CHAPTER 6

LEAF MASS LOSS ESTIMATED BY LITTER BAG TECHNIQUE

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1. INTRODUCTION

Leaf litter is a dominant component of coarse particulate organic matter in streams, and its decomposition has received considerable attention (Webster & Benfield 1986, Allan 1995, Gessner et al. 1999). Gessner & Chauvet (2002) proposed using leaf litter breakdown to evaluate functional stream integrity. In order to increase the sensitivity and robustness of the assay, 'noise' due to non-standardized procedures has to be minimized. Many studies have used pre-dried leaves or leaf disks enclosed in litter bags. Several aspects of this approach have been criticized as introducing artificial modifications of the natural process (Petersen & Cummins 1974, Wieder & Lang 1982, Boulton & Boon 1991, Bärlocher 1997). Mass loss in litter bags (1 mm mesh size) resembles that of loose, naturally entrained leaves in depositional zones, while mass loss in litter packs (leaves tied together and tethered in streams) is close to that of loose leaves in riffle areas (Cummins et al. 1980). On the other hand, the use of litter bags with different mesh sizes allows size-selective exclusion of macro-consumers.

The mass loss of leaves and needles as a function of time is most often approximated by an exponential decay model:

$$M_t = M_0 \cdot e^{-kt} \tag{6.1}$$

where M_t = mass at time t; M_0 = mass at time 0; k = exponential decay coefficient; and t = time in days. Based on their daily decay coefficient, leaves have been classified as "fast" (k > 0.01), "medium" (k = 0.005—0.001) and "slow" (k < 0.005) based on extensive work in a stream in Michigan (Petersen & Cummins 1974). However, the decomposition rate of a given leaf species can vary greatly among streams (Suberkropp & Chauvet 1995), suggesting that Petersen & Cummins' (1974)

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classification has limitations when applied uncritically. A few typical values of *k*, with number of days required for 50% mass loss, are listed in Table 6.1.

Table 6.1. Daily decay rates, k, and number of days to reach 50% mass loss (=T_{50}), of selected leaf species (data from Petersen & Cummins 1974, based on leaf packs).

Category	*Species*	*k*	T_{50}
Fast	*Fraxinus americana*	0.0120	58
	Tilia americana	0.0175	40
Medium	*Carya glabra*	0.0089	78
	Salix lucida	0.0078	89
Slow	*Fagus grandifolia*	0.0025	277
	Quercus alba	0.0022	315

The exponential decay equation is typically converted to a linear form before regression is performed.

$$\ln[M_t] = \ln[M_0 \cdot e^{-kt}] = \ln[M_o] - kt \tag{6.2}$$

rewritten as

$$Y = a + bX \tag{6.3}$$

Y is the dependent variable, corresponding to M_t. The independent variable *X* equals time in days. The linear regression procedure, which minimizes the sum of squares, determines the slope *b* (equals decay coefficient *k*) and the intercept *a* (calculated mass at time 0, which should be close to 100%). Most computer programs will also calculate R^2. This indicates how much of the variance among the data is due to the linear relationship between the *X* and *Y* values. For example, a value of 0.95 corresponds to 95%.

Linear regression calculations are only valid when the experimental uncertainty of replicate *Y* values is not related to the values of *X* or *Y* (Zar 1998). This is not usually the case after data transformation, which tends to enhance errors associated with small *Y* values. These points will be emphasized by linear regressions and points with large *Y* values will be relatively ignored. Thus, linearizing transformations is not an ideal procedure because it distorts experimental errors (Motulsky & Ransnas 1987, Motulsky & Christopoulos 2003).

The alternative is nonlinear regression analysis. This is defined as fitting data to any selected equation. As with linear regression, nonlinear regression procedures determine values of the parameters that minimize the sum of the squares of the distances of the data points to the curve. The approach is only appropriate when the experimental uncertainty is normally distributed, and not related to the values of *X* or *Y*. Nonlinear regression (or curve-fitting) must be solved iteratively, rather than analytically, and an initial estimate of each parameter must usually be provided.

During the fitting procedure, these values are modified to increasingly improve the fit (lower the sum of squared deviations) of the curve to the data. These iterations are continued until additional improvements are negligible.

Often two sets of data are fitted to the same model, and the question is whether the two sets of data differ significantly. For example, do eucalypt and alder leaves decay at significantly different rates? A good introduction to this topic can be found at http://www.graphpad.com/curvefit. The recommended approach is to repeat the experiment several times and compare the resulting estimates of the parameter k with a t-test, which compares a difference with the standard error of that difference. This method is labour-intensive and statistically conservative; the calculated p value may be too high. If the experiment has only been done once, the best-fit value of two groups can still be compared with a t-test by using the standard error reported by the curve-fitting program. This again assumes normal error distribution, which is approximately true for the exponential decay equation.

A commonly used approach is to analyze the two data sets separately as well as simultaneously. The question then is whether the separate fits are significantly better than the pooled fit. This method, known as Analysis of Covariance or ANCOVA, is again strictly valid only for a linear relationship between X and Y. The details of this test can be found in Zar (1998). Another approach is based on Akaike's Information Criterion (AIC), which answers the following questions: Which model is more likely to have generated the data? How much more likely? The theory behind AIC is quite difficult; it combines maximum likelihood, information theory, and the concept of information entropy. Fortunately, the computations and interpretation of the results are straightforward (Motulsky & Christopoulos 2003).

Finally, two (or more) data sets can be compared by permutation or randomization tests. The first step is to define a test statistic S, e.g., the difference between several estimates of the k values of alder and eucalypt. Calculate the value of S for the original data set. All values are then pooled and randomly assigned to alder or eucalypt. The difference is calculated. This is repeated many times, giving the distribution of all possible values of S. The final question is: How extreme is the S of the original data compared to all possible values? If it is more extreme than 5% (or 1%) of the population, H_0 is rejected. If we do not want to make the assumption of linear decay, we can choose another test statistic. For example, we can simply add up all data (% remaining mass on all sampling dates) separately for the two leaf species, and determine the difference. The null hypothesis is that low and high values are randomly distributed between alder and eucalypt. We again pool the data, randomly distribute them between alder and eucalypt, and calculate the new value of S. This is repeated many times, and we determine how extreme the original S is.

Sometimes, the single exponential model is clearly inappropriate (Wieder & Lang 1982). When the leaf consists of two clearly defined components decaying at different rates, a double exponential equation gives a better fit. When decomposition does not appear to proceed beyond a certain point, an asymptotic model provides a better fit (Sridhar et al. 2002). How does one decide which model gives a better fit? (Zen Koan of Statistics: the person with one watch knows what time it is; the person

with two watches is never sure.) A simple comparison of sum of squares (or R^2) values is inappropriate, since a curve with more parameters nearly always has a lower sum of squares because it has more inflection points (Kvålseth 1985). The question is whether this decrease is worth the "cost" of the additional variables, whose inclusion in the model results in a loss of degrees of freedom. Two approaches are commonly used: an *F* test (extra sum-of-squares test), or Akaike's Information Criterion. The appropriate procedures for making the decision are described in Motulsky & Christopoulos (2003).

2. EQUIPMENT, CHEMICALS AND SOLUTIONS

2.1. Equipment and Material

- Autumn-shed leaves (fresh and/or air-dried)
- Litter bags (10 x 10 cm, 1-mm mesh size; alternatively, 0.5-mm and 10-mm mesh size)
- Plastic labels (DYMO or laser printed numbers on transparencies)
- Drying oven (40—50°C)
- Balance (±1 mg precision)
- Statistics program or calculator

3. EXPERIMENTAL PROCEDURES

3.1. Sample Preparation

1. Leaves are collected and prepared as in Chapter 5 (Leaching). Depending on objectives, mass loss rates of different leaf species, of leaves in different mesh size bags or leaves placed in different rivers can be compared.
2. Place 2—3 preweighed leaves (or 3—4 g) in each labelled litter bag.
3. Prepare a sufficient number of bags to allow 4—6 replicates per sampling date plus two extra sets, one set to convert air-dry mass to oven-dry mass and another set to determine handling losses (see below).

3.2. Exposure of Litter Bags

1. Anchor leaf bags to the stream bed with bricks, steel pegs, etc. Depending on objectives, all bags may be placed in riffle or pool areas. Care must be taken not to place too many bags close to each other, because this may drastically change current patterns and thereby affect colonization by microorganisms and invertebrates. Alternatively, leaf bags may be attached to rebars anchored to the stream bed.

3.3. Recovery of Bags and Analysis

2. To correct air-dry mass of all leaf bags for humidity, dry a first set of bags at 40—50 °C for 2 days (or until mass remains constant) and weigh. Calculate an average correction factor, *D*.
3. *D* = (oven-dry mass) / (air-dry mass).
4. The initial oven-dry mass of each leaf pack brought to the streams is estimated by multiplying the measured air-dry mass by the average correction factor, *D*.
5. A second set of bags should be recovered immediately upon exposure in the stream. This allows an estimate of losses due to initial handling.
6. Subsequent samples are taken according to a preplanned schedule, for example after 3, 7, 14, 21, 28, and 35 days. With slowly decomposing leaves, an extended sampling schedule (up to 3 months or even longer) may be necessary.
7. Rinse leaves under running tap water, dry to constant mass and weigh.

3.4. Statistical Analysis

1. Express mass loss as percentage of original mass after correction for (1) humidity and (2) handling (100% = mass after corrections).
2. Run a regression analysis. (a) A linear regression with the original data (Time = independent variable; % Mass remaining = dependent variable). (b) A non-linear curve-fitting program (exponential decay; in most programs, you will have to type in the equation, and provide initial estimates of the parameters that are to be determined (in the exponential decay model, provide an estimate of *a* and *k*). (c) Transform [% Mass remaining] to ln[% Mass remaining] and run a linear regression (Time = independent variable; ln[% Mass remaining] = dependent variable).
3. Generally, the differences in slope estimates among the three models are small, but the non-linear curve-fit often provides the best estimate for the intercept (which, by definition, is 100%).
4. If data from more than one series have been collected (e.g., 2 or more leaf species), an analysis of covariance using Time as covariate can be run. This can be done in some computer programs, or as described in Zar (1998). Provided the estimated initial leaf mass of the two series are similar, the decay coefficients are significantly different if the *p* value for the interaction between time and series is < 0.05. Alternatively, an appropriate test statistic may be formulated and a corresponding permutation test performed (e.g. with the software Resampling Stats; see also Chapter 43).
5. If a pronounced steep decline during the early phase of decomposition is observed (which may be due to leaching), try fitting the data to a more complex model (e.g., double exponential decay). Motulsky & Christopoulos (2003) can be a useful to decide which model is more appropriate.

4. REFERENCES

Allan, J.D. (1995). *Stream Ecology. Structure and Function of Running Waters*. Chapman & Hall. London.

Bärlocher, F. (1997). Pitfalls of traditional techniques when studying decomposition of vascular plant remains in aquatic habitats. *Limnética*, 13, 1-11.

Boulton, A.J. & Boon, P.I. (1991). A review of the methodology used to measure leaf litter decomposition in lotic environments: time to turn over an old leaf? *Australian Journal of Marine and Freshwater Research*, 42, 1-43.

Cummins, K.W., Spengler, G.L., Ward, G.M., Speaker, R.M., Ovink, R.W., Mahan, D.C. & Mattingly, R.L. (1980). Processing of confined and naturally entrained leaf litter in a woodland stream ecosystem. *Limnology and Oceanography*, 25, 952-957.

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

Gessner, M.O. & Chauvet, E. (2002). A case for using litter breakdown to assess functional stream integrity. *Ecological Applications*, 12, 498-510.

Kvålseth, T.O. (1985). Cautionary note about R^2. *The American Statistician*, 39, 279-285.

Motulsky, H.J. (1995). *Intuitive Biostatistics*. Oxford University Press. Oxford.

Motulsky, H.J. & Rasnsnas, L.A. (1987). Fitting curves to data using nonlinear regression: a practical and non-mathematical review. *Journal of The Federation of American Societies for Experimental Biology*, 1, 365-374.

Motulsky, H.J. & Christopoulos, A. (2003). Fitting models to biological data using linear and nonlinear regression. A practical guide to curve fitting. San Diego: GraphPad Software, Inc., www.graphpad.com

Petersen, R.C. & Cummins, K.W. (1974). Leaf processing in a woodland stream. *Freshwater Biology*, 4, 343-368.

Sridhar, K.R., Krauss, G., Bärlocher, F., Raviraja, N.S., Wennrich, R. & Baumbach, R. (2002). Decomposition of alder leaves in two heavy metal polluted streams in Central Germany. *Aquatic Microbial Ecology*, 26, 73-80.

Wieder, R.K. & Lang, G.E. (1982). A critique of the analytical methods used in examining decomposition data obtained from litter bags. *Ecology*, 63, 1636-1642.

Zar, J.H. (1998). *Biostatistical Analysis*. Prentice-Hall. New York.