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A SIMPLE BIOASSAY FOR DETRITAL CONSUMER ACTIVITY IN STREAMS

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Introduction

The basic premise that streams in the temperate zone, like soil communities, are heterotrophic by evolutionary design is now fairly well documented (e.g. Fisher and Likens, 1972; Cummins et al., 1973; Petersen and Cummins, 1973). That is, community respiration exceeds photosynthesis, since the primary energy source is reduced carbon from the surrounding terrestrial environment. As processors of organic matter elaborated on the landscape, streams are most likely to be fundamentally altered by shifts to autotrophy, either by increased in-stream primary production or by changes in the normal allochthonous inputs. Therefore, measurement of the status of allochthonous organic matter processing in a given stream under a given set of conditions should provide a sensitive community monitoring strategy. "Leaf packs" can be used as the tool for such a monitoring procedure.

Leaf Pack Bioassay

The leaf pack bioassay procedure has the advantage that, even in its simplest form, it can provide information quite indicative of stream "health", that is, degree of perturbation. The processing of natural particulate organic matter, in the form of leaf litter, by the total stream community is measured. This can range from a qualitative assessment, in which the amount of leaf processing and type of loss over a given time interval are evaluated by eye, to detailed quantitative determinations of leaf weight loss and animal and microbial colonization. The exposed leaf packs are highly preferable to the mesh bags used by terrestrial (and some aquatic) ecologists to enclose leaves, since the latter gives unnaturally low rates of leaf processing.

Leaves from one or more of the common deciduous trees and shrubs in the drainage should be used to prepare the packs. Few drainages are devoid of broad-leaved deciduous trees or shrubs, but in such cases herbs, grasses or conifers can be used, although preparation of "packs" is more time consuming (e.g. conifer needles can be strung on monofilament like "beads on a chain"). Leaves should be collected just prior to abscission (or after abscission if accumulating nets are used), air dried for at least one week and stored dry until used.

Leaf packs (Fig. 1) are made of approximately 10 grams oven dry wt (50°C), the exact initial weight being determined if quantitative data on leaf weight loss are to be obtained (Fig. 2). If mixed species packs are used, the order of the species in the pack should be randomized and the positions and total weight of each leaf type recorded, allowing weight loss to be determined by species. Once the leaves are in the stream there is a rapid and significant (5-40%) weight loss due to leaching which is essentially complete within the first 24 hours (Fig. 3; Cummins et al., 1972; Petersen and Cummins, 1973). Once the leaves are in the stream, most of the differences between plants from which leaves were collected, between times of leaf collection relative to abscission, and in soaking during pack preparation are equalized during this one day leaching interval, the remaining differences observed being due primarily to such species-specific characteristics as thickness of cuticle, percent cellulose and lignin, etc.

Leaves are soaked at room temperature until softened (1-4 hrs) and the packs are laid loosely together and fastened with long shank "buttoners" (plastic "tees" made by Dennison Co.). Each pack is then tied on a brick with heavy monofilament line, together with an appropriate

plastic label, and placed in the stream with the pack facing into the current. Care should be taken to insure that the leaves are not too tightly bound (Fig. 2). Positioned in the stream in this fashion, the packs accurately simulate the way in which natural leaf packs (or "books") accumulate on the leading edge of natural obstructions such as logs, branches and cobbles.

A total of at least 12 packs are placed in the stream at each site to be evaluated. Packs are removed from the stream three (or more) at a time at least at 24 hours (leaching loss) and 2, 8 and 16 weeks. Additional times provide valuable information, but these four times allow reasonable evaluation of community heterotrophy. When a set of three packs is to be sampled, each brick is lifted carefully out of the water (preferably with a 250 μ or 75 μ mesh hand net placed around the brick), the monofilament cut and the pack dropped into a self-sealing plastic bag. The packs should be cut apart and spread out in white enamel trays for on-the-spot evaluation or, if quantitative data are to be obtained, placed in an ice chest and returned to the laboratory for analysis.

If only qualitative analyses are to be made, estimates of the amount (%) of leaf remaining, observations of the type of feeding activity that has occurred and animal counts can be accomplished in the field. The animals can be divided into four functional groups: 1) shredders (large particle detritivores); 2) collectors (fine particle detritivores); 3) grazers (periphyton scrapers); and 4) predators (Cummins, 1973). Table 1 lists some common taxa that fall primarily in the above categories.

Leaf processing is accomplished by three general types of biological activity. The shredders feed by eating sections of leaf between the major veins. The resulting leaves are characterized initially by large perforations through the leaf surface, and finally by almost complete skeletonization, as shown in Fig. 4. Collectors and grazers, in the absence of shredders, rasp away the surface tissues without perforation (Fig. 4). In the absence of any animal feeding, microbial activity alone renders the leaf tissues very soft; large perforations and the removal of surface tissues, except the cuticle in some cases, are not observed. After an extended period of microbial metabolism, fine fragmentation of the leaf occurs resulting in uniform "pinhole" perforations.

Procedures for detailed quantitative analyses of leaf packs are summarized in Fig. 5. After the leaf packs have been washed thoroughly through a stack of nested sieves to remove mineral sediments and dislodge the animals, the whole leaves together with the fragments retained on the 16 and 4 mm mesh screens (leaves, twigs, etc. that are obviously not part of the original leaf pack should be removed) are oven dried (50°C) for at least 48 hrs. In multiple species experiments, the remaining leaf material for each species should be weighed separately, if possible. The animals are sorted, at least to the functional groups described above, dried (50°C) and weighed. In addition to weight loss of the leaf packs over time and animal inhabitants, additional information on such aspects as microbial colonization, community respiration (excluding macroanimals), tracer uptake associated with the leaves, caloric content and chemical constituents (e.g. lignin to cellulose ratio Goering and Van Soest, 1970) can also be determined.

Leaf colonization by microorganisms can be qualitatively examined in a number of ways. Discs cut from leaves (e.g. with a 2.5 cm cork borer), spaced on insect pins between plastic beads (3 to 5 to a pin) and incubated in the stream by sticking the pins into a small piece of styrofoam fastened to a brick. Sets of discs removed at random are then microscopically examined and the relative abundance of various groups of organisms determined by scanning the periphery of replicate discs. Diatoms, protozoans and terrestrial and aquatic fungal spores can be enumerated, using wet mounts (stream water), at 400x. Discs can also be cultured on nutrient agar and later examined for microorganisms. Observation of bacteria on fresh or cultured leaf discs requires 100x phase contrast (preferably water immersion lens) or fluorescence microscopy (following acridine orange staining). Observation of aquatic hyphomycete fungal spores is facilitated by 24 hour incubation of leaves or discs from the stream in aerated distilled water for 24 hours.

Some examples of leaf weight loss rates are presented in Table 2. These results reflect the general pattern that has been observed in other woodland streams at widely separated locations in North America. The general pattern is the input of some leaf litter that is processed very rapidly, for example, oaks, beech, aspen and conifers) and some that is processed rapidly, for example, ashes, walnuts, elms, basswood, maples and alders. Species processed at intermediate rates are such forms as hickory and common riparian shrubs such as willows, alders, viburnums and dogwoods. In some stream systems willows are processed more rapidly and alders more slowly than the values shown in Table 2).

Summary

The leaf pack bioassay method provides an easy, yet sensitive, procedure for evaluating both qualitative and quantitative aspects of running water community structure and function. The bioassay allows a stream or section of stream to be compared to rates of organic processing and biotic activity typical of natural "healthy" systems in which organism diversity is dominated by species associated with the heterotrophic nature of lotic communities.

Fig. 1. Leaf packs, fastened together with "Buttoneer" plastic "T's" (Dennison Co.) and lashed to 10 hole bricks with monofilament line (see Fig. 2 for methods of preparation).

Fig. 2. Procedure for preparing preweighed leaf packs used in assessing leaf litter colonization and processing rates.

AUGUSTA CREEK

Fig. 3. Leaching weight loss of some representative leaf species in a small, hardwater, southern Michigan trout stream (Augusta Creek).

Fig. 4. Comparison of leaves fed upon by the "shredder" Tipula and the "collector" Stenonema.

Fig. 5. Procedures followed in leaf pack experiments, in both natural streams and the greenhouse channels.

Table 1. Common macroconsumer detritivores associated with leaf litter in Augusta Creek.

I. Large Particle Feeders

Plecoptera

Nemouridae

Taeniopteryx sp. (probably parvula)

Nemoura sp.

Pteronarcidae

Pteronarcys pictetii

Trichoptera

Limnephilidae

Pycnopsyche spp. (guttifer, lepida, scabripennis)

Hydatophylax argus

Platycentropus radiata

Phryganeidae

Ptilostomis ocellifera

Lepidostomatidae

Lepidostoma costalis

Diptera

Tipulidae

Tipula spp.

Chironomidae

Brillia sera

II. Fine Particle Feeders

Ephemeroptera

Ephemerellidae

Ephemerella spp. (subvaria, invaria)

Baetidae

Baetis spp.

Leptophlebiidae

Paraleptophlebia sp. (prob. debilis)

Leptophlebia sp. (prob. cupida)

Heptageniidae

Stenonema spp. (fuscum, tripunctatum, interpunctatum, canadense)

Hemiptera

Corixidae

Corixa sp.

Trichoptera

Hydropsychidae

Hydropsyche spp. (bronta, betteni, slossonae)

Cheumatopsyche spp.

Trichoptera (continued)

Molannidae

Molanna tryphena

Helicopsychidae

Helicopsyche borealis

Brachycentridae

Brachycentrus spp. (americanus, occidentalis)

Coleoptera

Elmidae

Stenelmis sp.

Optioservus sp.

Dubiraphia sp.

Psephenidae

Psephenus herricki

Ectopria sp.

Diptera

Tipulidae

Antocha, sp.

Empididae

Hemerodromia spp.

Simuliidae

Prosimulium sp.

Simulium sp.

Chironomidae

Orthocladinae

Corynoneura spp. (taris and others)

Chironominae

Paratendipes albimanus

Stictochironomus spp.

Polypedilum spp.

Amphipoda

Gammarus subfasciata

Table 2. Winter rates of leaf weight loss for some typical leaf species constituting detrital input to a woodland stream in southern Michigan (Augusta Creek, Kalamazoo County). Modified from Petersen and Cummins (1973).

Leaf Species	% Dry Weight Loss per Day	
	Head waters, heavily wooded areas	Middle section of drainage, less heavily wooded, some agriculture
<u>Carpinus carolinianum</u> (ironwood)	0.28	0.83
<u>Populus tremuloides</u> (quaking aspen)	0.35	0.58
<u>Populus grandidentata</u> (big-tooth aspen)	0.38	0.35
<u>Carya glabra</u> (pignut hickory)	0.56	0.81
<u>Quercus alba</u> (white oak)	0.56	0.43
<u>Quercus rubra</u> (red oak)	0.65	0.29
<u>Jugulans nigra</u> (black walnut)	0.70	0.72
<u>Acer platanoides</u> (maple)	0.74	0.76
<u>Salix lucida</u> (willow)	0.83	1.19
<u>Decodon verticillatus</u> (swamp loosestrife)	0.83	0.86
<u>Acer rubrum</u> (red oak)	0.93	0.62
<u>Acer saccharinum</u> (sugar maple)	1.07	0.66
<u>Cornus amomum</u> (silky dogwood)	1.27	1.06
<u>Fraxinus americana</u> (white ash)	1.43	1.37
<u>Tilia americana</u> (basswood)	1.75	2.34

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