The ability of selected aquatic hyphomycetes and terrestrial fungi to decompose leaves in freshwater

Manuel A. S. Graça & R. C. F. Ferreira

Departamento de Zoologia – Universidade de Coimbra, 3049 Coimbra codex, Portugal

Graça, M. A. S. & R. C. F. Ferreira (1995). The ability of selected aquatic hyphomycetes and terrestrial fungi to decompose leaves in freshwater. – Sydowia 47 (2): 167–179.

We determined the optimal growth temperatures for four aquatic hyphomycetes and four terrestrial fungi frequently associated with decaying submerged leaves as well as the ability of the same species to degrade leaf tissue under laboratory conditions. Both terrestrial fungi and aquatic hyphomycetes tested had optimum growth between 10 C and 25 C; *Mortierella* sp. and *Penicillium* sp. did not grow at 5 C. The aquatic hyphomycetes *Articulospora tetracladia*, *Lemonniera terrestris*, *Lemonniera aquatica* and *Heliscus lugdunensis* caused significantly higher weight loss than *Mortierella* sp., *Penicillium* sp., *Cladosporium* sp. and *Aureobasidium pullulans* at low (4 C) or high (20 C) temperatures. *Heliscus lugdunensis*, however, was less active than the other aquatic hyphomycetes. The results suggest that aquatic hyphomycetes have the potential to be the main agents responsible for degradation of submerged organic matter through a wide range of climatic conditions and that terrestrial fungi are unable to macerate leaf material when submerged.

Keywords: aquatic hyphomycetes, leaf maceration, decomposition, fungal growth.

Plant material produced in the riparian zone has been regarded as a major energy source for low order streams (Fisher & Likens, 1973; Vannote & al., 1980). Such material consists mainly of leaves and, to a lesser extent, of twigs, bark, seeds and flowers.

Senescent leaves are rich in highly energetic structural compounds (Cummins & Klug, 1979). However, this energy is not easily accessible to aquatic animals feeding on detritus. Fungi, on the other hand, can macerate the leaf matrix (Suberkropp & Klug, 1980; Suberkropp & al., 1983; Chamier, 1985; Zemek & al., 1985).

There is considerable experimental evidence that detritivores selectively feed on leaves previously colonized by fungi (Suberkropp, 1992; Graça, 1993). Moreover, higher growth rates and fecundity have been observed in animals feeding on a diet rich in fungi, when compared to controls feeding on poorly colonized leaves (Kostalos &

Seymour, 1976; Graça & al., 1993). Therefore, fungi seem to play a key role in the energy balance of low order stream systems.

Aquatic hyphomycetes are considered the dominant mycoflora associated with decaying leaves in streams (Bärlocher & Kendrick, 1974; 1981). Other fungal taxa, however, have also been isolated from submerged decaying leaves (Kaushik & Hynes, 1968; Park, 1980; Godfrey, 1983; Gupta & Mehrotra, 1989; Graça, 1990). It has been suggested that the predominance of aquatic hyphomycetes is based on their ability to remain active at low temperatures (Thornton, 1963; Bärlocher & Kendrick, 1974; Godfrey, 1983). Low temperatures, however, are seldom reached in tropical, subtropical and some temperate areas. If temperature were the only factor affecting activity of fungi in the water, we would expect that in warmer areas terrestrial fungi play an equally important role in the degradation of organic matter.

To investigate the role of terrestrial fungi in the degradation of submerged leaf material, we compared the ability of four aquatic hyphomycetes and four terrestrial fungi to grow on and degrade leaf susbstrates at high (20 C) and low (4 C) temperatures.

Material and methods

Fungal strains

Cultures of aquatic hyphomycetes were derived from single conidia found on submerged leaves collected in several streams in central Portugal (Rio Mondego, Estação de Gouveia; Ribeira de S. João, Lousã; Ribeira do Sobral Cid, Coimbra). To confirm the identity of pure cultures, small pieces of agar with mycelium were placed in Petri dishes with sterile artificial pond water (APW, for composition see below) to allow sporulation. Pure cultures were maintained at 4 C until needed. Isolates of *Articulospora tetracladia* Ingold, *Heliscus lugdunensis* Sacc. & Therry, *Lemonniera aquatica* de Wild, *Lemonniera terrestris* Tubaki were used in the experiments.

Terrestrial fungi growing on submerged leaves were obtained by vigorously shaking leaves with stream water in plastic bags under sterile conditions. Drops of the washing water were used as inoculum in Malt extract agar (MEA; Difco, 36.6 g L^{-1}). Specimens of *Mortierella* sp. *Cladosporium* sp. and *Penicillium* sp. were isolated using this process. Laboratory cultures of *Aureobasidium pullulans* (De Bary) Arnaud were also used in the experiment (mycological collection of the Department of Botany, University of Coimbra); this species is frequently found on submerged leaves (Kaushik & Hynes, 1968; Bärlocher & Kendrick, 1974; Godfrey, 1983; Graça & al., 1993).

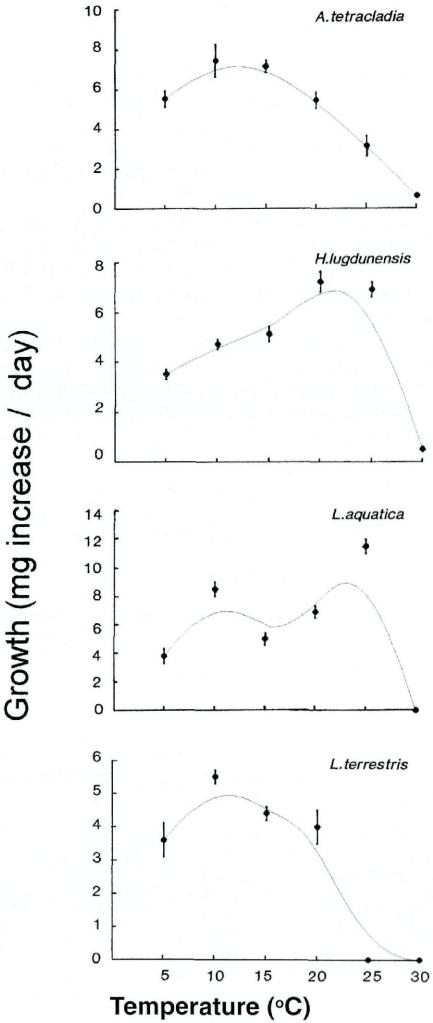

Fig. 1. – Growth of aquatic hyphomycetes (mg weight increase per day) in MEB at 5, 10, 20, 25 and 30 C; mean ± S.E.

Cladosporium sp. has been used in feeding trials with aquatic shredders (Rossi & al., 1983; Naylor & al., 1988).

Determination of optimal growth temperature

Growth rates were measured at 5, 10, 15, 20, 25 and 30 C. To measure growth, Petri dishes containing 20 ml of malt extract broth (MEB, Difco, 15 g L^{-1}) were inoculated by transferring a small piece of mycelium from a pure culture under sterile conditions. For each fungus and temperature, 24 Petri dishes were inoculated. Every 6 hours in the case of *Mortierella* sp. and *Penicillium* sp. growing at 25 C and 30 C, or every 3 to 6 days for other species and temperatures, three replicates of each culture were retrieved, filtered through a pre-weighted filter paper, dried in an oven at 50 C for 3 days and weighed to the nearest 0.1 mg.

Growth was expressed in terms of mass increase per day. This was given by the slope of a linear regression of mass (mg of dry mycelium) over time (days), during the linear (rapid) growth phase. Data for *A. pullulans* were excluded because this fungus did not grow well in the medium used.

Tab. 1. – The effect of fungal species and temperature (2-way ANOVA – Tukey test) on percentage of remaining weight of leaf substrates inoculated under laboratory conditions after 51 (± 1) days. Homogeneous groups: significant differences are indicated with different letters.

Source	d.f.	Mean-square	F-ratio	P
taxa	8	416.1	24.8	0.000
temperature	1	30.7	1.8	0.179
residual	11	16.8		
	4			

Taxa	Average residual weight	Homogeneous groups
Lemonniera aquatica	65.0	a
L. terrestris	65.7	a
Articulospora tetracladia	68.8	a b
Heliscus lugdunensis	72.3	b c
Aureobasidium pullulans	72.5	b c
Mortierella sp.	75.4	c d
Cladosporium sp.	77.4	d
Penicillium sp.	78.9	d
Control	79.8	d

Temperature (C)	Average residual weight	Homogeneous groups
20	71.0	a
4	73.0	a

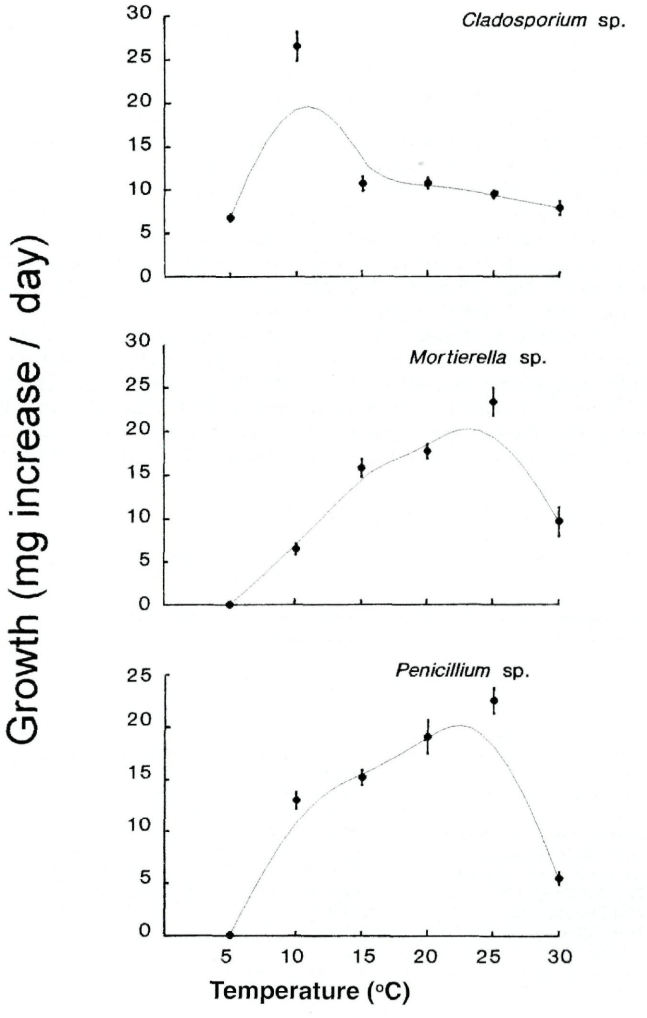

Fig. 2. – Growth of terrestrial fungi (mg weight increase per day) in MEB at 5, 10, 20, 25 and 30 C; mean ± S.E.

Leaf maceration

The ability of fungi to macerate organic matter was investigated by inoculating leaf discs with pure cultures of the eight fungi and determining mass loss and decreases in leaf resistance (see below). Senescent leaves of chestnut (*Castanea sativa* L.) from a single tree were used as substrate for fungi. Prior to the experiment, the leaves were leached in tap water for 5 days to eliminate soluble compounds which frequently account for 11% to 40% of leaf weight in angiosperms (Kaushik & Hynes, 1971; Blackburn & Petr, 1979). Leaf discs of similar size (8 ± 0.3 mg) were obtained according to Graça & al. (1993). They were allocated to 27 conical flasks (8 leaf discs each) containing 150 ml of artificial pond water (APW: Ca^{2+}, 80 mg L^{-1}; Cl, 145 mg L^{-1}; Mg^{2+}, 12 mg L^{-1}; Na^{+}, 247 mg L^{-1}; K^{+}, 121 mg L^{-1}; NO_3^{-}, 619 mg L^{-1}; PO_4^{3-}, 286 mg L^{-1}; pH 7.9) and autoclaved.

Twenty-four flasks (three replicates for each fungus) were inoculated with a 1.8 cm diameter mycelial pellet obtained from cultures growing in malt extract broth (MEB). Three uninoculated cultures were used to determine the initial tensile strength and dry mass, the others were incubated in an orbital shaker (100 rpm, 51 days). One experimental set was incubated at 20 C and the other at 4 C.

Tab. 2. – The effect of fungal species and temperature (2-way ANOVA – Tukey test) on percentage of initial tensile strenght of leaf substrates inoculated under laboratory conditions after 51 (±1) days. Homogeneous groups: significant differences are indicated with different letters.

Source	d.f.	Mean square	F-ratio	P
taxa	8	2477.8	32.4	.0000
temperature	1	158.2	2.1	.1530
residual	119	76.5		

Taxa	Average residual tensile strenght	Homogeneous groups			
Lemonniera aquatica	36.4	a			
L. terrestris	37.4	a			
Articulospora tetracladia	47.6		b		
Heliscus lugdunensis	58.7			c	
Aureobasidium pullulans	63.6			c	d
Mortierella sp.	63.9			c	d
Cladosporium sp.	64.9			c	d
Penicillium sp.	67.2			c	d
Control	71.2				d

Temperature (C)	Average residual tensile strenght	Homogeneous groups
20	53.2	a
4	56.7	a

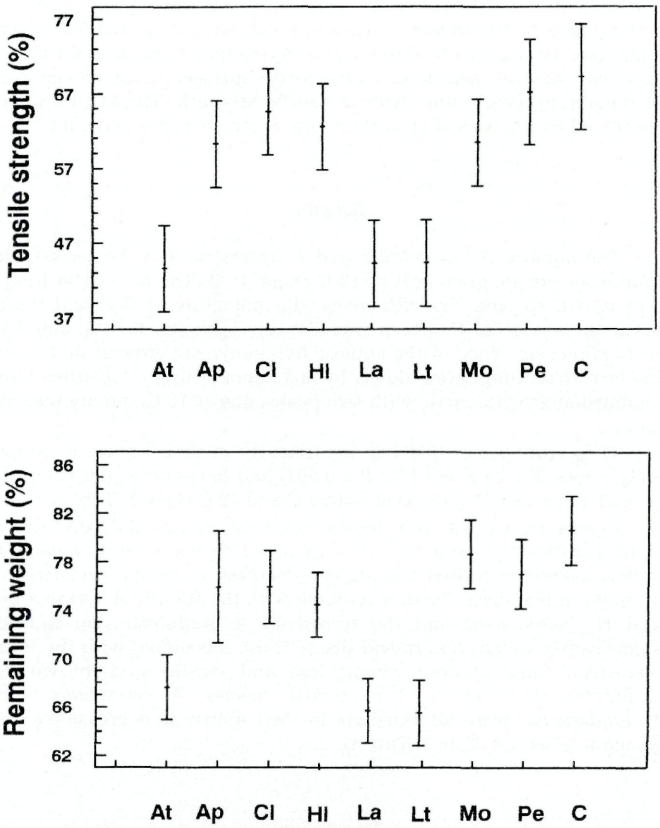

Fig. 3. – Tensile strength and percentage of remaining weight for leaf discs after 51 (±1) incubation days at 4 C. – At = *Articulospora tetracladia*; Ap = *Aureobasidium pullulans*; Cn = *Cladosporium* sp.; Hl = *Heliscus lugdunensis*; La = *Lemonniera aquatica*; Lt = *Lemonniera terrestris*; Mo = *Mortierella* sp.; Pe = *Penicillium* sp.; C = Control.

At the end of the incubation period leaf tensile strength was estimated. This was done by securing leaf discs with two pegs; one of the pegs was fixed whereas the other was connected to an aluminum cup (200 ml capacity) with a pulley. Sand was gradually added to the

cup until its weight caused the leaf disc break. The tensile strength was equated to the weight of the sand required to tear the leaf discs apart (see Graça, 1990). Finally, the dry weight (3 days at 50 C) of each leaf disc was measured. Values were expressed as the percentage of remaining weight and original tensile strength. Treatments were compared by analysis of variance of the arcsin-transformed values.

Results

The aquatic *A. tetracladia* and *L. terrestris* and the terrestrial *Cladosporium* sp. grew best at 10 C (Figs. 1, 2). The terrestrial fungi *Mortierella* sp. and *Penicillium* sp. did not grow at 5 C and their optimum was at 25 C, which was also the optimum for the aquatic *H. lugdunensis*. None of the aquatic hyphomycetes grew at 30 C and the terrestrial fungi grew slowly at this temperature. *L. aquatica* had an unusual growth curve, with two peaks, one at 10 C and another at 25 C.

The eight species differed significantly in their ability to cause weight loss ($F = 24.8$; $n = 115$; $P < 0.001$) and to lower tensile strength ($F = 32.4$; $n = 120$; $P < 0.001$) at both 4 C and 20 C (Tab. 1; Tab. 2).

Losses in weight and tensile strenght values did not differ significantly at 4 C and 20 C ($F < 1.8$; $n > 115$; $P > 0.05$). *L. aquatica* and *L. terrestris* caused the highest decrease in weight and tensile strenght of leaf discs. Discs inoculated with the aquatic *A. tetracladia* and *H. lugdunensis* and the terrestrial *A. pullulans* also caused significantly weight loss in leaf discs. Those inoculated with the four terrestrial fungi showed weight loss and tensile strenght values similar to the controls. The aquatic species, *A. tetracladia* and *H. lugdunensis* were intermediate in their ability to decrease tensile strenght (Figs 3, 4; Tab. 1, Tab. 4).

Discussion

Thornton (1963) reported that the optimum growth temperatures of eight aquatic hyphomycetes were 10–23 C, which was regarded to be lower than those reported for terrestrial fungi (Bärlocher & Kendrick, 1974). For *A. tetracladia*, Thornton (1963) found an optimum growth temperature of 20–25 C. For the same species, Koske & Duncan (1974) quoted a temperature of 15–20 C, Sridhar & Bärlocher (1993) a temperature of 20 C. Our results indicated 10–15 C; such discrepancies may reflect strain-related differences or are possibly dependent on the media used.

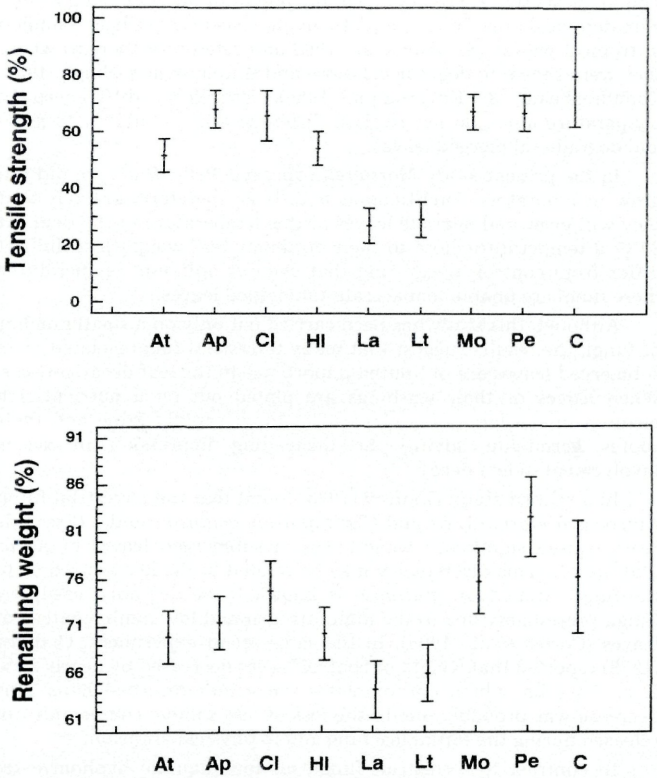

Fig. 4. – Tensile strength and percentage of remaining weight for leaf discs after 51 (±1) incubation days at 20 C. – At = *Articulospora tetracladia*; Ap = *Aureobasidium pullulans*; Cn = *Cladosporium* sp.; Hl = *Heliscus lugdunensis*; La = *Lemonniera aquatica*; Lt = *Lemonniera terrestris*; Mo= *Mortierella* sp.; Pe= *Penicillium* sp.; C= Control.

Our results confirm the ≤ 20 C optimum for 2 aquatic hyphomycetes, but also for one terrestrial fungus frequently found on submerged leaves. Two aquatic hyphomycetes also had a higher growth rate at 25 C.

Koske & Duncan (1974) reported that the optimum temperature for mycelial growth of 10 out of 12 aquatic hyphomycetes was 20 C.

This suggests that these taxa have the potential for colonizing a wide climatological area. In fact, aquatic hyphomycetes have been sampled in tropical waters (Sridhar & al., 1992 and references therein) where they were shown to degrade cellulose and simple sugars (Singh, 1982; Chandrashekar & Kaveriappa, 1988). Therefore, differences in temperature alone do not explain differences in the ability to grow and degrade submerged leaves.

In the present study *Mortierella* sp., and *Penicillium* sp. did not grow in laboratory conditions at 5 C. It is, therefore, unlikely that they will grow and degrade leaves at this temperature in the field. At 20 C, a temperature close to their optimum, leaf weight loss did not differ from controls suggesting that even at optimum temperatures these fungi are unable to macerate submerged leaves.

Although this study has been carried out only on a small number of fungi, the results suggest that many terrestrial fungi isolated from submerged leaves are of limited importance in the leaf decay process. When leaves or their washings are plated out on a nutrient-rich medium in the laboratory, terrestrial fungi rapidly grow and their spores germinate, giving the misleading impression of active involvement in leaf decay.

In a related study Godfrey (1983) found that the terrestrial fungi *Epicoccum nigrum* Link and *Cladosporium cladosporioides* (Fres.) de Vries caused significant weight loss in submerged leaves of *Alnus glutinosa* L. This discrepancy may be related to the leaves used or to the fungi studied. *A. glutinosa* is known to be degraded easily by fungi, presumably due to the high nitrogen and low lignin content of leaves (Cortes & al., 1994). In the same set of experiment, Godfrey (1983) reported that weight of control leaves decreased by nearly 20% after 7 weeks, which approximates the values recorded here. This decrease was probably due to the loss of less soluble compounds not released during the initial leaching and to physical abrasion.

In contrast to terrestrial fungi, all four aquatic hyphomycetes tested caused a significant loss in weight and tensile strenght in leaves. *L. aquatica* has been reported to have a high ability to degrade submerged substrates (Chergui & Pattée, 1991). *H. lugdunensis* ranked lower in the ability to degrade leaf tissues, which is consistent with other published data (e.g. Butler & Suberkropp, 1986).

In some cases terrestrial fungi have been identified as major components of the mycoflora colonizing submerged leaves (e.g. Rossi & al., 1983). This may be the case when the oxygen dissolved in the water is low, as shown by Chergui & Pattée (1988). These authors found higher degradation rates and higher number of species of aquatic hyphomycetes in leaf substrates in sections of rivers with well-oxygenated waters and lower degradations rates and higher

proportion of terrestrial fungi in a river section with low oxygen content.

The tetraradiate or sigmoid spores produced in submerged substrates provide aquatic hyphomycetes with an additional colonisation advantage over terrestrial fungi, which possess spores more suitable for air transportation (Webster & Descals, 1981). Moreover, there is some evidence that sporulation is favoured by temperatures lower than, or equal to, that allowing maximum mycelial growth (Thornton, 1963; Koske & Duncan, 1974) and by turbulence (Webster & Descals, 1981).

Our data support the view that terrestrial fungi colonizing leaves are soon replaced by aquatic hyphomycetes when submerged in well-oxygenated flowing waters. The number of strains used here was low, thus not allowing firm conclusions. More research using a wider variety of species and strains is still needed. Future research will have to examine the enzymatic abilities of fungi in submerged leaves and fungal species replacement on leaves falling in the streams.

Acknowledgments

This work was supported by J.N.I.C.T. grant number PEAM/C/CNT/31/91. We thank Enrique Descals for confirming the identifications of aquatic hyphomycetes, Felix Bärlocher for comments on an earlier version of the manuscript and Leonel Pereira for the cultures of *Aureobasidium pullulans*. The comments of two anonimous referees had a significant contribution to the final text.

References

Bärlocher, F. & B. Kendrick (1974). Dynamics of the fungal populations on leaves in a stream. – J. Ecol. 62: 761–791.

—— & —— (1981). Fungi on the food and in faeces of *Gammarus pulex*. – Trans. Br. Mycol. Soc. 76: 160–165.

Blackburn, W. & T. Petr (1979). Forest litter decomposition and benthos in a mountain stream in Victoria, Australia. – Arch. Hydrobiol. 86: 453–498.

Butler, S. & K. Suberkropp (1986). Aquatic hyphomycetes on oak leaves; comparison of growth, degradation and palatability. – Mycologia 78: 922–928.

Chamier, A.-C. (1985). Cell wall-degrading enzymes of aquatic hyphomycetes: a review. – Bot. J. Linn. Soc. 96: 67–81.

Chandrashekar, K. R. & K. M. Kaveriappa (1988). Production of extracellular enzymes by aquatic hyphomycetes. – Folia Microbiol. 33: 55–58.

Chergui, H. & E. Pattée (1988). The dynamics of hyphomycetes on decaying leaves in the network of the river Rhône (France). – Arch. Hydrobiol. 114: 3–20.

—— & —— (1991). An experimental study of the breakdown of submerged leaves by hyphomycetes and streams in Morocco. – Freshw. Biol. 26: 97–110.

Cortes, R. M. V., M. A. S. Graça & A. Monzón (1994). The replacement of alder by eucalypt along two streams with different characteristics: Differences on

decay rates and consequences to the system functioning. – Verh. Internat. Verein. Limnol. 25: 1697–1702.

Cummins, K. W. & M. J. Klug (1979). Feeding ecology of stream invertebrates. – Ann. Rev. Ecol. Syst. 10: 147–172.

Fisher, S. G. & G. E. Likens (1973). Energy flow in Bear Brook, New Hampshire: an alternative approach to stream metabolism. – Ecol. Monogr. 43: 421–439.

Godfrey, B. E. S. (1983). Growth of two terrestrial microfungi on submerged alder leaves. – Trans. Br. Mycol. Soc. 79: 418–421.

Graça, M. A. S. (1990). Observations on the feeding of two stream dwelling detritivores: *Gammarus pulex* (L.) and *Asellus aquaticus* (L.). Ph. D. thesis, University of Sheffield, Sheffield, U. K. 220 pp.

—— (1993). Patterns and processes in detritus-based stream systems. –Limnologica 23: 107–114.

—— , L. Maltby & P. Calow (1993). Importance of fungi in the diet of *Gammarus pulex* and *Asellus aquaticus* II: effects on growth, reproduction and physiology. – Oecologia 96: 304–309.

Gupta, A. K. & R. S. Mehrotra (1989). Seasonal peridiocity of aquatic fungi in tanks at Kurukshetra, India. – Hydrobiologia 173: 219–229.

Kaushik, N. K. & H. B. N. Hynes (1968). Experimental study on the role of autumn shed leaves in aquatic environments. – J. Ecol. 56: 229–245.

—— & —— (1971). The fate of dead leaves that fall into streams. – Arch. Hydrobiol. 68: 465–515.

Koske, R. E. & I. W. Duncan (1974). Temperature effects on growth, sporulation, and germination of some "aquatic" hyphomycetes. – Can. J. Bot. 52: 1387–1391.

Kostalos, M. & R. L. Seymour (1976). Role of microbial enriched detritus in the nutrition of *Gammarus minus* (Amphipod). – Oikos 77: 512–516.

Naylor, C., L. Maltby & P. Calow (1988). Scope for growth in *Gammarus pulex*, a freshwater benthic detritivore. – Hydrobiologia 188/189: 517–523.

Park, D. (1980). A two year study of numbers of cellulolytic *Pythium* in river water. – Trans. Br. Mycol. Soc. 74: 253–258.

Rossi, L., E. A. Fano, A. Basset, C. Fanelli & A. A. Fabri (1983). An experimental study of a microfungal community on plant detritus in a mediterranean woodland stream. – Mycologia 75: 887–896.

Singh, N. (1982). Cellulose decomposition by some tropical aquatic hyphomycetes. – Trans. Br. Mycol. Soc. 79: 560–561.

Sridhar, K. R. & F. Bärlocher (1993). Effect of temperature on growth and survival of five aquatic hyphomycetes. – Sydowia. 43: 377–387.

—— , K. R. Chandrashekar & K. M. Kaveriappa (1992). Research on the Indian subcontinent. In: Bärlocher, F. (Ed.), The Ecology of Aquatic Hyphomycetes. – Springer-Verlag. Ecological Studies 94: 182–211.

Suberkropp, K. (1992). Interactions with invertebrates. In: Bärlocher, F. (Ed.). The Ecology of Aquatic Hyphomycetes. – Springer-Verlag. Ecological Studies 94: 118–134.

—— , T. L. Arsuffi & J. P. Anderson (1983). Comparisons of degradative ability, enzymatic activity and palatability of aquatic hyphomycetes growing on leaf litter. – Appl. Environ. Microbiol. 46: 237–244.

—— & M. J. Klug (1980). The maceration of deciduous leaf litter by aquatic hyphomycetes. – Can. J. Bot. 50: 1025–1031.

Thornton, D. R. (1963). The physiology and nutrition of some aquatic hyphomycetes. – J. Gen. Microbiol. 33: 23–31.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell & C. E. Cushing (1980). The river continuum concept. – Can. J. Fish. Aquat. Sci. 37: 130–137.

Webster, J. & E. Descals (1981). Morphology, distribution, and ecology of conidial fungi in freshwater habitats. In: Cole, G. T. & B. Kendrick (Eds.), The Biology of Conidial Fungi. – Academic Press, N.York Vol. I: 295–355.

Zemek, K. J., L. Marvanová, L. Kuniak & B. Kadlecíková (1985). Hydrolytic enzymes in aquatic hyphomycetes. – Folia Microbiol. 30: 363–372.

(Manuscript accepted 17th April 1995)