karsten Rinke for their help with statistics and Sonja Raub for her comments and improving the English of an earlier version of the manuscript. We acknowledge Karen A. Brune for editing the English language of the manuscript. This study was supported by a grant of the German Research Foundation (DFG) to E. v. E. within the Collaborative Research Centre SFB 454 *Littoral Zone of Lake Constance*.

Chapter V

Effects of leaf litter and its fungal colonisation on the diet of *Limnomysis benedeni* (Crustacea: Mysida)

Christine Aßmann, Eric von Elert and René Gergs

Hydrobiologia, 636 (1), 439-447

Abstract

The strong invasive freshwater mysid *Limnomysis benedeni*, a detritivorous–herbivorous feeder, has a preference for small food particles, but also feeds on leaf litter. Here, we tested whether leaf litter consumption by *L. benedeni* depends on the tree species and leaf conditioning (two types of physical and biological leaf conditioning). At the physical leaf conditioning, *L. benedeni* was fed with shortly leached or extensively leached leaves of five tree species in laboratory food assays. The mysid consumed shortly leached leaves of Copper Beech, Lombardy Poplar, Common Oak, and especially White Willow, and did not feed on shortly leached Black Alder leaves. The consumption of extensively leached leaves by *L. benedeni* did not depend on the tree species. Overall, 74% of the variation of the leaf consumption by *L. benedeni* was explained by the significant interaction of the factors carbon content and polyphenol content of the leaves, caused the feeding strategy of *L. benedeni*. For the biological leaf conditioning, the mysids consumed to a high degree naturally conditioned leaves, followed by leaves colonised by one of three fungi, but oomycete-colonised leaf litter and autoclaved leaves were consumed at similar low levels. Our results indicate that *L. benedeni* feeds on different types of conditioned leaves to different extents, and therefore may affect leaf litter degradation in many invaded freshwaters.

Keywords: Invasive species, fungi, oomycetes, leaf litter conditioning, decomposition

1. Introduction

The introduction, establishment, and distribution of invasive species are promoted by the increasing global trade (reviewed by Chandra and Gerhardt 2008). The aquatic invaders in Europe in recent decades originated mainly from the Ponto-Caspian region (Bij de Vaate *et al.* 2002). Not only Amphipoda and Mollusca, but also Mysida have successfully invaded the upper regions of the Danube and Rhine river systems (Kurek 1992; Wittmann 2007). In particular, the mysid *Limnomysis benedeni* has spread rapidly through the Main-Danube canal into the Central Europe (Bij de Vaate *et al.* 2002). In the Rhine River system, the mysid has been recently recorded up to the pre-alpine Lake Constance (Fritz *et al.* 2006; Wittmann 2007). *L. benedeni* lives in close association with the benthos in lotic and lentic habitats (Wittmann 1995; Wittmann *et al.* 1999; Gergs *et al.* 2008). Most studies of the invasive mysid species is derived from field observations and descriptive studies in running water habitats (e.g. Wittmann 2007; Wittmann and Ariani 2009). *L. benedeni* is a detritivorous–herbivorous feeder with a preference for smaller food particles (Dediu 1966). Gergs *et al.* (2008) classified *L. benedeni* as omnivore–herbivore because the mysid fed in their laboratory study on green algae, epilithon, chironomids, and conditioned Black Alder leaves. The authors assumed that the food source was mainly fungi and bacteria associated with the biofilm on the leaves.

Allochthonous organic matter from the riparian vegetation is important for the food web in many aquatic systems with leaves as the largest portion of this organic input and thus constitutes a major allochthonous energy resource (Abelho 2001). In these ecosystems the transfer of fixed carbon to herbivores and detritivores represents a major pathway of energy flow (Reshi and Tyub 2007). Microorganisms and invertebrates integrate the energy and nutrients of leaves into secondary production (reviewed by Graça 2001). Aquatic shredders promote/ benefit via their leaf processing (ingestion, fragmentation, and formation of faeces) the energy flow from the allochthonous organic material (e.g., leaf litter) to the detrital food web (Gessner *et al.* 1999). Shredder and all

other by shredder-feeding promoted invertebrates (e.g., filterers and collectors), provide in this way food for higher trophic levels (e.g. fish; (Dahl and Greenberg 1996), creating an allochthonous material-based food chain in freshwaters. If typical shredder species (Cummins and Klug 1979) are missing, the decomposition by microorganisms is often slower and the litter persist longer (reviewed by Webster and Benfield 1986) and (Reshi and Tyub 2007)), indicating (Kaushik and Hynes 1971) that the leaf-consuming organisms perform a key step in the allochthonous material-based food chains.

Shredders like amphipods and trichopterans, feeding on coarse particulate organic material, discriminate between leaf species, differing in their nutritional content (Kaushik and Hynes 1971) and presence of deterring plant secondary components (e.g., polyphenols; Graça 2001) of certain species. Consequently, the different leaf species should differ with respect to their chemical contents for leaf litter consuming invertebrates.

When leaf litter falls into an aquatic system, organic and inorganic soluble substances are physically lost and this is called ‘‘leaching,’’ and leaves are rapidly colonised by bacteria and fungi (Abelho 2001); and this entire process is referred as conditioning. In the following manuscript the terms ‘‘leached’’ (physical conditioning) and ‘‘conditioned’’ (biological conditioning) leaves were used. Naturally conditioned leaves are more palatable for leaf consuming invertebrates and are preferred over unconditioned leaves (Graça *et al.* 1994b; Kiran 1996; Abelho 2001). Not only naturally leaf conditioning, but also the colonisation of the leaves by various fungal species leads to preferential consumption by shredders (Graça 2001).

Most leaf litter decomposition studies have focused on trichopterans, amphipods, and isopods, which were regarded as typical shredders (e.g., Arsuffi and Suberkropp 1988; Graça *et al.* 2001a; van Dokkum *et al.* 2002). However, when these typical shredder groups are of less importance or occur in very low numbers, the importance of other invertebrates as shredders can increase (e.g., as found in a Moroccan river in which gastropods act as shredders in the absence of plecoptera and trichoptera; reviewed by Graça 2001). Since *L. benedeni* has recently become an abundant member of the

benthic community in many freshwaters, we hypothesized that this mysid affects the leaf litter decomposition, thereby affecting those ecosystems. We tested this hypothesis in standardized laboratory experiments by feeding L. benedeni leaf litter of five different tree species physically conditioned by shortly leaching or by extensively leaching (autoclaving), simulating the initial and late stages of physical leaching during decomposition. We also tested the feeding activity on leaf litter naturally conditioned or by inoculation with one of three fungal strains or one oomycete strain.

2. Methods

2.1. Origin of mysids and leaf litter

Limnomysis benedeni was collected in the littoral zone of the eastern part of Lake Constance near the confluence of the Rhine River via kick-sampling with a dip net (mesh size 200 µm) at a water depth of approximately 0.5 m. For laboratory feeding experiments, the mysids were kept in a climate chamber at 15°C with a diurnal light rhythm of 12 h:12 h (day: night). Tanks for maintenance were kept in a flow-through system with water from Lake Constance, and the animals were fed with greenstuff tablets (Tetra PlecoMin®). In all experiments, adult individuals (>6 mm) of both sexes were used. Black Alder (*Alnus glutinosa* (L.) Gaertner) and Copper Beech (*Fagus sylvatica* L.) leaves were collected with a nylon net mounted above ground in autumn 2005. Lombardy Poplar (*Populus nigra italica*), Common Oak (*Quercus robur* L.), and White Willow (*Salix alba* L.) freshly fallen leaves were collected from the ground in autumn 2003. All leaves were air-dried and stored at room temperature in the dark until used.

2.2 Conditioning of leaf litter

Leaf litter of all five tree species was physically conditioned to simulate the initial and late stages of physical leaching during decomposition. The primarily leaching takes part within the first 24 and 48 h (Gessner and Schwörbel 1989;

Bärlocher 1991a; Bärlocher 2005b). Shortly leached leaf litter was prepared by soaking leaves in filtered (30 µm) lake water for 3.5 h. In order to simulate a extensively leaching of the leaves, leaf litter was autoclaved (taken in notice that autoclaving of leaves has an effect on the leaf chemistry). The leaves were incubated in tap water for 1 day and then autoclaved for 30 min at 121°C.

Black Alder leaf litter was naturally conditioned by exposing approximately 2 g dry wt. of briefly (30 min) pre-soaked alder leaves in cages to the littoral of Upper Lake Constance at 0.4 m water depth in contact to the sediment for 3 weeks.

The effect of fungi and oomycetes on the attractiveness of leaf litter was investigated by inoculating sterile alder leaf litter with mycelium of one of the following strains: the fungi *Fusarium sporotrichoides* (GenBank® accession number: EU637901), Ascomycete sp. PV So8 (EU669082), *Microdochium* sp. PV So2 (EU637902), and the oomycete *Pythium* sp. JN 1-b (EU637903). Leaves were shortly soaked in tap water and then autoclaved (30 min, 121°C). Single leaves were placed on a cellulose filter (Ø = 70 mm) saturated with a mineral solution (0.01 g $MgSO_4 \bullet 7H_2O$, 0.01 g $CaCl_2 \bullet 2H_2O$, 0.01 g KNO_3, 0.01 g K_2HPO_4, and 0.5 g 2-[N-morpholino] ethanesulfonic acid per liter, pH 6.0; Duarte *et al.* 2006) under sterile conditions in a petri dish (Ø = 90 mm). Each leaf was inoculated with a pinhead-sized piece of mycelium of an isolate (grown in malt extract agar, in the center of the leaf). Petri dishes with the fungi or oomycete inoculated leaves were incubated at 20°C with a diurnal light rhythm of 12 h:12 h (day: night). Leaves were used for the feeding assays after 14–21 days of incubation, when the whole surface was covered by mycelia of the fungus or oomycete (‘‘fully conditioned phase’’; Bärlocher 1985; determined visually).

2.3. Feeding experiments

The attractiveness of the leaves of five different treespecies as a food source for *L. benedeni* was tested using the leaves of each species, physically conditioned by shortly leaching or extensively leaching (autoclaving), in a no-choice experiment following the protocol of Gergs *et al* (2008), the control

lacked food. The effect of the type of leaf conditioning was tested using shortly leached, autoclaved, naturally conditioned, and fungus/oomycete-colonised alder leaves. For each test, 20 mysids were starved for 24 h in an aerated tank containing 4 l of 30 µm-filtered lake water. One food source was then added ad libitum. After 24 h, the feeding activity of each individual was classified as either ‘‘empty gut’’ or ‘‘filled gut’’ using a stereomicroscope (Zeiss Stemi 2000-C, Jena, Germany). Each test was replicated 10 times.

2.4. Measured leaf parameters

Subsamples from each experiment were taken and frozen at -80°C. The samples were then freeze dried, homogenized with mortar and pestle, and stored at -80°C. The particulate organic carbon and nitrogen contents were determined with an NCS-2500 analyzer (Carlo Erba Instruments, Milano, Italy). For determination of the particulate phosphorus content, samples were digested with 10% potassium peroxidisulfate/ 1.5% sodium hydroxide at 121°C for 60 min; soluble reactive phosphorous was determined using the molybdate-ascorbic acid method (Greenberg *et al.* 1985) and a Technicon autoanalyzer. The polyphenol content was measured photometrically (Bärlocher and Graça 2005). All measurements were replicated five times.

2.5 Statistical Analyses

For each of the replicates in the feeding experiments, the percentage of living animals with a filled gut at the end of the experiment was calculated. If within a replicate more than five individuals had died, the replicate was excluded from the statistical analysis. Those values used were arcsine($\sqrt{x}$) transformed; homogeneity of the variances was tested using Levene’s test. One-way analyses of variance (ANOVA) was used to test for differences in feeding on the leaf litter of the different tree species, both shortly leached and extensively leached (autoclaved), followed by posthoc comparison with Tukey’s HSD test. We compared the two physical leaf conditionings, shortly leached and autoclaved, with a two-way ANOVA, followed by post-hoc comparison with Tukey’s HSD test. Transformed data did not show homogeneity of variances,

but this violation of the ANOVA assumptions causes only minor errors in designs with large, balanced experiments, i.e., with more than about five treatments, with more than about six replicates (Underwood 1997). Differences in the feeding activity between naturally conditioned leaves, leaf litter inoculated with a single strain of a fungus or oomycete were analyzed using one-way ANOVA with Tukey's HSD post-hoc test for pair-wise comparison of treatments. The dependence of feeding of *L. benedeni* on the chemical composition of the leaves of different tree species was calculated by regression analyses. All variances and regressions were analyzed using the statistical package SPSS (version 15.0/2006; SPSS, Inc., Chicago, IL, USA). Statistical differences between the physical leaf conditioning (shortly leached and autoclaved) and tree species (for each conditioning type) were analyzed using PERMANOVA (version 1.6), which analyses multivariate data on the basis of any distance measure, according to any linear ANOVA model, using permutations (Anderson 2001; McArdle and Anderson 2001). Significance level of pairwise comparisons between treatments was adjusted to sequential Bonferroni correction (Rice 1988).

3. Results

The carbon, nitrogen, phosphorous, and polyphenol contents differed between the five tree species and the two physical conditioning types (shortly leached and extensively leached; Table V 1). Furthermore, the two physical leaf conditionings each led to significant tree species x conditioning term (PERMANOVA, P = 0.001) and differences in the chemical composition between the two physical conditioning types were significant for the leaves of all tree species (Table V 1). When the tree species and the type of physical conditioning were combined in the analysis, the feeding of *L. benedeni* was significantly negatively related to the carbon content and to the interaction of the carbon content with the polyphenol content of the different tree species (Fig. V 1, Table V 2). The carbon content explained 42% of the variation in the feeding

of the mysids, whereas the interaction of the carbon content with the polyphenol content explained 74% of the variation. Polyphenol content alone, the sum of the polyphenol and carbon contents, and all other measured leaf parameters of the tree species had no significant explanatory power for the feeding of *L. benedeni* (Table V 2).

We estimated the attractiveness of differently physical conditioned leaf litter for

Table V 1. PERMANOVA results comparing chemical composition (C, N, P, and polyphenol content) of tree species, conditioning type and their interaction. Tree species with the same capital letters are not significant different as the results of pair-wise tests with sequential Bonferroni correction. P values for each tree species in the interaction term are given for the comparison between conditioning types. Significant results are indicated by an asterisk.

Factor	F-value	d.f.	P-value	Willow	Poplar	Beech	Oak	Alder
Tree species	271.97	4	0.001*	AB	C	A	A	B
Conditioning	842.60	1	0.001*					
Tree species × conditioning	15.06	4	0.001*	p = 0.013*	p = 0.008*	p = 0.008*	p = 0.005*	p = 0.012*

L. benedeni by determining the percentage of individuals feeding on one food source, as indicated by gut filling. We found differences in feeding on shortly leached leaf litter of the five tree species (ANOVA, P<0.001; Table V 3, Fig. V 2a). Significantly more *L. benedeni* individuals fed on shortly leached leaf litter of all tree species except alder in comparison to the control. Most individuals fed on willow leaves, followed by poplar, beech, and oak leaves. Significantly more *L. benedeni* individuals fed on extensively leached (autoclaved) leaf litter of all tree species in comparison to the control, and the mysids fed on the leaves of the different tree species to the same extent (ANOVA, P<0.001; Table V 3, Fig. V 2b). The overall feeding of *L. benedeni* on leaf litter conditioned by shortly leaching did not differ from feeding on leaf litter conditioned by autoclaving (Table V 3, physical conditioning was significant, two-way-ANOVA). Specifically,

significantly more *L. benedeni* individuals fed on autoclaved alder leaves than on shortly leached alder leaves and on shortly leached willow leaves than on autoclaved willow leaves (Table V 3, Fig. V 2a, b). The type of physical conditioning (shortly leaching and autoclaving) of the leaf litter of each of the other tree species did not affect the feeding activity of *L. benedeni* (Fig. V 2). In our experiments comparing the influences of biological and physical conditioning of alder leaf litter on feeding, the feeding activity of *L. benedeni* was significantly higher on naturally conditioned leaf litter (exposed in the littoral) than on autoclaved leaf litter (Fig. V 3; one-way ANOVA, $F = 15.075$, $P<0.001$). Leaf litter colonised with the fungus *F. sporotrichoides*, *Microdochium* sp. PV So2, or Ascomycete sp. PV So8 was consumed by *L. benedeni* at an intermediate level between that of autoclaved leaf litter and naturally conditioned leaf litter.

Fewer *L. benedeni* individuals fed on leaf litter colonised with the oomycete *Pythium* sp. JN 1-b than on naturally conditioned leaves; oomycete-colonised leaf litter and autoclaved leaves were consumed at similar levels. In all experiments, the number of *L. benedeni* individuals offered food that had full guts was significantly higher than the number in the control in which food was not offered (Fig. V 3).

Table V 2. Multiple linear regressions with percentage of individuals with a filled gut as a function of the carbon and polyphenol contents of leaf litter. Significant regressions are indicated by an asterisk.

Factor	R^2	F-value	P-value
Carbon	0.42	5.81	0.042 *
Polyphenol	0.08	0.69	0.430
Carbon + Polyphenol	0.43	2.70	0.140
Carbon*Polyphenol	0.74	5.64	0.035 *

In all feeding experiments, in the control (*L. benedeni* kept without food), only 20% of the individuals had a filled gut. This might be explained by cannibalism, which is known for *P. rubra* in the North Adriatic Sea (Wittmann 1985), or perhaps the gut was not emptied completely, as is known for *Gammarus pseudolimnaeus* (Bärlocher and Kendrick 1975).

4. Discussion

Leaf litter in aquatic systems, as an important allochthonous energy resource, is processed in three phases: (1) leaching, (2) microbial colonisation, and (3) invertebrate feeding and physical abrasion (Abelho 2001; Gessner *et al.* 2003). Effected by these phases of processing the invasive mysid *L. benedeni* fed on shortly leached or extensively leached (autoclaved) leaf litter of several tree species in our experiments. Naturally conditioned alder leaves (exposed in the littoral) were more palatable to *L. benedeni* than autoclaved leaves and the palatability of alder leaf litter colonised by one fungal species lay in between. These results indicate that *L. benedeni* might affect leaf litter decomposition in invaded aquatic freshwater systems.

Table V 3. Analysis of variance (ANOVA) results comparing the effects of tree species and conditioning type, leached and extensively leached (autoclaved) leaf litter, on the feeding activity of *L. benedeni*. d.f., degrees of freedom. Significant results are indicated by an asterisk.

Factor	Conditioning	F-value	d.f.	P-value
Conditioning	Both	0.552	1	0.459
Conditioning	Leached	40.086	5	< 0.001*
Conditioning	Autoclaved	9.845	5	< 0.001*
Tree species	Both	14.678	4	< 0.001*
Tree species × conditioning	Both	17.096	4	< 0.001*

For most studies, in which detritivore insects (mostly trichopterans) or crustaceans (amphipods and isopods) have been tested in their feeding preference, the consumption rate was the estimated variable (reviewed by Suberkropp 1992 and Graça 2001). Here, we used a different methodological approach (from Gergs *et al.* 2008) to investigate the difference of leaf types and their conditioning status for the palatability to *L. benedeni* because the mysids mode of consumption differs from shredding insects or crustaceans. The structure of the feeding appendages of mysids contain paired mandibles, each with cutting, grinding, and macerating regions (Mauchline 1980), resulting in a consumption of *L. benedeni* mainly of food items with a small particle size, such as pythoplankton, epilithon, and detritus (Gergs *et al.* 2008).

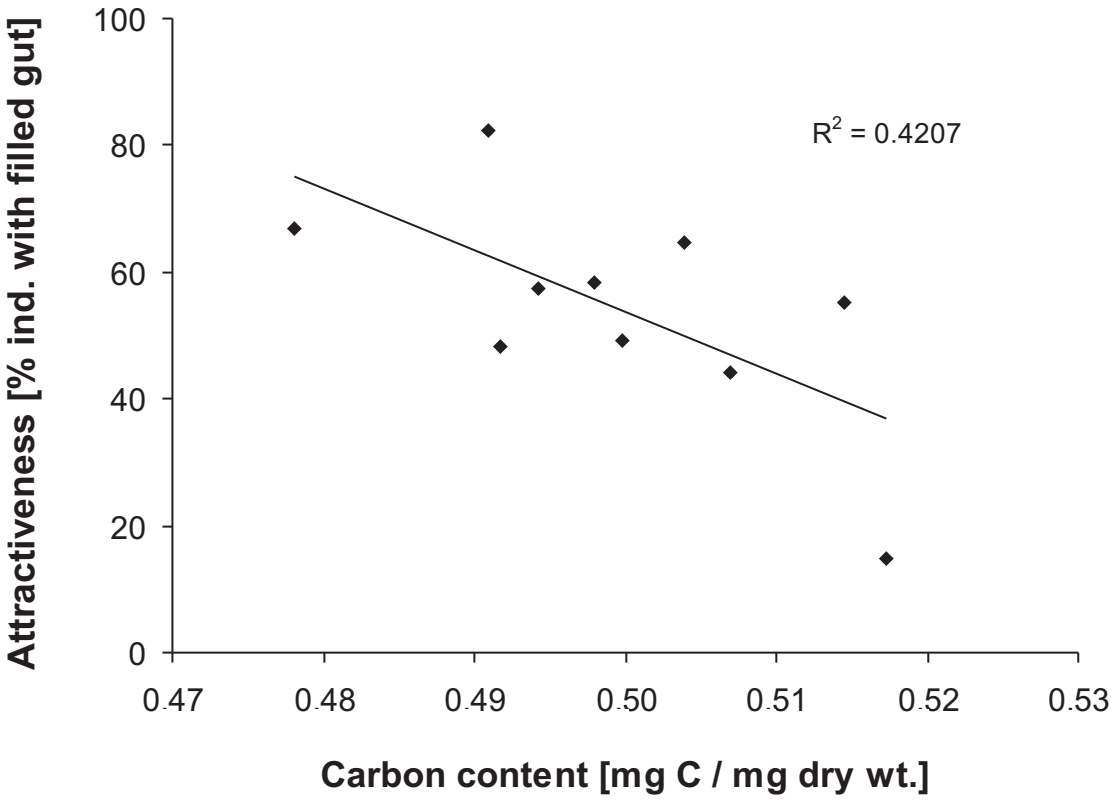

Figure V 1. Attractiveness of leaf litter for *L. benedeni* as a function of the carbon content (mg C/mg dry wt.) of the different leaf types. Attractiveness was determined as the percentage of *L. benedeni* individuals with filled guts. The line represents the linear regression (R^2 = 0.42, P = 0.042).

Therefore, the mysids presumably scrape with their feeding appendages on the leaf surface.

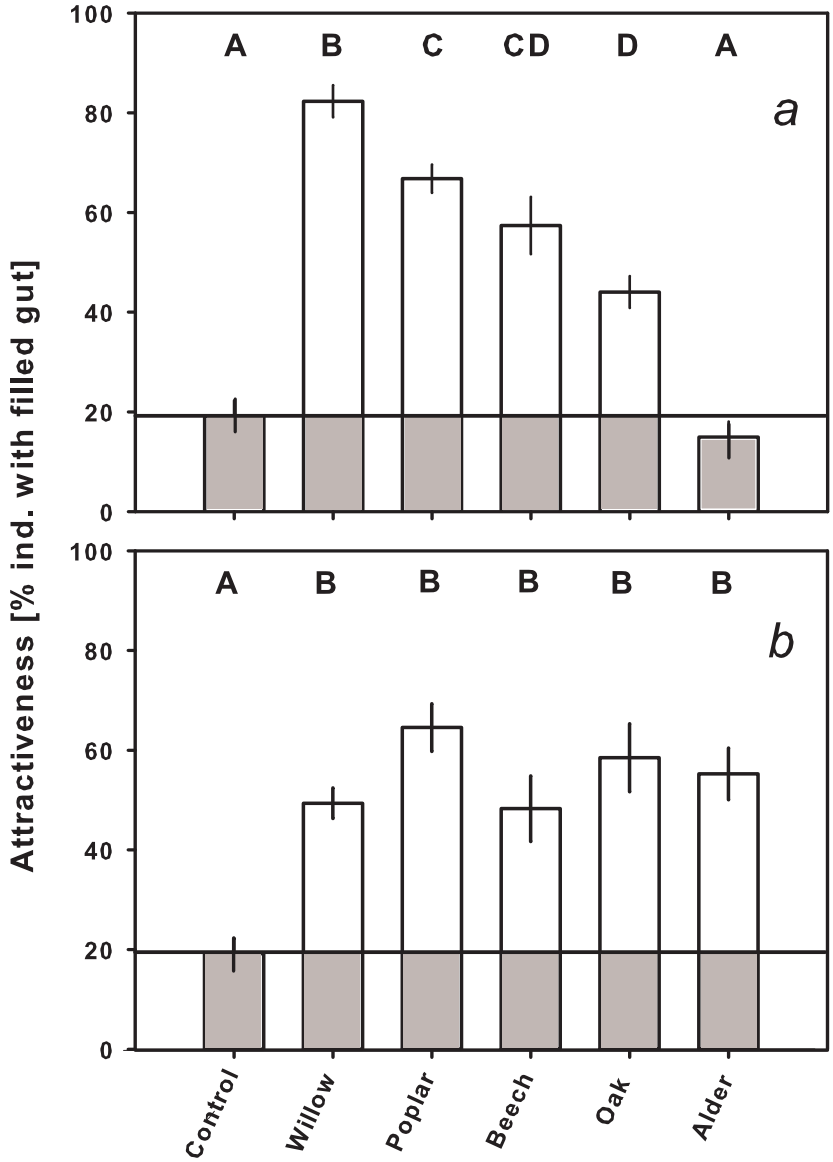

Figure V 2 Attractiveness of leaf litter of five different tree species as food sources for *L. benedeni*. Each type of leaf litter was (a) shortly leached for 3.5 h or (b) autoclaved. The dependent variable indicates the percentage of individuals feeding on a food source. The horizontal line denotes the control without food. Letters on the bars indicate homogeneous subgroups detected using ANOVA ($P < 0.001$) with a Tukey-HSD post-hoc test. Mean ± SE (n = 10).

In our experiments, *L. benedeni* fed on shortly leached leaf litter of poplar, beech, oak, and especially willow, but not of alder. This significant difference in

feeding on leaf litter of different tree species no longer occurred when *L. benedeni* was fed extensively leached (autoclaved) leaves. These results indicate that under natural conditions, *L. benedeni* consume leaf litter of all tree species in the later stages of decomposition. Shredders select leaves as food according to the concentration of nutrients and secondary components for chemical plant defense (reviewed by Graça 2001). Consistent feeding activity of *L. benedeni* was related to the chemical content of leaves of the different tree species. Carbon content of the leaves had a negative effect on the mysids feeding activity and explained 42% of the variation in the feeding activity (Table V 2).

Although polyphenols are known to repel grazing invertebrates (Pennings *et al.* 2000; Graça and Bärlocher 2005), the feeding activity of L. benedeni was not correlated to the polyphenol content of the leaves offered in our study. However, the interaction of the factors carbon content and polyphenol content explained 74% of the attractiveness of the leaf litter (Table V 2). This can be explained as follows: leaf compounds with high carbon content are, for example, waxes and cutins on the leaf surface and lignocellulose, which provide a physical barrier against the attack by decomposers (Webster and Benfield 1986). By scraping on the leaf surface, feeding of *L. benedeni* is hindered by the waxes and cutin of the cuticula and the lignocellulose structure of the leaf. In addition, release of polyphenols on the leaf surface increase the deterrence reaction. Owing to their high nitrogen content, alder leaves are preferred by many shredders over leaves of other tree species (Kaushik and Hynes 1971; Schmidt 1996), but the feeding activity of *L. benedeni* was not correlated with the nitrogen content of the leaves, maybe because the mysids were not able to reach the nitrogen-containing leaf components of the whole leaf matrix directly.

The biological conditioning by micro-organisms increases the palatability of leaf litter for invertebrate shredders (Graça *et al.* 1993b; Graça *et al.* 1994b; Abelho 2001). Likewise, the feeding activity of *L. benedeni* on alder leaves increased stepwise from shortly leached to extensively leached to naturally conditioned leaves. The palatability of naturally conditioned leaves is mainly caused by fungi; shredders, such as amphipods, for example, often prefer leaf litter

colonised with a single fungal strain over unconditioned leaves (Arsuffi and Suberkropp 1989). In our study, the three fungal species colonising the leaves elicited a feeding activity that was intermediate to that of littoral-exposed and autoclaved leaf litter. Leaves colonised with an oomycete had no effect on the feeding of the mysid, whereas the amphipod *Gammarus roeselii* was repelled by the extract of the same oomycete on leaves (Aßmann and von Elert 2009).

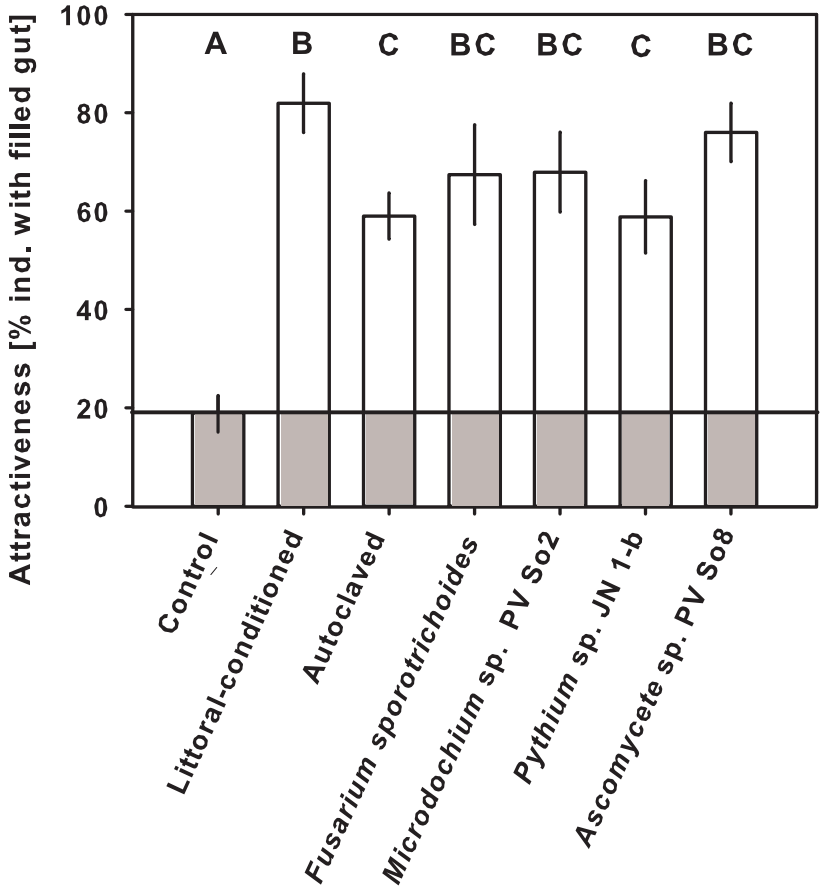

Figure V 3 Attractiveness of differently conditioned Black Alder leaves as a food source for *L. benedeni*. Alder leaves were conditioned naturally in the littoral zone, biologically by colonising with one of three fungal species or one oomycete species, or physically by autoclaving. Attractiveness indicates the percentage of individuals feeding on the food source indicated. The horizontal line denotes the control without food. Letters on the bars indicate homogeneous subgroups after ANOVA ($P < 0.001$) with a Tukey-HSD post-hoc test. Mean ± SE (n = 10).

These findings suggest that a natural assemblage of fungi or oomycetes make littoral exposed leaves more attractive to *L. benedeni* than colonisation by single species. Different combinations of fungal and oomycete species colonising leaves should be tested in future studies.

Activity and abundance of shredders account for the breakdown rate of leaf litter in freshwaters (Merritt *et al.* 1984; Kok and van der Velde 1994). Our study clearly shows that the invasive *L. benedeni*, with a more scraping than shredding feeding strategy, can be assigned to the leaf consumers of the benthic community. Because of its recent spread into many European freshwaters and occurrence in high abundances (Wittmann 1995; Bij de Vaate *et al.* 2002; Wittmann 2007; Wittmann and Ariani 2009), we assume that *L. benedeni* have an important stake on leaf litter decomposition in freshwater habitats. *L. benedeni* could substitute a missing shredder and/ or facilitate allochthonous leaf litter degradation and therefore increase the energy flow to higher trophic levels like predatory invertebrates and fish. This might not only result into an increase of the benthic secondary production, but probably also into a higher species diversity of an ecosystem invaded by this mysid species.

5. Acknowledgements

We thank Almut Hanselmann for providing *Limnomysis benedeni*; Christine Gebauer and Petra Merkel for technical assistance with analyses of leaf litter carbon, nitrogen, and phosphorous; Isabelle Eisele for assistance with the no-choice assays; and John Hesselschwerdt for providing beech leaf litter. We acknowledge Karen A. Brune for editing the English language of the manuscript. This study was supported by the German Research Foundation (DFG) within the Collaborative Research Centre SFB 454 ‘‘Littoral Zone of Lake Constance.’’

Chapter VI

General Discussion and Perspectives

General Discussion

Leaf litter from riparian vegetation represents a large input in freshwater systems and a major allochthonous energy resource for aquatic food webs (Webster and Benfield 1986; Abelho 2001). During decomposition, this coarse detritus (dead organic material; (Lampert and Sommer 1997) transfers, allochthonous carbon to aquatic microorganisms, herbivores and detritivores (Webster and Benfield 1986; Abelho 2001), which integrate the energy and the nutrients of leaf litter into secondary production (reviewed by Graça 2001).

Leaf litter decomposition has mainly been investigated in streams and rivers (reviewed by Abelho 2001), whereas this process has been documented in lakes in only a few reports (Federle and Vestal 1982; Federle *et al.* 1982; Sabetta *et al.* 2000). It has been estimated that detritus may provide up to two thirds of the total organic input into lakes (Webster and Benfield 1986). Littoral communities receive relatively high allochthonous leaf litter inputs along the shoreline. Shredders constitute a major fraction of these highly diverse communities (Bohman and Tranvik 2001; Mörtl 2004), however, the importance of the shredder *Gammarus* spp. as consumer of leaf litter in lakes is poorly understood (Sabetta *et al.* 2000; van Dokkum *et al.* 2002). It is therefore important to improve our understanding of the process of leaf conditioning and its effects on the benthic community in lakes. Therefore this thesis examined effects of i) the presence, and ii) identity of microbial colonisers on leaf litter consumption by *Gammarus roeselii* and shows that the leaf consumption of *G. roeselii* is mediated by physical and microbial processes. It could be demonstrated that single fungal and oomycete strains alter leaf parameters in a strain-specific manner and this results in a strain-specific consumption by *G. roeselii*. This suggests that the rate of leaf litter decomposition in the littoral may be affected by the relative abundance of different strains of fungi and oomycetes on the leaf litter.

Earlier it was assumed that fungal colonisers of leaf litter affect the consumption by shredders via attractants and repellents. In this thesis it could be shown that

the type of carbon source, on which fungi and oomycete were grown, affects the impact of fungal secondary products on the preference of gammarids.
Furthermore it was demonstrated that the mysid, *L. benedeni*, that only recently invaded Lake Constance, functions as a leaf decomposer and feeds on littoral conditioned leaves as it is known from other invertebrate shredders (Graça 2001), which suggests that *L. benedeni* could enhance litter decomposition in the littoral zone.

During leaf litter decomposition in freshwaters three phases of processing can be distinguished: (i) leaching, (ii) microbial colonisation and maceration, and (iii) fragmentation by invertebrates and physical forces (Abelho 2001). During these phases the physical structure of leaf litter and its chemical composition change, and it has been suggested that the increased preference of shredders for conditioned leaves is caused by these changes (Abelho 2001; Graça and Zimmer 2005). Corroborating earlier findings (Kaushik and Hynes 1971) consumption by *G. roeselii* was shown to increase with ongoing conditioning time of leaf litter (**chapters 2** and **3)**. Accordingly, in the two exposition experiments (**chapter 2** and **3**) the chemical, biological and physical parameters of littoral-exposed leaves changed markedly during exposition. In both experiments, the content of polyphenols, which have been shown to repel grazers (Rosset *et al.* 1982; Pennings *et al.* 2000; Abelho 2001; Graça and Bärlocher 2005), in the alder leaves decreased during exposition. The repellence by polyphenols is corroborated by the finding that the consumption of the amphipod was negatively correlated to the polyphenol content of the leaves (**chapters 2** and **3**). Surprisingly, the feeding activity of the mysid *L. benedeni*, a very recent invader of Lake Constance, was not correlated to the polyphenol content of the leaves. However, the interaction of the factors carbon content and polyphenol content explained 74% of the attractiveness of the leaf litter for the mysid. This suggested that feeding of *L. benedeni* is hindered by the waxes and cutin of the cuticula and by the lignocellulose structure of the leaf (Webster and Benfield 1986), and it may be hypothesized that the leaching out of

polyphenols on the very surface of the leaves, further increases the deterrence of the leaf litter to *L. benedeni*.

It has been postulated that the leaf litter contents of nitrogen and protein increase during decomposition, leading to enhanced preference by shredders (Bärlocher 1985; Suberkropp 1992). However, the results of the two exposition experiments described in this thesis, (**chapters 2** and **3**), do not support this notion. The N content as well as the protein content of the littoral-exposed leaves decreased over the first exposure period, and therefore it was hypothesised that proteins and other N-containing compounds were leached out of the leaves, and that the leaf colonising micro-organisms (fungi and oomycetes) could not compensate for this loss.

The results of the consumption and the parameters of the exposed leaves (in both experiments, **chapters 2** and **3**) point to the development of microbial biomass on the littoral-exposed leaves (Baldy and Gessner 1997; Hieber and Gessner 2002). This suggests that the preference by shredder organisms cannot easily be predicted from parameters such as protein content, but depends on the fungal species that colonise the leaf litter.

The primary decomposers of forest, soil and freshwater ecosystems are microorganisms (reviewed by Berg and McClaugherty 2003 and Abelho 2001), i.e. bacteria and mainly fungi. With the enzymatic capabilities of fungi cellulose, hemicellulose and different lignins can be degraded (Gessner *et al.* 2010). Therefore fungi are regarded as the most important microbial component on decaying leaves and leaf-associated fungal biomass accounts for up to 16% of total detrital litter mass in freshwaters (reviewed by Abelho 2001 and Gessner *et al.* 2007).

Several fungal taxa of the class of Hyphomycetes and phyla of Basidiomycota, Chytridiomycota, Zygomycota and Ascomycota were found on decaying leaves in freshwaters (Bärlocher *et al.* 1992; Bärlocher *et al.* 2008). But mostly aquatic hyphomycetes were considered as dominant species of the fungal communities on leaves (Bärlocher *et al.* 1992; Bärlocher 2009). In **chapters 2** and **3** together 9 ascomycete strains were found. Astonishingly, no aquatic hyphyomycete

species were identified here; this suggests that in lentic freshwaters aquatic fungi others than aquatic hyphomycetes, play a role in leaf litter degradation.

Ergosterol, a major cell membrane component of fungi, is frequently used to determine metabolically active eumycotic fungal biomass (Gessner and Newell 1997; Gessner 2005). In the exposition experiments carried out in this study, the ergosterol content of the littoral exposed leaves increased during exposure until day 28 (**chapter 2**) and 36 (**chapter 3**), indicating an increasing fungal biomass on leaf litter in both experiments. However, it is well known that ergosterol is absent from the cell membranes of oomycetes (Weete 1989) and that oomycete species are well represented in the early stages of decomposition of leaf litter (Bärlocher 1992a; Wielgoss *et al.* 2009). Oomycetes are well known saprophytes or plant parasites in soils and water and have been reported as pathogens of crustaceans (Nechwatal *et al.* 2005; Nechwatal and Mendgen 2006; Hirsch *et al.* 2008). In freshwater habitats oomycetes are frequently found (Nechwatal and Mendgen 2009) and are assumed to impact detritus degradation (Brasier *et al.* 2003). In the two exposition studies we isolated four different oomycete species that represent approximately one third of the total number of identified strains. For the first time it was demonstrated that oomycetes can positively affect *Gammarus*' consumption (**chapter 3**). The effects of oomycetes on leaf litter parameters were similar to those of other fungal strains. Due to the presence of oomycetes in the early stages of conditioning (Bärlocher 1992a; Wielgoss *et al.* 2009), these results suggest that oomycetes have a greater impact on the early leaf litter decomposition in freshwaters than hitherto assumed.

The two leaf exposition experiments were carried out in two different seasons, the first in summer (**chapter 2**) and the second in autumn (**chapter 3**). Six ascomycete strains were isolated in summer and another three in autumn. Though considerable taxonomic similarity between summer and autumn fungal isolates was expected, only a single fungal species of the genus *Cylindrocarpon* was isolated in summer and in autumn. However, all the oomycete species isolated in summer and autumn belonged to the genus *Pythium* (**chapters 2** and **3**). These pronounced taxonomic differences suggest that the fungal and

oomycete assemblages differ seasonally. It has been shown that the absence of summer species of aquatic hyphomycetes during the cold season was due to effects of temperature on growth (Suberkropp 1984). However, it remains unclear to what degree the isolates established here reflect the *in-situ* genetic diversity of the community of fungi or oomycetes.

I conducted three laboratory experiments with the aim to assess the impact of colonisation of leaves by the different strains of fungi and oomycetes on the leaf chemistry and the consumption by invertebrates, (**chapters 2**, **3** and **5**).

Consumption rates by *G. roeselii* varied significantly between leaves colonised with different fungi and oomycetes. Isolates on leaves were preferred (65%) over controls, and none was rejected. These results confirm the results of earlier studies, in which the preferences of amphipod shredders were shown to vary depending on the individual fungal species (Bärlocher and Kendrick 1973; Graça *et al.* 1993b; Graça *et al.* 1994b).

All leaf parameters were affected by colonisation with single isolates and the magnitude of the effects showed considerable variation depending on the type of isolate, demonstrating that the effects of microbial colonisers are strongly strain-specific. Bärlocher and Kendrick (1973) showed that the protein content of leaves inoculated with single fungi increased in comparison to the initial content and varied depending on the fungal species. In accordance with these results, the protein content of the single isolates on leaves was highly variable in this study and other leaf parameters also showed considerable variation within the single-strain experiment.

In the exposition experiment, leaf toughness and polyphenol content were important leaf parameters determining consumption. This result is in agreement with the finding of Graça (2001) that leaf toughness and content of secondary compounds are main factors determining shredder preference. Although leaf toughness may represent an important parameter in the field, it was not significant in the single isolates experiment, due to the fact that leaves were used that had been softened by autoclaving. Instead, in the single isolates experiment polyphenol and protein levels were major determinants of the consumption rate of *Gammarus*, which suggests that fungi and oomycetes

might indirectly steer consumption by altering the leaf litter content of protein and polyphenols. As leaf toughness declines substantially during the early phase of conditioning, it is reasonable to assume that the effects observed with autoclaved leaves are relevant for the later phase of conditioning. Hence, alteration of the content of polyphenol and protein by fungi and oomycetes may have a steering effect on the consumption of *Gammarus* in particular during later stages of conditioning in the field.

The effect of microbial colonisation on the protein and nitrogen content of the leaf litter was clearly visible in the single isolates experiment (**chapter 3**) which indicated that leaching constitutes a major loss of nitrogen and protein that is significant over several weeks in the field. Other studies of leaf conditioning observed increasing nitrogen and protein contents during microbial colonisation coinciding with increasing shredder consumption (reviewed by Suberkropp 1992). Though in the single isolates experiment I also found that protein content increased with microbial colonisation, the relationship to shredder consumption was less clear as the protein content of the colonised leaves was negatively correlated with consumption. These results are not in accordance with the finding that palatability for leaves colonised with fungi is steered by the nutrient concentration (Bärlocher 1985). In my experiments increasing protein content let to decreased consumption (e.g. *Fusarium sporotrichoides*, **chapter 3**), which suggests that high protein contents coincides with deterring fungal or oomycete biomass on leaves for some of the single strains. It is most likely, that other leaf characteristics than protein or nitrogen content determine the attractiveness of leaf material, and the production of these substances differs between strains of fungi and oomycetes.

As described in **chapters 2** and **3**, biological conditioning by micro-organisms increases the palatability of leaf litter for invertebrate shredders (Graça *et al.* 1993b; Graça *et al.* 1994b; Abelho 2001). The palatability of these leaves is mainly caused by fungi (see also **chapter 2**) and also by oomycetes (see **3rd chapter**) and shredders, such as amphipods, often prefer leaf litter colonised with a single fungal strain over unconditioned leaves (Arsuffi and Suberkropp 1989). Despite this, three fungal species on leaves elicited an intermediate

feeding activity by *L. benedeni* (**chapter 5**), compared to that of littoral-exposed and autoclaved leaf litter. This suggests a preferential feeding of *L. benedeni* on leaves colonised with a variety of aquatic fungi (**chapters 2** and **3**). *L. benedeni* feeds unselectively on the different microbial colonisers on decaying leaf litter. This might indicate that regardless of which conditioned leaf litter, is probably ingested by the mysid and that could facilitate leaf processing in the littoral zone of Lake Constance. It can be hypothesized that this unselective feeding of *L. benedeni* will lead to a general facilitation of leaf processing in the littoral invertebrate community.

In this thesis (**chapter 2** and **3**) it was shown that shredders discriminate between leaves colonised by different fungal and oomycete species corroborating results of others (e.g. Arsuffi and Suberkropp 1989; Graça *et al.* 1993b; Graça *et al.* 1994b), resulting in a strain-specific consumption of the colonised leaf litter. I further demonstrated that fungal and oomycete colonisers actively alter the chemical and physical parameters of the leaves. However, the mechanisms behind the mediation of preference by fungi are not well understood (Graça 2001). Hence I hypothesized, that the mediation of preference of *G. roeselii* is due to attractants or repellents that are constituents of fungi or oomycetes (**chapter 4**), an aspect that had been addressed in only a few studies before (Cargill *et al.* 1985a; Cargill *et al.* 1985b; Rong *et al.* 1995).
In **chapter 2** I showed that *Cylindrocarpon* sp. 94-2057 and *Ascomycete* sp. PV So8 on leaf litter were preferred and that the oomycete *Pythium* sp. JN 1-b was neither attractive nor repellent to *G. roeselii.* In contrast to these results, in the **4th chapter** it was observed, that methanol extracts of these two fungi and the oomycete clearly repelled the amphipod, which suggested that compounds others than those extracted by methanol mediated the preference of *G. roeselii*.
The quality of food for grazers and shredders does not only depend on the elemental ratios, but also on other constituents such as proteins, carbohydrates, and lipids. Food selection of aquatic insects is mostly influenced by their nutritional needs because their food often contains low amounts of proteins, carbohydrates, and lipids (reviewed by Cargill *et al.* 1985a). Cargill *et al.*

(1985a) assumed that lipids from aquatic fungi are important intermediates in the energy transfer form dead organic material to detritivores, and lipid extracts of three aquatic hyphomycetes led to the same patterns of leaf consumption by *Gammarus tigrinus* as leaf litter inoculated with those fungi (Rong *et al.* 1995). However, in this thesis fungal and oomycete lipid extracts on leaves did not elicit preference by *G. roeselii*, which strongly indicated that the preference of these food organisms by *G. roeselii* was not mediated by their lipids.

It is common practice to use algal, bacterial and fungal cultures grown on synthetic media in experimental setups (Cargill *et al.* 1985a; Cargill *et al.* 1985b; Rong *et al.* 1995). Therefore in the experiments of **chapter 4**, oomycetes and the fungi were also grown in a synthetic medium with sucrose prior to extraction. None of the extracts were attractive to *G. roeselii*. This let to the assumption that growth on sucrose in a synthetic medium might result in the absence of attractants, therefore subsequently the fungi and oomycete were grown in leaf extract medium (more natural carbon source). In contrast to the methanol extracts of *Cylindrocarpon* sp. 94-2057 and *Ascomycete* sp. PV So8, the extracts of these two fungi grown on leaf extract medium did not repel *G. roeselii,* which indicated that the type of carbon source affects the biological activity of the repellent compounds in these two fungi.

In addition to defence against invertebrate shredders and grazers (Bärlocher 1980); successful competition with bacteria and other fungi is a major determinant for the fitness of aquatic fungi and oomycetes. It can be hypothesized that these competitive relationships may have led to the production of antibiotics (Mille-Lindblom *et al.* 2006a). This type of interactions between bacteria and fungi or oomycetes interaction was not investigated in this thesis.

Only recently Lake Constance was invaded by a few benthic invertebrate species (e.g. Werner and Mörtl 2004; Rey *et al.* 2005), and one of the most recent invaders was *L. benedeni* (Fritz *et al.* 2006); of which the impacts for the lake community are currently unknown. In this thesis I showed that *L. benedeni*, formerly known as detritivorous-herbivorous with a preference for smaller food

particles (e.g. pythoplankton, epilithon, and detritus; Dediu 1966; Gergs *et al.* 2008), fed on abscised leaf litter. Due to the morphology of its feeding appendages (Mauchline 1980) the mode of consumption of *L. benedeni* differs from that of shredders in such that the mysid scrapes the biofilm from the leaf surface.

L. benedeni was found to feed on shortly leached as well as on extensively leached leaf litter of several tree species (**chapter 5**). Naturally conditioned leaves were more palatable to *L. benedeni* than autoclaved leaves, which confirmed earlier studies (Abelho 2001; Graça 2001).

In **chapter 5** I demonstrated that the invasive mysid *L. benedeni* with its scraping feeding strategy, can be assigned to the leaf consumers of the benthic community of Lake Constance. It is assumed that the mysid might facilitate allochthonous leaf litter degradation in Lake Constance, which may result in an increase in secondary production.

Perspectives

Leaf litter decomposition by invertebrates is well studied in running waters. This thesis is one of only a few studies that consider leaf litter decomposition in the littoral zone of a large pre-alpine lake, and it therefore provides only a little snapshot on leaf litter degradation in a large lake. For a better understanding of the processes of leaf litter degradation in the littoral and its possible impacts on the whole lake ecosystem further investigations are needed.

Here only a small selection of fungi and oomycetes colonising leaf litter in the littoral habitat were investigated. Therefore the diversity of fungi and oomycetes in Lake Constance requires further investigations. This could be done by a leaf litter exposition time series and in which the community on the decomposing leaves could be assessed on the level of PCR products obtained with fungal and oomycete specific primers and subsequent analysis with denaturing gradient gel electrophoresis (DGGE) or terminal restriction fragment length

polymorphism (T-RFLP). These types of analyse should indicate the diversity of the fungal and oomycete communities on leaf litter and their changes over time and would not ignore fungi and oomycetes that may be present as dormant or non-reproducing mycelia.

In this study it was shown for the first time that aquatic oomycetes on leaf litter were fed upon and preferred by a littoral amphipod shredder. Ergosterol is a widely used proxy for living fungal biomass that does not detect oomycetes as they do not contain ergosterol. Because of the absence of a determinable parameter, the function of oomycetes on leaf decomposition is not well known, and thus requires further investigation. One possibility to determine the living oomycete biomass on leaf litter is via real-time PCR, which has been used previously to determine oomycete biomass in terrestrial soils (Kernaghan *et al.* 2008). This approach will help elucidate the importance of oomycetes for leaf litter decomposition in lakes.

From a chemical point of view the identity of substances from aquatic fungi and oomycetes that mediate shredder preference remains unclear. Other solvents could be applied, and bioassay-guided analyses of the fungal and oomycete extracts could elucidate the chemical identity of the biologically active compounds.

The Lake Constance shore line is strongly anthropogenically affected, and the question arises of how the increasing number of neophytal leaves is degraded in the lake littoral and if these leaves are suitable food compared to the native leaf tree species.

To investigate the impacts on the littoral and lake ecosystem and to estimate possible effects on leaf litter decomposition of the successful invasion of *L. benedeni*, data should be collected in several field samplings over a longer period. In order to assess the impact of *L. benedeni* on leaf litter decomposition, an enclosure leaf exposition study should be done, in which degradation rates due to this invasive mysid should be determined.

Leaf litter decomposition is a well studied field in running water and terrestrial habitats, but it remains unclear if all decomposition processes in littoral ecosystems are equal to those of the two other ecosystems. Here in my thesis

only a few points of the wide field of microbial and invertebrate leaf litter decomposition could be adressed and there are many open questions which must be answered in the allochthonous energy pathway in relation to the littoral and entire lake ecosystem.

Literature cited

Abdelzaher HMA, Ichitani T, Elnaghy MA. (1994) A technique for isolating bacteria-free *Pythium* spp. from pond water. *Mycoscience*, **35**, 433-434.

Abelho M. (2001) From litterfall to breakdown in streams: a review. *The Scientific World Journal*, **1**, 656-680.

Anderson MJ. (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, **26**, 32-46.

Arsuffi TL, Suberkropp K. (1988) Effects of fungal mycelia and enzymatically degraded leaves on feeding and performance of caddisfly (Trichoptera) larvae. *Journal of the North American Benthological Society*, **7**, 205-211.

Arsuffi TL, Suberkropp K. (1989) Selective feeding by shredders on leaf-colonizing stream fungi: comparison of macroinvertebrate taxa. *Oecologia*, **79**, 30-37.

Aßmann C, Nechwatal J, Rinke K, von Elert E. (in press) The impact of axenic strains of fungi and oomycetes on the preference of *Gammarus roeselii* for leaf litter. *Fundamental and Applied Limnology*.

Aßmann C, von Elert E. (2009) The impact of fungal extracts on leaf litter on the food preference of *Gammarus roeselii*. *International Review of Hydrobiology*, **94**, 484-496.

Aßmann C, von Elert E, Gergs R. (2009) Effects of leaf litter and its fungal colonization on the diet of *Limnomysis benedeni* (Crustacea: Mysida). *Hydrobiologia*, **636**, 439-447.

Baerlocher MO. (2005) Total protein. In: *Methods to study litter decomposition: a practical guide*, 61-68, Springer, Dordrecht.

Baldy V, Gessner MO. (1997) Towards a budget of leaf litter decomposition in a first-order woodland stream. *Comptes Rendus de L'Academie des Sciences - Serie Iii, Sciences de la Vie*, **320**, 747-758.

Baldy V, Gessner MO, Chauvet E. (1995) Bacteria, fungi and the breakdown of leaf litter in large river. *Oikos*, **74**, 93-102.

Bärlocher F. (2007) Decomposition and fungal community structure in aquatic environments. In: *Manual of Environmental Microbiology*, 469-478, ASM Press, Washington.

Bärlocher F. (1992b) Effects of drying and freezing autumn leaves on leaching and colonization by aquatic hyphomycetes. *Freshwater Biology*, **28**, 1-7.

Bärlocher F. (2005a) A primer for statistical analysis. In: *Methods to study litter decomposition: a practical guide*, 305-312, Springer, Dordrecht.

Bärlocher F. (2005b) Leaching. In: *Methods to study litter decomposition: a practical guide*, 33-36, Springer, Dordrecht.

Bärlocher F. (1985) The role of fungi in the nutrition of stream invertebrates. *Botanical Journal of the Linnean Society*, **91**, 83-94.

Bärlocher F. (1980) Leaf-eating invertebrates as competitors of aquatic hyphomycetes. *Oecologia*, **47**, 303-306.

Bärlocher F. (2009) Reproduction and dispersal in aquatic hyphomycetes. *Mycoscience*, **50**, 3-8.

Bärlocher F. (1992a) Community Organisation. In: *The ecology of aquatic hyphomycetes.*, 38-76, Springer Verlag, Heidelberg & New York.

Bärlocher F. (1991a) Fungal colonization of fresh and dried leaves in the River Teign (Devon, England). *Nova Hedwigia*, **52**, 349-357.

Bärlocher F. (1991b) Intraspecific hyphal interactions among aquatic hyphomycetes. *Mycologia*, **83**, 82-88.

Bärlocher F, Canhoto C, Graça MAS. (1995) Fungal colonization of alder and eucalypt leaves in two streams in Central Portugal. *Hydrobiologia*, **133**, 457-470.

Bärlocher F, Chamier AC, Chandrashekar KR, Jones EBG, Kaveriappa KM, Moss ST, Read SJ, Shearer CA, Sridhar KR, Suberkropp K, Webster J. (1992) *The ecology of aquatic hyphomycetes*, Springer Verlag, Heidelberg & New York.

Bärlocher F, Graça MAS. (2005) Total phenolics. In: *Methods to study litter decomposition: a practical guide*, 97-100, Springer, Dordrecht.

Bärlocher F, Kendrick B. (1973) Fungi and food preferences of *Gammarus pseudolimnaeus*. *Archiv Für Hydrobiologie.*, **72**, 501-516.

Bärlocher F, Kendrick B. (1975) Assimilation efficiency of *Gammarus pseudolimnaeus* feeding on fungal mycelium or autumn-shed leaves. *Oikos*, **26**, 55-59.

Bärlocher F, Seena S, Wilson KP, Williams DD. (2008) Raised water temperature lowers diversity of hyporheic aquatic hyphomycetes. *Freshwater Biology*, **53**, 368-379.

Baumgärtner D, Mörtl M, Rothhaupt KO. (2008) Effects of water-depth and water-level fluctuations on the macroinvertebrate community structure in the littoral zone of Lake Constance. *Hydrobiologia*, **613**, 97-107.

Berg B, McClaugherty C. (2003) *Plant litter. Decomposition, humus formation, carbon sequestration*, Springer.

Bhat TK, Singh B, Sharma OP. (1998) Microbial degradation of tannins – A current perspective. *Biodegradation*, **9**, 343-357.

Bij de Vaate A, Jazdzewski K, Ketelaars HAM, Gollasch S, van der Velde G. (2002) Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe. *Canadian Journal of Fisheries & Aquatic Sciences*, **59**, 1159-1174.

Blair JM, Parmelee RW, Beare MH. (1990) Decay rates, nitrogen fluxes, and decomposers communities of single- and mixed-species foliar litter. *Ecology*, **71**, 1976-1985.

Bohman IM, Tranvik LJ. (2001) The effects of shredding invertebrates on the transfer of organic carbon from littoral leaf litter to water-column bacteria. *Aquatic Ecology*, **35**, 43-50.

Boulton AJ, Boon PI. (1991) A review of methodology used to measure leaf litter decomposition in lotic environments time to turn over an old leaf. *Australian Journal of Marine & Freshwater Research*, **42**, 1-44.

Boye P. (2003) Neozoen. In: *Biologische Invasionen: Neophyten und Neozoen in Mitteleuropa*, 264-282, Ulmer Verlag, Stuttgart (Hohenheim).

Brasier CM, Cooke DEL, Duncan JM, Hansen EM. (2003) Multiple new phenotypic taxa from trees and riparian ecosystems in *Phytophthora gonapodyides-P*. megasperma ITS Clade 6, which tend to be high-temperature tolerant and either inbreeding or sterile. *Mycolgical Research*, **107**, 277-290.

Canhoto C, Graça MAS. (1996) Decomposition of Eucalyptus globulus leaves and three native leaf species (*Alnus glutinosa*, *Castanea sativa* and *Quercus faginea*) in a Portuguese low order stream. *Hydrobiologia*, **333**, 79-85.

Cargill ASII, Cummins KW, Hanson BJ, Lowry RR. (1985a) The role of lipids as feeding stimulants for shredding aquatic insects. *Freshwater Biology*, **15**, 455-464.

Cargill ASII, Cummins KW, Hanson BJ, Lowry RR. (1985b) The role of lipids fungi and temperature in the nutrition of a shredder Caddisfly *Clistoronia magnifica*. *Freshwater Invertebrate Biology*, **4**, 64-78.

Chamier AC, Dixon PA, Archer SA. (1984) The spatial distribution of fungi on decomposing alder leaves in a freshwater stream. *Oecologia*, **64**, 92-103.

Chandra S, Gerhardt A. (2008) Invasive species in aquatic ecosystems: issue of global concern. *Aquatic Invasions*, **3**, 1-2.

Chergui H, Pattee E. (1993) Fungal and invertebrate colonization of *Salix* fresh and dry leaves in a Moroccan river system. *Archiv Für Hydrobiologie.*, **127**, 57-72.

Cross WF, Benstead JP, Frost PC, Thomas SA. (2005) Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. *Freshwater Biology*, **50**, 1895-1912.

Cummins KW, Klug MJ. (1979) Feeding ecology of stream invertebrates. *Annual Review of Ecology and Systematics*, **10**, 147-172.

Dahl J, Greenberg L. (1996) Impact on stream benthic prey by benthic vs. drift-feeding predators: a meta-analysis. *Oikos*, **77**, 177-181.

Dediu II. (1966) Répartition et caractéristique écoloque des Mycides des bassins des rivièrs Dniestr et Pruth. *Revue roumaine de Biologie / Serie de Zoologie*, **11**, 239.

Dix NJ, Webster J. (1995) Aquatic fungi. In: *Fungal Ecology*, 225-265, Chapman & Hall, London.

Duarte S, Pascoal C, Cássio F, Bärlocher F. (2006) Aquatic hyphomycete diversity and identity affect leaf litter decomposition in microcosms. *Oecologia*, **147**, 658-666.

Edwards CA. (1974) Macroarthropods. In: *Biology of plant litter decomposition*, 533-554, Academic Press, New York.

Fano AE, Rossi L, Basset A. (1982) Fungi in the diet of 3 invertebrate species. *Bollettino di Zoologia*, **49**, 99-106.

Federle TW, McKinley VL, Vestal JR. (1982) Physical determinants of microbial colonization and decomposition of plant litter in an Arctic Lake. *Microbial Ecology*, **8**, 127-138.

Federle TW, Vestal JR. (1982) Evidence of microbial succession on decaying leaf litter in an arctic lake. *Canadian Journal of Microbiology*, **28**, 686-695.

Fink P, Peters L, von Elert E. (2006) Stoichiometric mismatch between littoral invertebrates and their periphyton food. *Archiv Für Hydrobiologie.*, **165**, 145-165.

Fritz B, Nisch A, Wittkugel C, Mörtl M. (2006) First record of *Limnomysis benedeni* Cerniavsky (Crustacea: Mysidacea) in Lake Constance. *Lauterbornia*, **58**, 157-160.

Gardes M, Bruns TD. (1993) ITS primers with enhanced specifity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Molecular Ecology*, **2**, 113-118.

Gergs R, Hanselmann AJ, Eisele I, Rothhaupt KO. (2008) Autecology of *Limnomysis benedeni* Czerniavsky, 1882 (Crustacea: Mysida) in Lake Constance, Southwestern Germany. *Limnologica*, **38**, 139-146.

Gergs R, Rothhaupt KO. (2008) Feeding rates, assimilation efficiencies and growth of two amphipod species on biodeposited material from zebra mussels. *Freshwater Biology*, **53**, 2494-2503.

Gervais M. (1835) Note sur deux especes de Crevettes qui vivent aux environs de Paris. *Annules des science naturelles*, **2**, 127-128.

Gessner MO. (1991) Difference in processing dynamics of fresh and dried leaf litter in a stream ecosystem. *Freshwater Biology*, **26**, 387-398.

Gessner MO. (2005) Ergosterol as a measure of fungal biomass. In: *Methods to study litter decomposition: a practical guide*, 189-196, Springer, Dordrecht.

Gessner MO, Bärlocher F, Chauvet E. (2003) Qualitative and quantitative analyses of aquatic hyphomycetes in streams. In: *Freshwater Mycology*, 127-157, Koeltz Scientific Books, Koenigstein, Germany.

Gessner MO, Chauvet E. (1993) Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. *Applied & Environmental Microbiology*, **59**, 502-507.

Gessner MO, Chauvet E, Dobson M. (1999) A perspective on leaf litter breakdown in streams. *Oikos*, **85**, 377-384.

Gessner MO, Gulis V, Kuehn KA, Chauvet E, Suberkropp K. (2007) Fungal decomposers of plant litter in aquatic ecosystems. In: *The Mycota, Volume IV: Microbial and Environmental Relationships*, 301-324, Springer-Verlag, Berlin.

Gessner MO, Newell SY. (1997) Bulk quantitative methods for the examination of eukaryotic organoosmotrophs in plant litter. In: *Manual of Environmental Microbiology*, 295-308, ASM Press, Washington, D.C., USA.

Gessner MO, Schwörbel J. (1989) Leaching kinetics of fresh leaf litter with implications for the current concept of leaf-processing in streams. *Archiv Für Hydrobiologie*, **115**, 81-90.

Gessner MO, Swan CM, Dang CK, McKie BG, Bardgett RD, Wall DH, Hättenschwiler S. (2010) Diversity meets decomposition. *Trends in Ecology & Evolution*, **doi:10.1016/j.tree.2010.01.010**.

Gherardi F. (2007) Biological invasions in inland waters: an overview. In: *Biological invaders in inland waters: profiles, distribution, and threats*, 3-25, Springer, Dordrecht.

Golladay SW, Webster JR, Benfield EF. (1983) Factors affecting food utilization by a leaf shredding aquatic insect: leaf species and conditioning time. *Holarctic Ecology*, **6**, 157-162.

Graça MAS. (2001) The role of invertebrates on leaf litter decomposition in streams - A review. *International Review of Hydrobiology*, **86**, 383-393.

Graça MAS. (1993) Patterns and processes in detritus-based systems. *Limnologica*, **23**, 107-114.

Graça MAS, Bärlocher F. (2005) Radial diffusion assay for tannins. In: *Methods to study litter decomposition: a practical guide*, 101-108, Springer, Dordrecht.

Graça MAS, Cressa C, Gessner MO, Feio MJ, Callies KA, Barrios C. (2001a) Food quality, feeding preferences, survival and growth of shredders from temperate and tropical streams. *Freshwater Biology*, **46**, 947-957.

Graça MAS, Ferreira RCF, Coimbra CN. (2001b) Litter processing along a stream gradient: The role of invertebrates and decomposers. *Journal of the North American Benthological Society*, **20**, 408-420.

Graça MAS, Maltby L, Calow P. (1994a) Comparative ecology of Gammarus pulex (L.) and Asellus aquaticus (L.) I: population dynamics and microdistribution. *Hydrobiologia*, **281**, 155-162.

Graça MAS, Maltby L, Calow P. (1993a) Importance of fungi in the diet of *Gammarus pulex* and *Asellus aquaticus.* II. Effects on growth, reproduction and physiology. *Oecologia*, **96**, 304-309.

Graça MAS, Maltby L, Calow P. (1993b) Importance of fungi in the diet of *Gammarus pulex* and *Asellus aquaticus*: I. Feeding strategies. *Oecologia*, **93**, 139-144.

Graça MAS, Maltby L, Calow P. (1994b) Comparative ecology of *Gammarus pulex* (L.) and *Asellus aquaticus* (L.): II. Fungal preferences. *Hydrobiologia*, **281**, 163-170.

Graça MAS, Zimmer M. (2005) Leaf toughness. In: *Methods to study litter decomposition: a practical guide*, 121-125, Springer, Dordrecht.

Greenberg AE, Trussel RR, Clesceri LS. (1985) *Standard methods for the examination of water and waste-water*, American Public Health Association (APHA).

Grigorovich IA, MacIsaac HJ, Shadrin NV, Mills EL. (2002) Patterns and mechanisms of aquatic invertebrate introductions in the Ponto-Caspian region. *Canadian Journal of Fisheries & Aquatic Sciences*, **59**, 1189-1208.

Gulis V, Suberkropp K. (2003) Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshwater Biology*, **48**, 123-134.

Hanselmann AJ, Gergs R. (2008) First record of *Crangonyx pseudogracilis* Bousfield 1958 (Amphipoda, Crustacea) in Lake Constance. *Lauterbornia*, **62**, 21-25.

Heneghana L, Colemana DC, Zoub X, Crossley DA Jr., Haines BL. (1998) Soil microarthropod community structure and litter decomposition dynamics: A study of tropical and temperate sites. *Applied Soil Ecology*, **9**, 33-38.

Hesselschwerdt J, Necker J, Tscharner T, Wantzen KM. (2009) A local gammarid uses kairomones to avoid predation by the invasive crustaceans *Dikerogammarus villosus* and *Orconectes limosus*. *Biological Invasions*, **11**, 2133-2140.

Hesselschwerdt J, Necker J, Wantzen KM. (2008) Gammarids in Lake Constance: habitat segregation between the invasive *Dikerogammarus villosus* and the indigenous *Gammarus roeselii*. *Fundamental and Applied Limnology*, **173**, 177-186.

Hieber M, Gessner MO. (2002) Contribution of stream detrivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology*, **83**, 1026-1038.

Hirsch PE, Nechwatal J, Fischer P. (2008) A previously undescribed set of Saprolegnia spp. in the invasive spiny chee crayfish (*Orconectes limosus*, Rafinesque). *Fundamental and Applied Limnology*, **172**, 161-165.

Holm S. (1979) A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics*, **6**, 70.

Kaushik NK, Hynes HBN. (1971) The fate of the dead leaves that fall into streams. *Archiv Für Hydrobiologie*, **68**, 465-515.

Kendrick B. (2005) *The Fifth Kingdom*, 1-386, Focus Pub/R Pullins Co.

Kernaghan G, Reeleder RD, Hokk SMT. (2008) Quantification of *Pythium* populations in ginseng soils by culture dependent and real-time PCR methods. *Applied Soil Ecology*, **40**, 447-455.

Kiran U. (1996) Fungi and food preferences of aquatic invertebrates. *National Academy Science Letters*, **19**, 188-190.

Klironomos JN, Widden P, Deslandes I. (1992) Feeding preferences of the collembolan *Folsomia candida* in relation to microfungal successions on decaying litter. *Soil Biology & Biochemistry*, **24**, 685-692.

Kok CJ, van der Velde G. (1994) Decomposition and macroinvertebrate colonization of aquatic and terrestrial leaf material in alkaline and acid still water. *Freshwater Biology*, **31**, 65-75.

Kominkova D, Kuehn KA, Busing N, Steiner D, Gessner MO. (2000) Microbial biomass, growth, and respiration associated with submerged litter of *Phragmites australis* decomposing in a littoral reed stand of a large lake. *Aquatic Microbial Ecology*, **22**, 271-282.

Kostalos M, Seymour RL. (1976) Role of microbial enriched detritus in the nutrition of *Gammarus minus* (Amphipoda). *Oikos*, **27**, 512-516.

Kowarik I. (2003) *Biologische Invasionen: Neophyten und Neozoen in Mitteleuropa*, 1-380, Ulmer Verlag, Stuttgart (Hohenheim).

Kurek A. (1992) Neue Tiere im Rhein - Die Wiederbesiedlung des Stroms und die Ausbreitung der Neozoen. *Naturwissenschaften*, **79**, 533-540.

Lampert W, Sommer U. (1997) *Limnoecology: The ecology of lakes and streams*, 1-382, Oxford University Press.

Macneil C, Dick JT, Elwood RW. (1997) The trophic ecology of freshwater *Gammarus* spp. (Crustacea: Amphipoda): Problems and perspectives concerning the functional feeding group concept. *Biological Reviews of the Cambridge Philosophical Society*, **72**, 349-364.

Marano AV, Pires-Zottarelli CLA, Barrera MD, Steciow MM, Gleason FH. (2010) Diversity, role in decomposition, and succession of zoosporic fungi and straminipiles on submerged decaying leaves in a woodland stream. *Hydrobiologia*, **doi 10.1007/s10750-009-0006-4**.

Maraun M, Martens H, Migge S, Theenhaus A, Scheu S. (2003) Adding to 'the enigma of soil animal diversity': fungal feeders and saprophagous soil invertebrates prefer similar food substrates. *European Journal of Soil Biology*, **39**, 85-95.

Mauchline J. (1980) *The biology of mysids and euphausiids*, 1-681, Academic Press.

McArdle BH, Anderson MJ. (2001) Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology*, **82**, 290-297.

Merritt R, Cummins KW, Burton TM. (1984) The role of aquatic insects in the processing and cycling of nutrients. In: *The ecology of aquatic insects.*, 134-163, Praeger, New York, Philadelphia.

Mille-Lindblom C, Fischer H, Tranvik LJ. (2006b) Litter-associated bacteria and fungi - a comparison of biomass and communities across lakes and plant species. *Freshwater Biology*, **51**, 730-741.

Mille-Lindblom C, Fischer H, Tranvik LJ. (2006a) Antagonism between bacteria and fungi: substrate competition and a possible tradeoff between fungal growth and tolerance towards bacteria. *Oikos*, **113**, 233-242.

Mörtl M. (2004) *Biotic interactions in the infralittoral of Lake Constance*, PhD thesis, University of Konstanz, Konstanz.

Nechwatal J, Mendgen K. (2006) *Pythium litorale* sp. nov., a new species from the littoral of Lake Constance, Germany. *FEMS Microbiology Letters*, **255**, 96-101.

Nechwatal J, Mendgen K. (2009) Evidence for the occurrence of natural hybridization in reed-associated *Pythium* species. *Plant Pathology*, **58**, 261-270.

Nechwatal J, Wielgoss A, Mendgen K. (2005) *Pythium phragmitis* sp. nov., a new species close to *P. arrhenomanes* as a pathogen of common reed (*Phragmites australis*). *Mycological Research*, **109**, 1337-1346.

Nechwatal J, Wielgoss A, Mendgen K. (2008) Diversity, host and habitat specificity of oomycete communities in declining reed stands (*Phragmites australis*) of a large freshwater lake. *Mycological Research*, **112**, 689-696.

Nehring S, Klingenstein F. (2009) Aquatic alien species in Germany - Listing system and options for action. *Neobiota*, **7**, 33.

Neubert K, Mendgen K, Brinkmann H, Wirsel SGR. (2006) Only a few fungal species dominate highly diverse mycofloras associated with the common reed. *Applied & Environmental Microbiology*, **72**, 1118-1128.

Nikolcheva LG, Bärlocher F. (2004) Taxon-specific fungal primers reveal unexpectedly high diversity during leaf decomposition in a stream. *Mycological Progress*, **3**, 41-49.

Nikolcheva LG, Bourque T, Bärlocher F. (2005) Fungal diversity during initial stages of leaf decomposition in a stream. *Mycological Research*, **109**, 246-253.

Ostendorp J, Schmieder K, Jöhnk K. (2004) Assessment of human pressures and their hydromorphological impacts on lakeshores in Europe. *International Journal of Ecohydrology & Hydrobiology*, **4**, 379-395.

Otto H-J. (1994) *Waldökologie*, 1-391, Eugen Ulmer GmbH & Co., Stuttgart-Hohenheim.

Pabst S, Scheifhacken N, Hesselschwerdt J, Wantzen KM. (2008) Leaf litter degradation in the wave impact zone of a pre-alpine lake. *Hydrobiologia*, **613**, 117-131.

Pace M, Cole J, Carpenter S, Kitchell J, Hodgson J, Van de Bodert M, Bade D, Kritzberg E, Bastviken D. (2004) Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. *Nature*, **427**, 240-243.

Pennings SC, Carefoot TH, Zimmer M, Danko JP, Ziegler A. (2000) Feeding preferences of supralittoral isopods and amphipods. *Canadian Journal of Zoology*, **78**, 1918-1929.

Reshi Z, Tyub S. (2007) *Detritus and decomposition in ecosystems*, 1-269, New India Publishing Agency, New Delhi.

Rey P, Mürle U, Ortlepp J, Mörtl M, Scheifhacken N, Werner S, Ostendorp W, Ostendorp J. (2005) *Wirbellose Neozoen im Bodensee– neu eingeschleppte invasive Benthos-Arten – Monitoringprogramm Bodenseeufer 2004*, 1-44, Landesanstalt für Umweltschutz Baden-Württemberg (LfU), Karlsruhe.

Rice WR. (1988) Analyzing tables of statistical test. *Evolution*, **43**, 223-225.

Rong Q, Sridhar KR, Bärlocher F. (1995) Food selection in three leaf-shredding stream invertebrates. *Hydrobiologia*, **316**, 173-181.

Rosset J, Bärlocher F, Oertli JJ. (1982) Decomposition of conifer needles and deciduous leaves in two Black Forest and two Swiss Jura streams. *Internationale Revue der gesamten Hydrobiologie (International review of hydrobiology)*, **67**, 695-711.

Rossi L. (1985) Interactions between invertebrates and microfungi in freshwater ecosystems. *Oikos*, **44**, 175-184.

Sabetta L, Costantini ML, Maggi O, Persiani AM, Rossi L. (2000) Interactions between detritivores and microfungi during the leaf detritus decomposition in a volcanic lake (Lake Vico - central Italy). *Hydrobiologia*, **439**, 49-60.

Schmidt E. (1996) *Ökosystem See. Der Uferbereich des Sees*, Quelle&Meyer Verlag, Wiesbaden.

Schofield JA, Hagerman AE, Harold A. (1998) Loss of tannins and other phenolics from willow leaf litter. *Journal of Chemical Ecology*, **24**, 1409-1421.

Shearer CA, Descals E, Kohlenmeyer J, Maranová L, Padgett D, Porter D, Raja HA, Schmidt JP, Thorton HA, Voglmayr H. (2007) Fungal biodiversity in aquatic habitats. *Biodiversity and Conservation*, **16**, 49-67.

Siessegger B. (1969) Vorkommen und Verbreitung von "*Dreissena polymorpha* Pallas" im Bodensee. In: *Gwf - wasser/abwasser*, 414-415.

Suberkropp K. (1992) Interactions with invertebrates. In: *The ecology of aquatic hyphomycetes*, 118-134, Springer Verlag, Heidelberg & New York.

Suberkropp K. (1984) Effect of temperature on seasonal occurence of aquatic hyphomycetes. *Transactions of the British Mycological Society*, **82**, 53-62.

Suberkropp K, Arsuffi TL, Anderson JP. (1983) Comparison of degradative ability, enzymatic activity, and palatability of aquatic hyphomycetes grown on leaf litter. *Applied & Environmental Microbiology*, **46**, 237-244.

Swift MJ, Heal OW, Anderson JM. (1979) *Decomposition in terrestrial ecosystems*, 1-372, University of California Press, Berkeley.

Tuite J. (1969) *Plant Pathology Methods. Fungi and Bacteria*, Burgess, Minneapolis.

Underwood AJ. (1997) *Experiments in Ecology: their logical design and interpretation using analysis of variance*, 1-524, Cambridge University Press, Cambridge.

Van Dokkum HP, Slijkerman DME, Rossi L, Costantini ML. (2002) Variation in the decomposition of *Phragmites australis* litter in a monomictic lake: The role of gammarids. *Hydrobiologia*, **482**, 69-77.

Von Elert E, Stampfel P. (2000) Food quality for *Eudiaptomus gracilis*: the importance of particular highly unsaturated fatty acids. *Freshwater Biology*, **45**, 189-200.

Waring RH, Schlesinger WH. (1985) *Forest ecosytems: concepts and managment*, 1-340, Academic Press, Inc., Orlando, Florida.

Webster JR, Benfield EF. (1986) Vascular plant breakdown in freshwater ecosystems. *Annual Review of Ecology and Systematics*, **17**, 567-594.

Webster JR, Simmons GM Jr. (1978) Leaf breakdown and invertebrate colonization on a reservoir bottom. *Verhandlungen - Internationale Vereinigung für Theoretische und Angewandte Limnologie*, **20**, 1587-1596.

Weete JD. (1989) Structure and function of sterols in fungi. *Advances in lipid research*, **23**, 115-167.

Weete JD, Gandhi SR. (1996) Biochemistry and molecular biology of fungal sterols. In: *The Mycota. Biochemistry and molecular biology*, 421-438, Springer-Verlag KG, Berlin, Germany.

Weish P, Türkay M. (1975) *Limnomysis benedeni* in Austria with considerations on its colonization history (Crustacea: Mysidacea). *Archiv Für Hydrobiologie/Suppl.*, **44**, 480-491.

Werner S, Mörtl M. (2004) Erstnachweis der Fluss-Körbchenmuschel *Corbicula fluminea* im Bodensee. *Lauterbornia*, **49**, 93-97.

White TJ, Bruns T, Lee S, Taylor J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *A Guide to Methods and Applications*, 315-322, Academic Press Inc, New York.

Wielgoss A, Nechwatal J, Bogs C, Mendgen K. (2009) Host plant development, water level and water parameters shape Phragmites australis-associated oomycete communities and determine reed pathogen dynamics in a large lake. *FEMS Microbiology Ecology*, **69**, 255-265.

Wirsel SGR. (2002) Four or more species of *Cladosporium* sympatrically colonise *Phragmites australis*. Fungal genetics and Biology. *Fungal Genetics and Biology*, **35**, 99-114.

Wirsel SGR, Leibinger W, Ernst M, Mendgen K. (2001) Genetic diversity of fungi closely associated with common reed. *New Phytologist*, **149**, 589-598.

Wittmann KJ. (1985) Freiland Untersuchungen zur Lebensweise von *Pyroleptomysis rubra* , einer neuen bentho-pelagischen Mycidacee aus dem Mittelmeer und aus dem Roten Meer. *Crustaceana*, **48**, 153-166.

Wittmann KJ. (1995) On the immigration of potamophilous Malacostraca into the Upper Danube River: *Limnomysis benedeni* (Mysidacea), *Corophium*

curvispinurn (Amphipoda), and *Atyaephyra desmaresti* (Decapoda). *Lauterbornia*, **20**, 77-85.

Wittmann KJ. (2007) Continued massive invasion of Mysidae in the Rhine and Danube river systems, with first records of the order Mysidacea (Crustacea: Malacostraca: Peracarida) for Switzerland. *Revue Suisse de Zoologie*, **114**, 65-86.

Wittmann KJ, Ariani AP. (2009) Reappraisal and range extension of non-indigenous Mysidae (Crustacea, Mysida) in continental and coastal waters of eastern France. *Biological Invasions*, **11**.

Wittmann KJ, Theiss J, Banning M. (1999) The drift of Mysidacea and Decapoda and its significance for the dispersion of neozoans in the Main-Danube system. *Lauterbornia*, **35**, 53-66.

Zare-Maivan H, Shearer CA. (1988) Wood decay activity and cellulase production by freshwater lignicolous fungi. *International Biodeterioration and Biodegradation*, **24**, 459-474.

Zhang N, Castlebury LA, Miller AN, Huhndorf SM, Schoch CL, Seifert KA, Rossman AY, Rogers JD, Kohlmeyer J, Volkmann-Kohlmeyer B, Sung G. (2006) An overview of the systematics of the Sordariomycetes based on a four-gene phylogeny. *Mycologia*, **98**, 1076-1078.

Zhao XR, Lin Q, Brooks PC. (2005) Does ergosterol concentration provide a reliable estimate of soil fungal biomass? *Soil Biology & Biochemistry*, **37**, 311-317.

Record of Achievement/ Abgrenzung der Eigenleistung

Chapter 2 and 3:	The concepts of these chapters were developed by Prof. Dr. Eric von Elert und me. All experimental work and generation and analysis of data were exclusively performed by myself except for the statistical analyses that were contributed by Dr. Karsten Rinke. Dr. Jan Nechwatal introduced me to the establishment of stock-cultures and to the identification of aquatic fungi and oomycetes.
Chapter 4:	The concept of this chapter was developed by Prof. Dr. Eric von Elert und me. All experimental work and generation and analysis of data were exclusively performed by myself.
Chapter 5:	The concept of this chapter was developed by Dr. René Gergs und me. I performed and supervised almost all laboratory experiments; the statistical analysis was contributed by Dr. René Gergs.

Ich erkläre hiermit, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Die benutzten Quellen und Hilfsmittel sind vollständig angeben und die Stellen der Arbeit - einschließlich Abbildungen -, die anderen Werken in Wortlaut oder dem Sinn nach entnommen wurden, sind in jedem Einzelfall als Entlehnung kenntlich gemacht. Die Dissertation wurde weder im In- noch im Ausland in gleicher oder ähnlicher Form einer Prüfungsbehörde vorgelegt. Die von mir vorgelegte Dissertation ist vonProf. Dr. Eric von Elert betreut worden.

Konstanz, April 2010

Christine Aßmann

Curriculum Vitae

Name	**Christine Aßmann**
Date of birth	31.10.1978
Place of birth	Esslingen am Neckar
Nationality	German

Scientific career

2005–2010: **Doctoral thesis** entitled „*Direct and indirect effects of fungi and oomycetes on leaf litter degradation by freshwater macroinvertebrates*“ at the Limnological Institute, University of Konstanz, supervisor Prof. Dr. Eric von Elert.

2004: **Diploma thesis** entitled „*Furagierkairomone bei aquatischen Organismen*“ at the University of Hohenheim under supervision of Dr. Beate Baier and Prof. Dr. Johannes L.M. Steidle.

University education

2004: **Diploma-degree** in the Institute of Zoology at the University of Hohenheim, Germany.

2001–2003: Studies of Zoology, Parasitology and Biophysic. Additional courses in Physiology, Bienenkunde and Versuchstierkunde at the University of Hohenheim, Germany.

2001: Vordiplom-degree in Biology, University of Hohenheim, Germany.

1999–2001: Basic studies of Biology, University of Hohenheim, Germany.

Selected Conference Contributions

AßMANN, CHRISTINE, Rinke Karsten, Nechwatal, Jan & *von Elert, Eric*. The steering role of fungi and oomycetes for leaf litter consumption by *Gammarus*. Talk. SFB 454 Symposium: The Role of Littoral Processes in Lake Ecology, Konstanz-Hegne, January 2010.

AßMANN, CHRISTINE & von Elert, Eric. The impact of aquatic fungi on leaf litter to the food preference of *Gammarus roeselii*. Talk. 5th International Meeting on Plant Litter Processing in Freshwaters, Coimbra, Portugal, July 2008.

List of Publications

Peer-reviewed*:*

CHRISTINE AßMANN, Karsten Rinke, Jan Nechwatal & Eric von Elert (Under revision). Consequences of the colonisation of leaves by fungi and oomycetes on leaf consumption by a gammarid shredder. Freshwater Biology.

CHRISTINE AßMANN, Jan Nechwatal, Karsten Rinke & Eric von Elert (In press). The impact of axenic strains of fungi and oomycetes on the preference of *Gammarus roeselii* for leaf litter. Fundamental and Applied Limnology.

CHRISTINE AßMANN, Eric von Elert & René Gergs (2009). Effects of leaf litter and its fungal colonisation on the diet of Limnomysis benedeni (Crustacea: Mysida). Hydrobiologia, 636 (1), 439-447.

CHRISTINE AßMANN & Eric von Elert (2009). The impact of fungal extracts on leaf litter on the food preference of *Gammarus roeselii*. International Review of Hydrobiology, 94 (4), 484-496.

Kirsten Kreuter, Beate Baier, **CHRISTINE AßMANN** & Johannes L.M. Steidle (2008). Prey location and prey choice by the freshwater leech *Erpobdella octoculata* using foraging kairomones. Freshwater Biology, 53 (8), 1524-1530.

<u>Non-peer-reviewed</u>:

René Gergs, Almut J. Hanselmann, **CHRISTINE AßMANN** & Karl-Otto Rothhaupt (2009). Syn- und autökologische Untersuchungen von *Limnomysis benedeni* im Bodensee. Tagungsbericht, Deutsche Gesellschaft für Limnologie (DGL) - Jahrestagung 2008 (Konstanz). 382-385.

CHRISTINE AßMANN & Eric Von Elert (2008). Die Rolle von aquatischen Pilzen auf Laub für die Nahrungspräferenz von *Gammarus roeselii*. Tagungsbericht, Deutsche Gesellschaft für Limnologie (DGL) - Jahrestagung 2007 (Münster): 126-130.

CHRISTINE AßMANN & Eric Von Elert (2007). Die Rolle von aquatischen Pilzen für die Futterqualität von Laub für *Gammarus roeselii*. Tagungsbericht, Deutsche Gesellschaft für Limnologie (DGL) - Jahrestagung 2006 (Dresden): 85-88

CHRISTINE AßMANN & von Elert, Eric (2006) Impact of aquatic fungi on the leaf litter degradation by *Gammarus roeselii*. Bulletin of the North American Benthological Society, 23 (1), 337.

CHRISTINE AßMANN & Eric Von Elert (2006). Einfluss von aquatischen Pilzen auf den Laubabbau von *Gammarus roeselii* im Bodensee-Litoral. Tagungsbericht, Deutsche Gesellschaft für Limnologie (DGL) - Jahrestagung 2005 (Karlsruhe): 312-315.

Acknowledgements

Mein besonderer Dank gilt Allen die mir in meiner Arbeit geholfen und mich unterstützt haben!

Zuerst möchte ich meinem Betreuer Eric von Elert, dem ich diese Doktorarbeit verdanke, für die Vergabe des Themas, große fachliche Hilfe und seine Betreuung bedanken. Danke Eric, dass Du Dir immer Zeit für lange Diskussionen und die häufig sehr lange Telefonate genommen hast und immer ein offenes Ohr für meine Fragen und Probleme hattest.

Karl-Otto Rothaupt möchte ich danken dass er mir einen Platz im Limnologischen Institut bereitgestellt hat, für die Unterstützung nach dem Ausscheiden von Eric von Elert aus der Limnologie, für das Übernehmen des Erstgutachtens meiner Arbeit und für das immer offne Ohr bei jeglichen Fragen und Schwierigkeiten.

Jan Nechwatal möchte ich danken für die Aufklärung meiner Unwissenheit im Bezug auf Oomyceten und Pilze, Unterstützung bei der mikro- und molekularbiologischen Arbeit, Offenheit für Fragen, jeden Rat und Tipp den Du mir bereitwillig gegeben hast.

Meinen Co-Autoren Karsten Rinke, Jan Nechwatal und René Gergs möchte ich für die große Unterstützung in meinen Publikationen danken.

Bei René Gergs, Hanno Seebens, Lars Peters und Patrick Fink möchte ich mich für die Hilfe bei statistischen Fragen bedanken. Danke Patrick für Deine Tipps und fürs' Zuhören.

Ich möchte mich bei Elisabeth M. Gross und Dominik Martin-Creuzburg bedanken, dass die beiden mir nach dem Weggang Erics, die Nutzung Ihrer Laborräume in Z8 und in der Limnologie ermöglicht haben.

René und Almut – danke für die schöne Zeit mit Euch und Ihr werdet mir fehlen. Die Zusammenarbeit mit Euch im Büro, Labor, Klimaraum oder Litoral war spitze und hat immer sehr viel Spaß gemacht! Danke auch fürs Zuhören, den Austausch und das Diskutieren - Ihr seid mehr als nur Kollegen für mich!

Anja Zitt möchte ich für die Unterstützung in meiner Anfangsphase und bei allen meinen Fragen Molekularbiologie und die nette Zeit im Büro danken.

Allen meinen Mitdoktorranden und Diplomanden aus dem Limnologischen Institut möchte ich für die schöne Zeit hier danken!!

Ich möchte mich bei allen Institutsmitarbeitern bedanken die zu Entstehung und zum Gelingen dieser Arbeit beigetragen haben. Vielen Dank für die gute Arbeitsatmosphäre und für die schöne Zeit.
Vor allem möchte ich mich aber bei Christine Gebauer und Petra Merkel für Ihre Einweisung, Unterstützung und Messungen im Labor bedanken. Christine und Petra, Ihr hattet immer aufmunternde Worte für mich und habt mich nach dem Ausscheiden Erics immer unterstützt!! Danke!

Martin Wolff möchte ich Danken für seine technische Unterstützung und Kreativität, ebenso wie Beatrix Rosenberg.

Dem ganzen Institutssekretariat möchte für die Unterstützung in Verwaltungs-angelegenheiten und Organisation Danken, vor allem aber Frau Berger die immer Zeit und jede Menge Tipps für mich hatte.

Ohne meine fleißigen Hiwi's: Isabelle Eisele, Corinna Geiss, Silke Pitlik, und Dominika, währe ein Teil der Experimente und Laborarbeit nicht möglich gewesen, deshalb möchte ich mich bei Euch für Eure Unterstützung und Hilfe bedanken!!!

Melanie, danke dass Du Dich um ein Projekt bei Ully Koch und mir bemüht hast. Leider wurde es sehr plötzlich durch eine schwere Krankheit beendet. Danke für Dein Interesse und die Arbeit im Projekt.

Für die Englisch Korrekturen der Kapitel und des Summarys möchte ich mich bei Frederik Bartlett, Karen A. Brune und bei Eveline O'Brien bedanken. Außerdem möchte ich mich bei Carolin Aßmann für die Korrektur der deutschen Zusammenfassung bedanken.

Außerdem möchte ich allen meinen Kollegen im *RIVA* danken da sie die mich offnen Armen aufgenommen haben, ein offenes Ohr und Verständnis für meine ‚frei-Wünsche' hatten.

Ich möchte mich bei meinen Freunden Anke, Ully, Ralf und vor allem Jutta bedanken (und alle die ich vergessen habe), dass sie mir Mut zu gesprochen, mit mir Sorgen geteilt und mich oft zu lachen gebracht haben. Danke dass Ihr immer für mich da seid!!

Danke Beate und Meziane für Euer Interesse und Eure Unterstützung!

Bei Rita Aßmann möchte ich mich ganz herzlich für die die Unterstützung und das Vertrauen das Du immer in mich gelegt hast bedanken.

Meinem Großvater, Robert Holz (Opa Hegensberg ☺), möchte ich danken, dass er schon früh mit mir die Natur erkundet hat, sein naturkundliche Neugier und Interesse an mich weiter gegeben hat. Und so in mir meinen späteren Berufswunsch geweckt hat.

An dieser Stelle möchte ich mich bei meiner lieben Großmutter -Elfriede Aßmann- bedanken! Sie hat mich immer gefördert, unterstützt und vor allem immer an mich geglaubt -Omi Du bist die Beste!

Ganz besonders herzlich möchte ich mich bei meinen Eltern, Hans-Werner und Adelheid, sowie meiner Schwester Carolin danken, die mit mir alles mitgemacht haben, immer Interesse gezeigt und mich in jeder Situation unterstützt haben – Danke für Eure Hilfe und Vertrauen!!!

Zum Schluss möchte ich Marc Hamitou danken für seine Liebe, Geduld, Unterstützung und vieles vieles mehr.

Research on this project was supported by a grant to E. v. E. from the German Research Foundation (DFG) within the Collaborative Research Centre SFB 454 - *Littoral Zone of Lake Constance*.