DOI 10.1007/s10750-013-1666-7

Relative influence of shredders and fungi on leaf litter decomposition along a river altitudinal gradient

Barry R. Taylor · Eric E. Chauvet

Abstract We compared autumn decomposition rates of European alder leaves at four sites along the Lasset–Hers River system, southern France, to test whether changes in litter decomposition rates from upstream (1,300 m elevation) to downstream (690 m) could be attributed to temperature-driven differences in microbial growth, shredder activity, or composition of the shredder community. Alder leaves lost 75–87% of original mass in 57 days, of which 46–67% could be attributed to microbial metabolism and 8–29% to shredder activity, with no trend along the river. Mass loss rates in both fine-mesh (excluding shredders) and coarse-mesh (including shredders) bags were faster at warm, downstream sites (mean daily temperature 7–8°C) than upstream (mean 1–2°C), but the difference disappeared when rates were expressed in heat units to remove the temperature effect. Mycelial biomass did not correlate with mass loss rates. Faster mass loss rates upstream, after temperature correction, evidently arise from more efficient shredding by Nemourid stoneflies than by the *Leuctra*-dominated assemblage downstream. The influence of water temperature on decomposition rate is therefore expressed both directly, through microbial metabolism, and indirectly, through the structure of shredder communities. These influences are evident even in cold water where temperature variation is small.

Keywords Mass loss rates · Streams · Fungi · Shredders · Temperature · Elevation gradient

B. R. Taylor (✉)
Department of Biology, St. Francis Xavier University, 2320 Notre Dame Avenue, Antigonish, NS B2G 2W5, Canada
e-mail: btaylor@stfx.ca

E. E. Chauvet
EcoLab (Laboratoire Ecologie Fonctionnelle et Environnement), INP-UPS, Université de Toulouse, 118 route de Narbonne, 31062 Toulouse, France

Introduction

Terrestrial litter, especially fallen leaves from riparian trees, is an essential source of energy and carbon for freshwater rivers and streams (Minshall et al., 1985; Webster & Benfeld, 1986; Tank et al., 2010). Because of the central role of organic detritus in stream energetics, the process of leaf litter decomposition and the important determinants of decomposition rates have been thoroughly investigated (e.g., Webster & Benfeld, 1986; Gessner et al., 1999; Graça, 2001). It is generally concluded that decomposition of leaf litter in streams may occur through a combination of three mechanisms: physical leaching by flowing water, microbial metabolism, largely by microfungi, and feeding by leaf-shredding invertebrates (Webster & Benfeld, 1986; Graça et al., 2001; Hieber & Gessner, 2002). The mechanisms are interdependent; accumulation

of microbial biomass and conditioning of the leaves by exoenzymes increase nutrient concentrations and reduce leaf toughness, making the leaves more attractive to shredders (Bärlocher, 1985; Graça, 2001; Chung & Suberkropp, 2009; Assmann et al., 2011). Maceration of the leaves by shredders, in turn, may release more leachable material and encourage further fungal colonization on frass (Giller & Malmqvist, 1998; Gessner et al., 1999).

For any given type of litter, water temperature is a key control on decomposition rate because microbial metabolism (and invertebrate metabolism) depends on temperature (Rajashekar & Kaveriappa, 2000; Fernandes et al., 2009; Boyero et al., 2011, Ferreira & Chauvet, 2011a, b). Thus, decomposition tends to proceed more quickly in warmer seasons than cold seasons at a particular site (Chergui & Pattee, 1993; Stockley et al., 1998; Graça et al., 2001; Bergfur, 2007), and at sites with warmer water within a river system compared with sites where water is cooler (Benfield et al., 1979; Stockley et al., 1998; Taylor & Dykstra, 2005; Imberger et al., 2008). Nevertheless, several investigators have observed lower rates of litter decomposition in large rivers than in headwater streams (Minshall et al., 1983; Collier & Winterbourn, 1986; Chauvet et al., 1993; MacDonald & Taylor, 2008), even though water temperatures in larger rivers tends to be higher than upstream.

This inverted pattern is generally attributed to the effect of shredding invertebrates, which are more numerous in the cool waters of leaf-filled tributaries than in larger rivers where the litter supply is more dilute (Minshall et al., 1983; Collier & Winterbourn, 1986; Baldy et al., 1995). Many of the most abundant shredders are members of the Plecoptera and Trichoptera, which are largely restricted to cool-water habitats (Wiggins & Mackay, 1978). In small river systems of Nova Scotia, Canada, rapid decomposition in cool headwater streams compared with warmer mainstem rivers has been attributed specifically to the stenothermal, leaf-shredding stonefly *Leuctra*, which is numerically dominant in cool streams (MacDonald & Taylor, 2008). *Leuctra* is absent in warm rivers, presumably because summer temperatures there exceed its thermal tolerance (Elliott, 1987), but is not replaced by an effective warm-water shredder.

In contrast to these findings, Fabre & Chauvet (1998) report that decomposition rates of alder leaves increased substantially with declining elevation and increasing channel size (first order to third order) in the Lasset–Hers River system of southern France. They attribute the trend to the distribution of shredding invertebrates and direct action by fungal decomposers. However, their conclusions are based on correlations and have not been tested experimentally. Since the results reported by Fabre & Chauvet (1998) run contrary to those found elsewhere, the Lasset–Hers River system is an appropriate place to test hypotheses concerning factors influencing decomposition rates, in particular the supposition of MacDonald & Taylor (2008) that Plecopteran shredders in cooler upstream reaches are principally responsible for the faster decomposition rates observed there.

This research had two broad objectives: first, to test whether differences in litter decomposition rates along a single river system could be mainly attributed to temperature-driven differences in microbial growth or shredder activity; second, to test whether a change in shredder community structure along a river system would lead to a reduction in the decomposition rate downstream, or at least to a smaller increase than would be expected based on microbial growth in warmer water. We anticipated that the shredder community would be dominated by Plecoptera at the cool upstream sites and by Diptera and Trichoptera in the warmer downstream river. We compared decomposition rates from a single litter type (alder leaves) at a set of four sites along the Lasset–Hers River system in fine-mesh bags (excluding shredders) and coarse-mesh bags (including shredders) to separate effects of shredders and microorganisms. Specifically, we hypothesized that:

(1) Decomposition in fine-mesh bags would be faster downstream than upstream, but decomposition rate in coarse-mesh bags would show a smaller difference between upstream and downstream, or none;
(2) Correcting for water temperature would reduce the decomposition rate downstream relative to the rate upstream in both fine-mesh and coarse-mesh bags;
(3) Decomposition rates in fine-mesh bags would correspond with fungal standing crops on the litter, but decomposition rates in coarse-mesh bags would correspond with both fungal standing crops and densities of shredders (or composition of the shredder assemblage).

Methods

Study sites

The sites used in this study were distributed along the Hers River and its tributary, the Lasset River (Fig. 1). The Lasset is one of the last undisturbed streams in southwestern France, especially regarding micro-hydroelectric power plants (Fabre & Chauvet, 1998). It arises as a steep-gradient mountain stream draining the northern slopes of the Pyrenees, eventually joining the lower-gradient Hers River, itself a tributary of the Ariège River in the Garonne River basin. The Lasset–Hers River system is first order in the mountains, increasing to third order by the lowest site on the Hers.

The montane portion of the drainage basin typically supports beech (*Fagus sylvatica* L.) forest on crystalline rocks, but dominant riparian trees are alder (*Alnus glutinosa* (L.) Gaertn.) and hazel (*Corylus avellana* L.). The lower portion of the basin, below the confluence with the Hers, is arable land on mainly calcareous soils, with a riparian zone dominated by alder, hazel, and ash (*Fraxinus excelsior* L.), frequently interrupted by clearing for agriculture. Leaf litter inputs from riparian forest are substantial along the length of the river, except for the highest alpine reaches. Leaf-fall typically occurs from mid-October at the upper limit of deciduous trees (~1,300 m altitude) to mid-November below 600 m altitude (Fabre & Chauvet, 1998).

Fabre & Chauvet (1998) used 14 sites distributed along a 1,400-m elevational gradient and 20 km of river length. The four sites used in this study were selected to represent the same three geophysical regions: Alpine, site A (high elevation, herbaceous riparian vegetation, pH near 6, low alkalinity); Forest, site B (intermediate elevation, forested riparian zone, mostly *Corylus*, pH 7–7.5, moderate alkalinity); and Plains, sites D and E (low elevation, agricultural land use, pH 8–8.5, high alkalinity) (Table 1). The four sites correspond to sites S3, S7, S11–12, and S13 in Fabre & Chauvet (1998). The lowest site is wider and has a lower gradient than the others (Table 1). A fifth site, C, was disturbed by a spate.

Mean slope for each site was calculated from the difference in elevation and linear distance between the sites immediately upstream and downstream in Fabre & Chauvet (1998). On each of two dates in November and December, 2007, wetted width was measured twice and depth was measured at 3–5 points evenly spaced across the channel. Water velocity was measured 3–5 times on each of three or four dates from

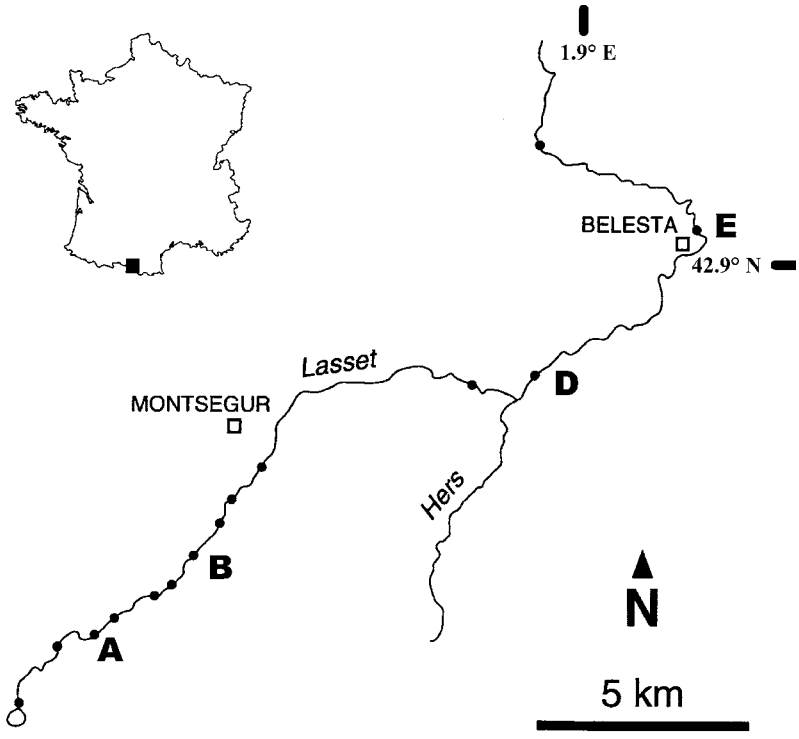

Fig. 1 Location of the study sites along the Lasset–Hers river system in southwestern France. *Unmarked circles mark* study sites used by Fabre & Chauvet (1998)

Table 1 Means (±1 SD) of physical and chemical characteristics of the study sites

Variable	Site			
	A	B	D	E
Elevation (m)	1,300	1,050	550	490
Mean slope (%)	20.8	9.5	13	0.8
Wetted width (m)	4.4	2.8	5.1	12
Depth (cm)	14 (8)	30 (8)	13 (5)	26 (12)
Water velocity (m/s)	0.32 (0.22)	0.77 (0.66)	0.43 (0.16)	0.64 (0.44)
pH (units)	6.57 (0.36)	7.08 (0.18)	8.25 (0.26)	8.12 (0.14)
Alkalinity (mg/l)	4.1 (3.90)	11.7 (4.2)	61.9 (10.9)	107.0 (18.8)
Ammonium, N	0.011 (0.022)	0.002 (0.003)	0.001 (0.003)	0.008 (0.010)
Nitrate, N	0.015 (0.025)	0.028 (0.047)	0.31 (0.06)	0.52 (0.06)
Orthophosphate, P	0.005 (0.001)	0.005 (0.001)	0.006 (0.001)	0.005 (0.001)

November 2007 to January 2008 using a current velocity meter or by timing the transit time of a neutrally buoyant object. Water samples for background chemistry were collected on each sampling date ($n = 3$ or 4) (Table 1). Dissolved oxygen tension was 10 mg/l or more at all sites.

Leaf litter mass loss

Litter decomposition was estimated using the litter bag method. Alder leaf litter was used in the decomposition experiment to be consistent with Fabre & Chauvet (1998) and because alder is the most consistently frequent tree along the entire length of the Lasset–Hers system. Freshly fallen alder leaves were collected in the riparian zone near sites B and C in early November 2007 and stored in sealed plastic bags at 4°C for no more than 3 days before being placed in litter bags. Then 5-g portions (±0.01 g) were placed in 15 × 15 cm, numbered bags with a mesh size of either 9 mm (coarse) or 0.5 mm (fine). Coarse-mesh bags permitted most invertebrates free access to the leaves; the fine-mesh bags prevented almost all macro-invertebrates from reaching the leaves, except for a few early-instar chironomids. Ten additional samples of fresh leaves were used to calculate fresh to oven-dry and oven-dry to ash-free dry mass (AFDM) conversion factors.

Twelve litter bags of each mesh size (plus one extra to allow for losses) were placed at each site, permitting three collections of four replicates each. Pairs of fine-mesh and coarse-mesh litter bags were tethered to rebar stakes driven into the center of the channel and held in place with rocks. Litter bags were set out on November 12, 2007. Four replicate bags of each mesh size were collected after 14, 28, and 57 days at sites A and B, and after 14, 35, and 57 days at sites D and E. High water delayed the second collection at the downstream sites. The extra coarse-mesh bag at site E was also collected after 57 days. Only two coarse-mesh bags were retrieved from site B on the third date; data from two damaged, coarse-mesh bags at site B and site D were discarded.

During collection, litter bags were removed from the water, sealed in plastic bags with stream water, and transported to the laboratory in a cooler. The litter bags were processed immediately. Leaves were washed onto a 500-μm sieve to remove silt and invertebrates, dried at 80°C for 48 h and weighed to determine dry mass remaining. Then leaves were ashed in a muffle furnace at 550°C for 4 h, cooled in a desiccator and re-weighed to determine ash-free dry mass.

Water temperature was recorded over the duration of the experiment using TinyTag Underwater thermistors (Gemini Data Loggers, Chichester, UK), set to record at 2-h intervals. The thermistors were placed in coarse-mesh litter bags secured to rebar stakes in the same manner as the other bags. The temperature readings, accurate to ±0.1°C, were later converted to daily means.

All invertebrates retained on the 500-μm sieve during washing of the leaves were preserved in 70% ethanol, and later identified to the lowest practical level, usually genus, using Tachet et al. (2006). Invertebrates were classified into functional feeding groups according to Tachet et al. (2006) and information

in the literature. Head capsule width and body length were measured in randomly chosen subsamples of shredders from each genus. Number of animals measured varied according to their abundance, from 6 (*Capnia*) to 88–89 (*Leuctra*, *Nemoura*), but included all specimens of larger organisms (Limnephilinae, *Taeniopteryx*). The total volume of shredding invertebrates, used as a surrogate for biomass, was calculated assuming the invertebrates are cylindrical.

Mycelial biomass

Five leaf disks, 12.5 mm diameter, were extracted from different leaves in each litter bag, avoiding veins, with a cork borer, freeze-dried, and weighed. Ergosterol extracted from the disks was used as an indicator of mycelial biomass (Gessner & Chauvet, 1993; Suberkropp et al., 1993; Graça et al., 2005). Mycelial biomass was not estimated on leaves in coarse-mesh bags on the last collection (57 days) because too few intact leaves remained.

Leaf disks were immersed in 5 ml KOH/methanol (8 g/l) at 80°C for 30 min to extract and saponify lipids. The extract was then purified by solid-phase extraction (Waters Oasis HLB 3-cc cartridges, Waters Corp., Milford, MA, USA; Graça et al., 2005). Ergosterol in the purified extract was quantified by HPLC (HPLC pump 422, HPLC detector 432, HPLC autosampler 360; Kontron Inst., Neufahru, Germany) by measuring absorbance at 282 nm. The HPLC detector was equipped with an FLT 0.5-mm A-316 precolumn (Upchurch Science, Oak Harbor, WA, USA) and a LispRP 18-5 250 × 4.6-mm column (Thermo-Hypersil Keystone, Bellefonte, PA, USA) maintained at 33°C. The mobile phase was 100% methanol and the flow rate was 1.4 ml/min. Ergosterol concentrations were multiplied by 182 to convert to units of mycelial biomass, expressed as milligrams of mycelial biomass per gram of leaf litter dry mass (Gessner & Chauvet, 1993). The mass of the extracted disks was added back to the mass remaining of the litter.

Statistics

Data on leaf litter AFDM remaining were fitted to a negative exponential model ($M_t/M_0 = e^{-kt}$) of percent mass remaining (M) over time (t). Linear regressions on ln-transformed percent AFDM remaining yielded estimates of decomposition rate constants (k, d^{-1}). The fit of significant regressions was judged by the R^2 value and how closely the intercept approached 100% (see "Results" section). Percent AFDM remaining was also regressed against cumulative heat units (degree-days) to test whether differences in mass loss rates among sites persisted after compensating for temperature differences. Cumulative heat units were calculated as the mean daily temperatures recorded by the thermistors, summed for each sampling interval.

Widely differing intercepts in the exponential models for fine-mesh bags versus coarse-mesh bags made direct comparisons of slopes among sites insensitive. Instead, mass loss rates were compared among sites using ANCOVA on ln-transformed AFDM remaining, using time or cumulative heat units as covariates, followed by Tukey's post hoc test. Linear regression was used to evaluate whether decomposition rates (k values) could be predicted from water temperatures, average numbers of shredders, or mesh size of the litter bags. Mesh size was entered as a dummy variable taking the value of 0 for fine-mesh bags and 1 for coarse-mesh bags (Zar, 2010).

Preliminary analysis showed no difference in fungal biomass (ergosterol content) between leaves in coarse-mesh and fine-mesh bags (t test, $P > 0.20$). Therefore, data from both types of litter bags were combined to provide eight replicates for each collection at each site. Fungal biomass was then compared among sites using ANOVA in a two-way factorial design, with collection time and site as main factors, followed by Tukey's post hoc test.

Data on shredders colonizing litter bags were non-normally distributed (Shapiro–Wilk test, $P < 0.01$) because of many low values and zeros. Therefore, differences among sites in numbers of shredders on each litter bag were determined using the Kruskal–Wallis test and a non-parametric post hoc test. Relationships between shredder abundances or biovolumes on each litter bag and various measures of decomposition rate were explored using Spearman's rank correlation. All of the above analyses were carried out using Statistix 9 (Analytical Software, Tallahassee, FL). Finally, detrended correspondence analysis (MVSP, Kovach Computing Services, Wales, UK) on ln-transformed shredder abundances was used to detect spatial associations of shredder taxa and

attempt to identify different shredder assemblages at different sites along the river system.

Results

Daily mean temperature ranged from 1.4 ± 1.3°C at site A to 7.6 ± 0.8°C at site E ($n = 57$). The difference in mean temperature between sites A and B was small (0.3°C) compared with all other site differences (Fig. 2).

A strong spate following 3 days of heavy rain in the mountains at the scheduled time of the second litter bag collection (8 December) delayed access to the downstream sites and removed some litter bags completely. For the extant samples, the fit to the negative exponential model was reasonably close at all sites ($r^2 = 0.66$–0.96, except site D, fine-mesh, $r^2 = 0.42$). However, the regression intercept for data from coarse-mesh bags always exceeded 100%, (range 107–117%) while the intercepts from fine-mesh bags were always <100% (range 81–96%).

Decomposition rate (k) for coarse-mesh bags over all four sites was significantly greater than k for fine-mesh bags ($F_{3,87} = 28.9$, $P < 0.001$), being on average twice as high (range 1.4–3.1 times). ANCOVA on ln-transformed mass remaining revealed a significant downstream trend in both coarse-mesh bags ($F_{3,38} = 5.5$, $P < 0.01$) and fine-mesh bags ($F_{3,42} = 3.9$, $P < 0.05$); in both situations, decomposition rate was significantly greater at site E than at site A, with site B or site D intermediate (Fig. 3). While this result

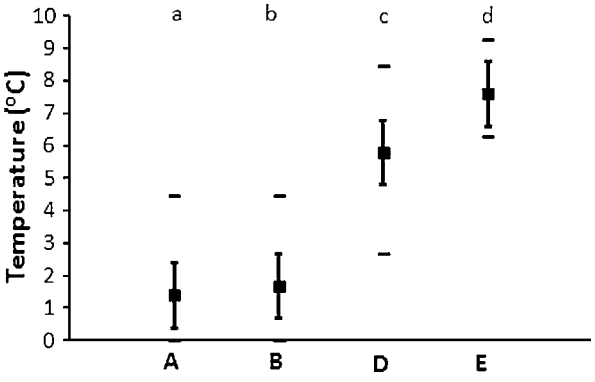

Fig. 2 Summary of temperature data at the study sites. Data are means (*squares*), standard deviations (*error bars*), maxima and minima (*dashes*). $n = 57$ daily means based on two-hourly thermistor measurements. All site differences are significant at $P < 0.01$

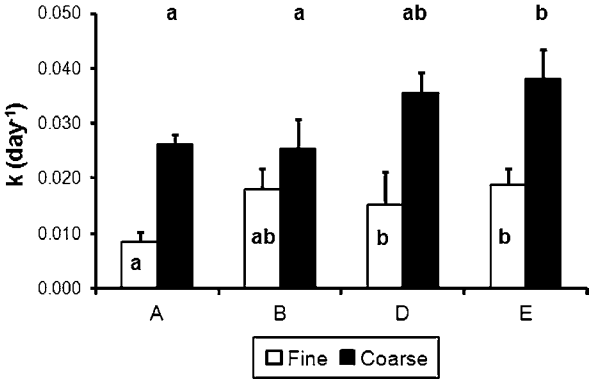

Fig. 3 Decomposition rates (±SE) of alder leaves in the Lasset–Hers river system in coarse-mesh (*solid bars*) or fine-mesh (*open bars*) bags. *Different letters* denote significant differences in mass remaining indicated by ANCOVA with time as a covariate ($P < 0.05$, $n = 10$–13, except *site D*, coarse-mesh: $n = 8$)

was predicted for litter in fine-mesh bags, the result for coarse-mesh bags refutes our first hypothesis. When decomposition rates were expressed in terms of degree-days in place of days, correcting for temperature differences among sites, the rates in coarse-mesh bags were 2–4 times faster ($F_{3,38} = 7.6$, $P < 0.001$) at sites A and B than at sites D and E (Fig. 4). There were also significant differences among rates in fine-mesh bags ($F_{3,42} = 4.4$, $P < 0.01$), with site B sustaining a higher rate than any of the remaining sites (Fig. 4), but no clear patterns related to position along the water course. Hence, removing the effect of temperature

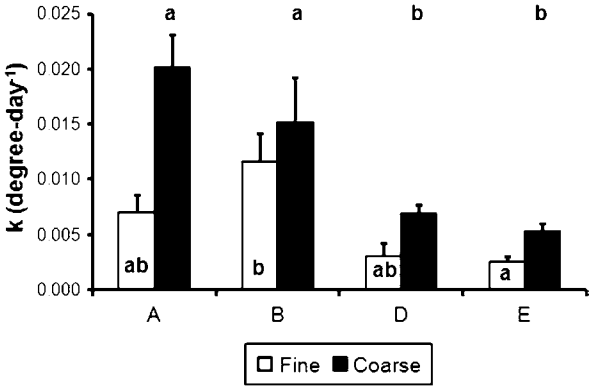

Fig. 4 Decomposition rates (±SE) of alder leaves in the Lasset–Hers river system in coarse-mesh (*solid bars*) or fine-mesh (*open bars*) bags, expressed in terms of heat units. *Different letters* denote significant differences in mass remaining indicated by ANCOVA with time as a covariate ($P < 0.05$, $n = 10$–13, except *site D*, coarse-mesh: $n = 8$)

removed or reversed the relatively faster decomposition downstream, in accordance with our second hypothesis.

ANOVA on ergosterol data from sites A, B, D, and E revealed significant differences among sites ($F_{3,62} = 7.5$, $P < 0.001$) and time in the stream ($F_{3,62} = 5.3$, $P < 0.01$) and a strong interaction between them ($F_{6,62} = 6.6$, $P < 0.001$). The interaction arises because there was a significant increase in mycelial biomass at site A ($r^2 = 0.60$, $P = 0.0001$, $n = 20$) and site B ($r^2 = 0.38$, $P < 0.001$, $n = 18$) over the 57 days that litter was decomposing in the stream, but no detectable change at sites D and E (Fig. 5). Over the whole experiment, however, leaf litter from sites A and B supported significantly more mycelial biomass than litter from sites D and E (Fig. 5). In contradiction to our third hypothesis, there was no significant correlation ($P > 0.10$) between AFDM remaining in fine-mesh or coarse-mesh litter bags and mycelial biomass, indicated by ergosterol content. There was also no significant correlation between mycelial biomass and mass loss during each sampling interval, nor between mean mycelial biomass and k ($P > 0.10$, $n = 8$).

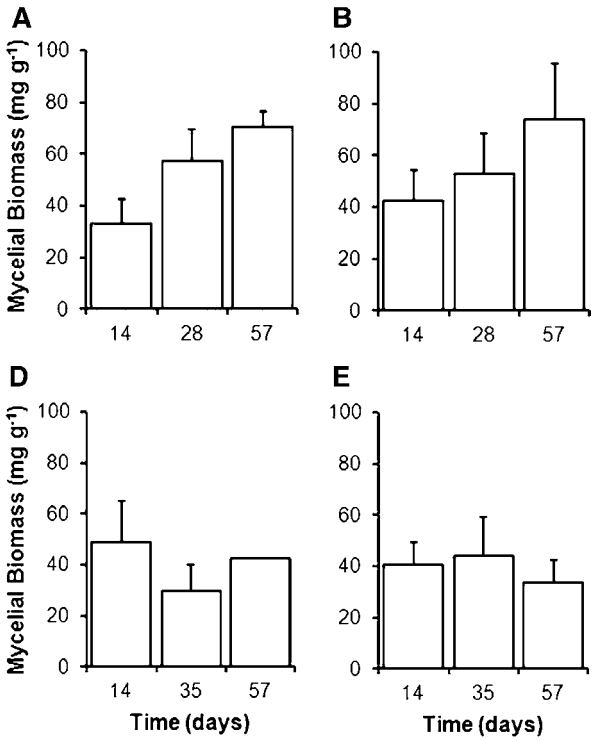

Fig. 5 Mycelial biomass on leaf litter decomposing at sites **A**, **B**, **D** and **E** in the Lasset–Hers river system. *Each bar* is the mean of eight replicates (four on day 57), combining coarse-mesh and fine-mesh bags, except where some bags were lost. *Error bar* represents standard deviation. *Note* change in date of second collection. Sites **A** and **B** are significantly different from sites **D** and **E** (Tukey's post hoc test, $P < 0.05$)

A total of 17 shredder genera were captured in coarse-mesh litter bags, of which ten were too infrequent for quantitative analysis (each <1.5% of total animals captured) and were not considered individually. The eight abundant shredder taxa were overwhelmingly Plecoptera. *Leuctra* was the most common shredder (>30% of total animals), followed by the family Nemouridae (*Nemoura*: 30%, *Protonemura*: 13%, *Amphinemura*: 3%) and *Capnia* (2.6%). The remaining common shredders were the Trichopteran *Micrasema* (10%) and the amphipod *Gammarus* (3.7%). Numbers varied widely among litter bags and collections, but showed few temporal patterns.

Across the entire experiment, there was no significant difference in total shredder densities or biovolumes among the study sites ($P > 0.10$, $n = 41$). Individual taxa, however, were more or less abundant upstream or downstream, leading to noticeably different assemblages at different sites (Table 2). Litter bags at sites A and B were dominated by Nemourid stoneflies, with lesser numbers of *Micrasema*. Litter bags at site E were not only strongly dominated by *Leuctra* but also supported *Gammarus* and some *Capnia.* Site D tended to be intermediate between the upstream sites and site E (Table 2).

Table 2 Median numbers of shredders in each coarse-mesh litter bag over the three collections of the 57-day experiment

Taxon	Site			
	A ($n = 12$)	B ($n = 8$)	D ($n = 10$)	E ($n = 13$)
All shredders	40^{a}	32^{a}	42.5^{a}	47^{a}
Leuctra	2.0^{a}	0.5^{a}	9.5^{ab}	39^{b}
Nemoura	9.0^{a}	9.5^{a}	24.5^{a}	0^{b}
Protonemura	13.5^{a}	7.5^{b}	0^{b}	0^{b}
Micrasema	7.5^{a}	5^{ab}	2.5^{bc}	1^{c}
Gammarus	0	0	0^{ab}	2^{b}
Amphinemura	2.5^{a}	0^{ab}	0^{b}	0^{b}

Different superscript letters denote significant differences within rows (Kruskal–Wallis test and non-parametric post hoc test, $P < 0.10$)

Analysis of biovolumes produced identical patterns of site differences

At the level of individual bags or means for each sampling interval, there were few significant correlations between densities (or biovolumes) of shredders in litter bags and measures of decomposition rate, calculated as AFDM remaining, average mass loss per sampling interval, or mass loss per degree-day. There were, however, negative nonlinear correlations between densities of *Leuctra* and *Nemoura* ($r_s = -0.56$, $P = 0.038$), *Protonemura* ($r_s = -0.54$, $P = 0.048$), and total Nemouridae ($r_s = -0.50$, $P = 0.07$), suggesting a marked spatial separation of Leuctridae and Nemouridae (cf. Fig. 6).

Detrended correspondence analysis accounted for 31.4% of the variation in shredder densities on the first axis and 12.2% on the second axis. While the first axis reiterated the strong directional pattern imposed on the data by the experimental design, it illustrated the distinctly different shredder communities found at different sites. Shredder assemblages at sites A and B were overlapping and indistinguishable, but the assemblages at sites D and E were each largely separated from each other and from the upstream sites, and clearly distinguishable along the first axis (Fig. 7). The ordinations confirm that the shredder assemblage changes in a consistent manner along the course of the Lasset–Hers River system.

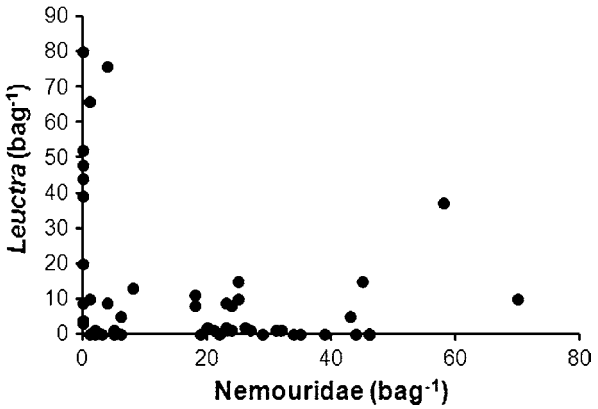

Fig. 6 Scatterplot showing relationship between densities of leaf-shredding stoneflies Nemouridae and *Leuctra* on litter bags over the course of the study. *Each point* represents a single litter sample

The difference between estimated mass remaining after 57 days (by regressions) in coarse-mesh bags and in fine-mesh bags represents the mass loss attributable to shredder activity. By this measure, shredders were responsible, on average, for removing 19% of original mass, compared with 61% for microbes; the low contribution of shredders at site B is based on fewer samples than at the other sites (Table 3). Shredders were proportionately more important at site A than at sites downstream, but otherwise there was no consistent downstream trend (Table 3).

A multiple linear regression of k against mean water temperature and mesh size of the litter bag accounted for 90% of the variation in values of k along the length of the Lasset–Hers system ($F = 22.7$, $P = 0.003$, $n = 8$). Essentially the same result can be obtained from regressions of k on densities of *Leuctra* and Nemouridae in the litter bags ($r^2 = 0.87$, $P = 0.007$) or *Leuctra* and the genus *Nemoura* alone ($r^2 = 0.89$, $P = 0.004$), without including temperature

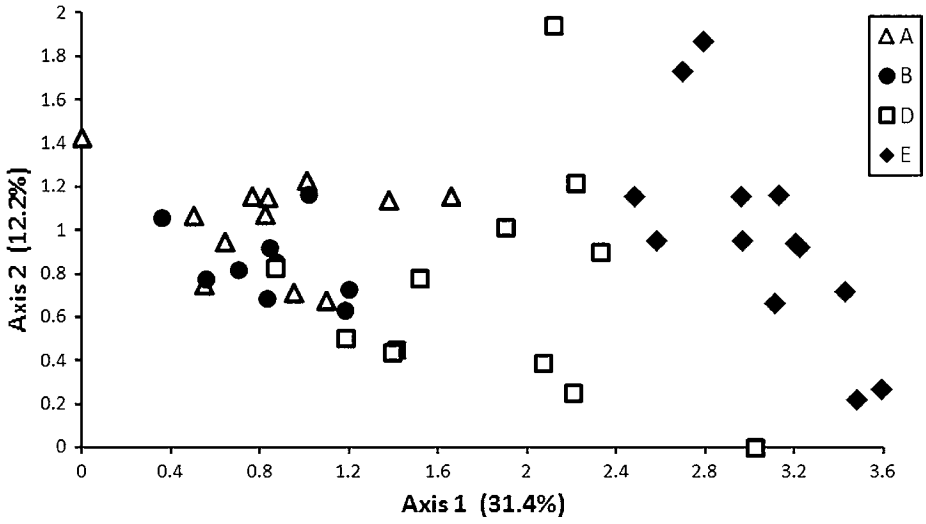

Fig. 7 Detrended correspondence analysis biplot comparing shredder assemblages in coarse-mesh litterbags from sites *A* (*open triangles*), *B* (*filled circles*), *D* (*open squares*), and *E* (*filled diamonds*) on the Lasset–Hers river system. *Each point* represents one site and one collection time, separated by the dissimilarity of the shredder assemblages

Table 3 Proportions of mass loss from decomposing alder leaves attributable to microbial metabolism or leaf-shredding invertebrates

Site	Predicted mass loss in 57 days (%)			
	Total	Microbes	Shredders	Ratio
A	74.6	46.0	28.6	1.6
B	74.7	65.7	9.0	7.3
D	84.6	66.0	18.5	3.6
E	86.8	67.1	19.7	3.4

Values were calculated as: predicted mass loss from coarse-mesh bags (total), predicted mass loss from fine-mesh bags (microbes), and the difference between them (shredders). Ratio is the contribution of microbes over the contribution of shredders. Values for microbes include leaching losses; values for shredders include losses from leaching and physical fragmentation

as a variable. No other shredding taxon, or combination of taxa, produced regressions of similar predictive strength.

Discussion

Our first hypothesis, that more active or efficient invertebrate feeding upstream would counter the faster microbial decomposition at warmer downstream sites, was not supported. Rather, leaf litter in both fine-mesh and coarse-mesh bags showed a modest but detectable increase in mass loss downstream, commensurate with the relatively small change in mean temperature. This result runs contrary to work on larger rivers elsewhere (Minshall et al., 1983; Collier & Winterbourn, 1986; Chauvet et al., 1993; MacDonald & Taylor, 2008) but is consistent with earlier work on this river system by Fabre & Chauvet (1998). However, when the effect of warmer water downstream was removed through regressions based on heat units, decomposition rates upstream were substantially greater than downstream, in accordance with our second hypothesis. The downstream trend of increasing temperature is positively correlated with trends in water quality (nutrient and calcium contents, pH) which could also influence decomposition rates (Suberkropp & Chauvet, 1995; Dangles & Guérold, 2001a, b; Woodward et al., 2012). However, the reversal of the downstream trend in k when time was replaced by heat units strongly suggests that it is temperature itself, and not other variables correlated with temperature, that is responsible for the more rapid decay at downstream sites.

Whether the effect of invertebrate feeding is sufficient to reverse the downstream increase in decay rate arising from warmer water would depend on the temperature difference and whether the temperature downstream precluded stenothermal shredders. In the Lasset–Hers system, the temperature difference between sites A and E averaged $\sim$6°C in autumn, suggesting that microbial metabolism, and therefore the decomposition process, is highly sensitive to water temperature, as others have concluded (Irons et al., 1994; Bergfur, 2007; Ferreira & Chauvet, 2011a). Therefore, while invertebrate feeding was decidedly more efficient upstream, it was not sufficient to overcome the effect of warmer water downstream. Moreover, the farthest downstream site in this study, while situated in a low-gradient river flowing through pasture land, is only third order and still relatively small compared with riverine sites in the studies cited above. Consequently, sites D and E supported abundant shredder communities, of the same numerical abundance as upstream sites. This situation differs from studies in larger rivers where insect shredders were essentially absent downstream (Minshall et al., 1983; Collier & Winterbourn, 1986; Chauvet et al., 1993).

Our small-scale results run closely parallel with the global study of Boyero et al. (2011), who compared decomposition of alder leaves in fine-mesh and coarse-mesh bags over a wide range of latitudes. As in the current work, they found that mass loss rates in fine-mesh bags increased with water temperature, but the effect was removed in regressions on heat units, indicating strict temperature dependence of microbial growth. They also found that shredders became more important to decomposition with increasing latitude, much as they became more important upstream in the Lasset–Hers system. Hence, elevational gradients in leaf litter decomposition may reiterate latitudinal gradients to the degree that both are controlled by water temperature.

Based on the work of Fabre & Chauvet (1998), we anticipated that shredder communities at site D and especially site E would be dominated by Tipulidae and Limnephilidae. Unexpectedly, site D was dominated by the stoneflies *Nemoura* and *Leuctra*, and site E was dominated by *Leuctra* alone. Species in these genera are cold stenotherms; *Nemoura* is widespread in low-order streams at high altitudes or high latitudes (Prenda & Gallardo-Mayenco, 1999; Ramchunder

et al., 2011) and can tolerate below-zero temperatures (Walters et al., 2009). *Leuctra* is slightly more temperature-tolerant. *Leuctra nigra*, the common European species, shows sharply reduced survival above 14°C (Elliott, 1987). In eastern Canada, *Leuctra* (three species) rarely occurs in streams with mean temperatures above 17°C (MacDonald & Taylor, 2008; B. Taylor, unpublished data). Both these genera are commonly encountered in cold water, however, so there should be no impediment to *Leuctra* or *Nemoura* colonizing the entire Lasset–Hers system in autumn. The negative correlation between *Leuctra* and members of the family Nemouridae strongly suggests competitive exclusion of *Leuctra* from the coldest upstream sites, and Nemouridae from the warmest downstream site. It would be of interest to explore the distribution of these insects in summer when water temperatures at the downstream sites may exceed *Leuctra*'s thermal tolerance.

The displacement of *Leuctra* by Nemouridae may be central to the more efficient shredding at the upstream sites. More rapid mass loss upstream, at constant temperature, cannot be attributed to a greater density of shredders (in refutation of our third hypothesis), because total counts and biovolumes in litter bags were similar everywhere. Biovolume, our surrogate for biomass, followed the same patterns as counts because all the principal shredders were about the same size, except for Limnephilinae and *Taeniopteryx*, which were too infrequent to produce a noticeable effect. More rapid consumption of litter upstream than downstream (by each individual shredder) would not be expected because fungal standing crops, and therefore food quality of litter, were greater upstream. Water velocity (promoting fragmentation) did not differ greatly among sites and thus could not contribute to the observed upstream–downstream pattern.

Therefore, the replacement of Nemouridae upstream with *Leuctra* downstream remains as the only explanation for the difference in temperature-corrected mass loss rates along the Lasset–Hers system. This conclusion again contrasts with earlier work in Nova Scotia and northern Norway, where the presence of *Leuctra* is strongly associated with rapid decomposition (MacDonald & Taylor, 2008; Taylor & Andrushchenko, 2013). Nemourids are rare in the low hills of eastern Canada, however, and larger rivers support few shredders. Therefore, the upstream sites (second and third order) in MacDonald & Taylor (2008) correspond more closely with downstream sites on the Lasset–Hers system. If this supposition is correct, there may be a three-phase pattern of litter shredders, in which Nemouridae are replaced by *Leuctra*, which are in turn replaced by potamic species (*Gammarus*, Limnephilidae, Tipulidae) along a gradient of declining elevation (mountains to plain) and increasing stream order (first to fifth). The corresponding decline in the relative importance of shredders to leaf litter decomposition observed here may not be general, because the potamic species may be influential where their numbers are substantial.

Microbial metabolism was evidently the largest source of variation in decomposition in this system. The contribution of shredders, while substantial everywhere, did not change greatly along the length of the river, except that shredders were responsible for a greater proportion of total mass loss at site A. This constancy is a reflection of the small change in shredder abundances along the system. In a third-order stream in Germany, Hieber & Gessner (2002) estimated that shredders were responsible for 64% (of 91%) mass loss from decomposing *Alnus glutinosa* leaves, with fungi and bacteria contributing the remaining 27%, the reverse of the relative contributions estimated in our study. However, they calculated a shredder contribution of 8–35% in previous studies on alder and willow leaves (Hieber & Gessner, 2002), encompassing the early suggestion of 21–24% for hickory leaves (Petersen & Cummins, 1974).

The finding that 90% of the variation in decomposition rates along the length of the stream can be accounted for by linear regression, including only the presence of shredders and water temperature, illustrates the primal importance of these factors in determining rates of mass loss. Regressions of comparable strength using only *Leuctra* and *Nemoura* are evidence of the central role played by these shredders in particular, whose spatial distribution approximately matches the temperature gradient. Evidently, water temperature is a key control on decomposition rates even in cold water and where temperature variation is small.

Acknowledgments The authors are grateful to Didier Lambrigot and Sylvain Lamothe for their technical help in field and laboratory, and to Amanda Lowe and Greg Walsh for help in enumerating invertebrates. This manuscript also benefitted from constructive comments by two anonymous reviewers.

References

Assmann, C., K. Rinke, J. Nechwatal & J. von Elert, 2011. Consequences of the colonisation of leaves by fungi and oomycetes for leaf consumption by a gammarid shredder. Freshwater Biology 56: 839–852.

Baldy, V., M. O. Gessner & E. Chauvet, 1995. Bacteria, fungi and the breakdown of leaf litter in a large river. Oikos 74: 93–102.

Bärlocher, F., 1985. The role of fungi in the nutrition of stream invertebrates. Botanical Journal of the Linnean Society 91: 85–94.

Benfield, E. F., R. W. Paul Jr. & J. R. Webster, 1979. Influence of exposure technique on leaf breakdown rates in streams. Oikos 33: 386–391.

Bergfur, J., 2007. Seasonal variation in leaf-litter breakdown in nine boreal streams: implications for assessing functional integrity. Fundamental and Applied Limnology 169: 319–330.

Boyero, L., R. G. Pearson, M. O. Gessner, L. A. Barmuta, V. Ferreira, M. A. S. Graça, D. Dudgeon, A. J. Boulton, M. Callisto, E. Chauvet, et al., 2011. A global experiment suggests climate warming will not accelerate litter decomposition in streams but might reduce carbon sequestration. Ecology Letters 14: 289–294.

Chauvet, E., N. Giani & M. O. Gessner, 1993. Breakdown and invertebrate colonization of leaf litter in two contrasting streams: significance of Oligochaetes in a large river. Canadian Journal of Fisheries and Aquatic Science 50: 488–495.

Chergui, H. & E. Pattee, 1993. Fungal and invertebrate colonization of *Salix* fresh and dry leaves in a Moroccan river system. Archiv für Hydrobiologie 127: 57–72.

Chung, N. & K. Suberkropp, 2009. Effects of aquatic fungi on feeding preferences and bioenergetics of *Pycnopsyche gentilis* (Trichoptera: Limnephilidae). Hydrobiologia 630: 257–269.

Collier, K. J. & M. J. Winterbourn, 1986. Processing of willow leaves in two suburban streams in Christchurch, New Zealand. New Zealand Journal of Marine and Freshwater Research 20: 575–582.

Dangles, O. & F. Guérold, 2001a. Influence of shredders in mediating breakdown rates of beech leaves in circumneutral and acidic forest streams. Archiv für Hydrobiologie 151: 649–666.

Dangles, O. & F. Guérold, 2001b. Linking shredders and leaf litter processing: insights from an acidic stream study. International Review of Hydrobiology 86: 395–406.

Elliott, J. M., 1987. Temperature-induced changes in the life cycle of *Leuctra nigra* (Plecoptera: Leuctridae) from a Lake District stream. Freshwater Biology 18: 177–184.

Fabre, E. & E. Chauvet, 1998. Leaf breakdown along an altitudinal stream gradient. Archiv für Hydrobiologie 141: 167–179.

Ferreira, V. & E. Chauvet, 2011a. Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. Global Change Biology 17: 551–564.

Ferreira, V. & E. Chauvet, 2011b. Future increase in temperature more than decrease in litter quality can affect microbial litter decomposition in streams. Oecologia 167: 279–291.

Fernandes, I., B. Uzun, C. Pascoal & F. Cássio, 2009. Responses of aquatic fungal communities on leaf litter to temperature-change events. International Review of Hydrobiology 94: 410–418.

Gessner, M. O. & E. Chauvet, 1993. Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. Applied and Environmental Microbiology 59: 502–507.

Gessner, M. O., M. Dobson & E. Chauvet, 1999. A perspective on leaf litter breakdown in streams. Oikos 85: 377–384.

Giller, P. S. & B. Malmqvist, 1998. The Biology of Streams and Rivers. Oxford University Press, Oxford, UK: 296 pp.

Graça, M. A. S., 2001. The role of invertebrates on leaf litter decomposition in streams: a review. International Review of Hydrobiology 86: 383–394.

Graça, M. A. S., R. C. F. Ferreira & C. N. Coimbra, 2001. Processing along a stream gradient: the role of invertebrates and decomposers. Journal of the North American Benthological Society 20: 408–420.

Graça, M. A. S., F. Bärlocher M. & O. Gessner, 2005. Methods to Study Litter Decomposition: A Practical Guide. Springer, the Netherlands.

Hieber, M. M. & O. Gessner, 2002. Contribution of stream detrivores, fungi, and bacteria to leaf breakdown based on biomass estimates. Ecology 83: 1026–1038.

Imberger, S. J., C. J. Walsh & M. R. Grace, 2008. More microbial activity, not abrasive flow or shredder abundance, accelerates breakdown of labile leaf litter in urban streams. Journal of the North American Benthological Society 27: 549–561.

Irons, J. G., M. W. Oswood, R. J. Stout & C. M. Pringle, 1994. Latitudinal patterns in leaf litter breakdown – is temperature really important. Freshwater Biology 32: 401–411.

MacDonald, E. E. & B. R. Taylor, 2008. Factors influencing litter decomposition rates in upstream and downstream reaches of river systems in eastern Canada. Fundamental and Applied Limnology 172: 71–86.

Minshall, G. W., R. C. Petersen, K. W. Cummins, T. L. Bott, J. R. Sedell, C. E. Cushing & R. L. Vannote, 1983. Interbiome comparison of stream ecosystem dynamics. Ecological Monographs 53: 1–25.

Minshall, G. W., K. W. Cummins, R. C. Petersen, C. E. Cushing, D. A. Bruns, J. R. Sedell & R. L. Vannote, 1985. Developments in stream ecosystem theory. Canadian Journal of Fisheries and Aquatic Sciences 42: 1045–1055.

Petersen, R. C. & K. W. Cummins, 1974. Leaf processing in a woodland stream. Freshwater Biology 4: 343–368.

Prenda, J. & A. Gallardo-Mayenco, 1999. Distribution patterns, species assemblages and habitat selection of the stoneflies (Plecoptera) from two Mediterranean river basins in southern Spain. International Review of Hydrobiology 84: 595–608.

Rajashekar, M. & K. M. Kaveriappa, 2000. Effects of temperature and light on growth and sporulation of aquatic hyphomycetes. Hydrobiologia 441: 149–153.

Ramchunder, S. J., L. E. Brown, J. Holden & R. Langton, 2011. Spatial and seasonal variability of peatland stream ecosystems. Ecohydrology 4: 577–588.

Stockley, R. A., G. S. Oxford & R. F. G. Ormond, 1998. Do invertebrates matter? Detrital processing in the River Swale-Ouse. Science of the Total Environment 210(211): 427–435.

Suberkropp, K. & E. Chauvet, 1995. Regulation of leaf breakdown by fungi in streams: influences of water chemistry. Ecology 76: 1433–1445.

Suberkropp, K., M. O. Gessner & E. Chauvet, 1993. Comparison of ATP and ergosterol as indicators of fungal biomass associated with decomposing leaves in streams. Applied and Environmental Microbiology 59: 3367–3372.

Tachet, H., P. Richoux, M. Bournaud & P. Usseglio-Polatera, 2006. Invertébrés d'eau douce: systématique, biologie, écologie. CNRS Editions, Paris, France: 587 pp.

Tank, J. L., E. J. Rosi-Marshall, N. A. Griffiths, S. A. Entrekin & M. L. Stephen, 2010. A review of allochthonous organic matter dynamics and metabolism in streams. Journal of the North American Benthological Society 29: 118–146.

Taylor, B. R & I. V. Andrushchenko, 2013. Interaction of water temperature and shredders on leaf litter breakdown: a comparison of streams in Canada and Norway. Hydrobiologia. doi:10.1007/s10750-013-1650-2.

Taylor, B. R. & A. N. Dykstra, 2005. Effects of hot ground water on a small swamp-stream in Nova Scotia, Canada. Hydrobiologia 545: 129–144.

Walters, K. R., T. Sformo, B. M. Barnes & J. G. Duman, 2009. Freeze tolerance in an arctic Alaska stonefly. The Journal of Experimental Biology 212: 305–312.

Webster, J. R. & E. F. Benfeld, 1986. Vascular plant breakdown in freshwater ecosystems. Annual Review of Ecology and Systematics 17: 567–594.

Wiggins, G. B. & R. J. Mackay, 1978. Some relationships between systematics and trophic ecology in Nearctic aquatic insects, with special reference to Trichoptera. Ecology 59: 1211–1220.

Woodward, G., M. O. Gessner, P. S. Giller, V. Gulis, S. Hladz, A. Lecerf, B. Malmqvist, B. McKie, S. D. Tiegs, H. Cariss, et al., 2012. Continental-scale effects of nutrient pollution on stream ecosystem functioning. Science 336: 1438–1440.

Zar, J. H., 2010. Biostatistical Analysis, 5th ed. Pearson Prentice Hall, Upper Saddle River, NJ.