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Case Report

Unfinished Business of an Emeritus Stream Ecologist

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Introduction

At my age, I feel there are three research areas that represent unfinished business. By presenting these, I hope those involved in active research will consider the issues.

Number 1: Stream Macroinvertebrate Functional Feeding Groups (FFG)

The macroinvertebrate Functional Feeding Group (FFG) procedure for evaluating stream ecosystem condition has been used widely since its inception in the 70s [1-3]. The FFG has been modified (e. g. [4]) and Validated (e. g. [5-8]).

The issue of obligate vs facultative FFGs requires direct validation. It is well established that a limited number of macroinvertebrate adaptations for acquiring food can be matched to a corresponding limited number of food resource categories (Table 1). The fitness of obligate FFGs resides with their maximum efficiency in converting ingested food to growth, directly measured as Relative Growth Rate (RGR). For example, the obligate scraper Heptageniidae mayfly nymphs are adapted to remove attached non-filamentous algae from hard substrates like cobbles in riffles. When they are held in laboratory streams, on fungal conditioned riparian leaf litter or Fine Particulate Organic Matter (FPOM) as their only food available, they scrape the surface of the leaves or ingest FPOM and survive. However, they lose weight and never emerge into normal sized adults (Cummins and Petersen unpublished). By contrast, *facultative* FFGs feed on more than one food resource, but at a lower RGR. Fitness is served because by survive and mature on changing or patchily distributed food resources. FFG Gathering collectors are all facultative FFGs and the usual food resource is the ubiquitous FPOM (Table 1). Consequently, gathering collectors, and the predators they support, usually dominate the macroinvertebrate fauna in impacted streams [7]. In "Aquatic Insects of North America" [8], ecological tables assign essentially all North American genera to a functional group. For those designated as facultative, the first listed of the alternatives are the most widely reported condition in the literature. This facultative designation is almost always based on gut analyses and not RGR.

Functional Feeding Group (FFG)	Food Resource Category (FRC)
Scrapers (SC)	Attached non-filamentous algae (especially diatoms)
Herbivore Shredders (HSH)	Rooted aquatic vascular plants
Detrital Shredders (DSH	Leaf litter of riparian origin conditioned by Hyphomycete fungi (CPOM)
Gathering Collectors (GC)	Fine particulate organic matter on or in the bottom sediments (BFPOM)
Filtering Collectors (FC)	Fine particulate organic matter in transport in the water column in the current (TFPOM)
Predators (P)	Live invertebrate prey

Table 1: Macroinvertebrate Functional Feeding Groups (FFG) and associated Food resource categories that match the FFG adaptations for acquiring the food. CPOM = coarse particulate Organic matter particles > 1 mm, FPOM = particles < 1mm. Modified from Cummins (1974), Cummins and Klug (1979). Merritt et al. 2017.

As shown by Anderson and Cummins [9], for the larvae of the scraper caddisfly *Glossosoma nigrior,* gut contents were not a predictor of food acquisition adaptation. The obligate scraper *G. nigrior* from a stream with mostly FPOM and not attached filamentous algae or rock surfaces had gut contents dominated by FPOM and produced prepupae of significantly reduced biomass.

Thus, the acquisition of food is based on the abundance of any food resource for which the macroinvertebrates have the adaptations to acquire it. Enzymatic differences between obligate and facultative FFGs would be a useful line of inquiry.

A general advantage of using FFG analysis of stream ecosystem condition concerns the taxonomic resolution required. Taxonomy at the species level will likely be the territory of genetic

coding in the future. However, I can envision no scenario in which it will be of use for the rapid higher-level taxonomy needed to determine FFGs. For example, all stream snails are obligate scrapers (class Gastropod), all clams are filtering collectors (class Bivalvia), all dragon- and damselfly nymphs are predators (Order Odonata), all stone case-bearing caddisfly larvae (e. g. the families Glossosomatidae, Helicopsychidae) are scrapers, and only larvae in the genus *Tipula* of the dipteran family Tipulidae are detrital shredders, all the rest are predators.

Ratios of FFGs have been used as surrogates for quickly measured stream ecosystem attributes [4,6,10,11]. It is useful that as dimensionless numbers, the ratios are essentially independent of sample size.

Determining if a stream ecosystem is autotrophic or heterotrophic is arguably the most fundamental characteristic attribute of any stream ecosystem. Measured directly (e. g. [12,13]) this P/R ratio is gross primary production to total community respiration and the threshold for autotrophy is P/R = > 1.0. The corresponding surrogate FFG ratio is: scrapers + herbivore shredders to detrital shredders + gathering collectors + filtering collectors. The corresponding threshold for autotrophy is P/R = > 0.75 (e.g. [10]).

I believe that the FFG surrogate ratio procedure [4] can be used in the field as a major tool for very rapid bio assessment of stream ecosystem condition. A fertile area of future research would be linking FFG assessments with more direct measures of stream ecosystem parameters. So far, they have been largely related to observational estimates of corresponding stream ecosystem attributes. Also, other FFG ratios remain to be developed and validated. Much is left to be done.

Number 2: Microbial Modification of Bottom Up or Top-Down Trophic Structure

Stream ecologists have often described the trophic structure of macroinvertebrate communities as controlled by bottom up, that is regulation by food supply, or top down, that is regulation by predation (e.g. [14]).

To investigate these controls, Peggy Wilzbach and I we conducted a study that combined laboratory and field enclosures with an in- stream manipulation (Cedar Creek in Maryland) using larvae of the caddisfly *Pycnopsyche guttifer* [15].

In the laboratory larvae were reared individually after hatching. Each larva had excess food, no competition and no predation. Molting (collection of cast skins) and mortality (dead larvae) were tracked throughout the growth period from instar one to terminal 5th instar

Gelatinous *P. guttifer* egg masses, which averaged 250 eggs per mass. IIn a Cedar Creek field study, growth boxes were stocked with one egg mass each and fungal conditioned leaf litter food. The population in each box was monitored from hatching to final 5th instar. These confined larvae had excess food, no predation, and only intraspecific competition and natural stream water conditions.

The species *P. guttifer* occurred in regional Maryland streams, but not in Cedar Creek. The local species *P. scaripennis* larvae in Cedar Creek could be distinguished from *P.* guttifer in stream benthos leaf litter collections on the basis of case construction. *P. guttifer* Egg masses were stocked every meter along 10 meters of stream bank. Leaf litter transects along the 10-meter section were taken weekly, stating down-stream, and moving up a meter a time to census the survival of the introduced *P. guttifer* larval population.

In all treatments, including a free ranging stream population, there was no significant difference between the patterns of mortality [15]. In this study when food limitation, competition, and predation were excluded, mortality followed the pattern of a natural field population. The only alternative to top down or bottom-up control seems to be microbial (inside out) control. When larvae molt, they shed the entire skin except for the lining of the midgut. We proposed that lesions that can develop at the junction of midgut with foregut and hind gut. This would allow resident gut bacteria to enter the hemolymph, changing from anaerobic to aerobic form. These bacteria, located mostly in the hindgut, where they contribute to the digestion of recalcitrant compounds, The digested products are refluxed forward to the midgut [16].

Further evidence of microbial related mortality in stream macroinvertebrates was found in drifting animals. A drift – benthos partitioning sampling device was used which allowed drift and benthos to be collected from the same area of stream bottom. Macroinvertebrates in the drift showed significantly greater mortality than those in the benthos. In each trial, benthos and drift macroinvertebrates were censused and then held for 24 hours to determine mortality. It appears that drift may not be largely a mechanism for population dispersal to new more favorable habitats, but rather a discarding mortality. Again, microbial mortality is the Likely agent. These initial studies seem exciting enough to warrant a great deal more research using laboratory studies and field enclosure; definitely unfinished business.

Number 3: Estimating Stream Macroinvertebrate Biomass in the Field

Typically, collections of stream macroinvertebrates are analyzed numerically. Expressing the same data in biomass terms can significantly change the interpretation of the information. For example, a terminal instar *Tipula* larva can be 70 times the biomass of a single chironomid midge larva [4]. Determining biomass of the stream macroinvertebrates collected is much more difficult than just counting them.

Realistic mg dry biomass values using preserved collections is not possible (Leuven et al. 1985). Variable weight loss of preserved specimens and mm measurement of soft bodied larvae are both problematic [17]. Therefore, to establish a data base of

dry biomass for any given macroinvertebrate FFG taxon, fresh specimens must be used. This entails measuring total length (X) of each fresh specimen, oven-drying and weighed it on a microbalance. Because the data base from this procedure is sparse, and only represents a fraction of the biota in a typical stream, a great deal more data are needed.

Because the direct measures of macroinvertebrate biomass are scarce, the nearly universal method for estimating dry biomass uses regression analysis. The regression used is, $Y = aX^b$, where Y = mg dry biomass, X = mm total body length, coefficient a = Y intercept of Y on X, and coefficient b = slope of Y on X.

A procedure for rapidly measuring body lengths of fresh collections in the field has been developed that leads to estimating dry biomass. The first step, sorting a sample of stream macroinvertebrates into FFGs has been covered above. The next step is taking mm measurements of the individuals that have been collected (the X in the regressions). On the inside back cover of [8] is a series of nine circles with diameters that increase in 5 mm increments from 5 mm to 45 mm.

A printed copy to exact scale of the circles from the book back cover is made and laminated. When the laminated sheet is placed on the bottom of an enamel tray and covered with thin layer of water, it is the length estimator. The individuals in each taxonomic-FFG category are introduced one by one to the tray. These individuals are moved to the circle in which they fit. The final number in each circle is the number in that 5 mm increment group. These are tabulated for use as X in the formula $Y = aX^b$. Because the specimens in a given circle represent a range of those that fit in the circle, for example, 0.5 -5 mm, the median value of the circle (= radius) is used for X of each circle size (e. g. 5 mm = 2.5 mm, 10 mm = 5 mm, 15 mm = 7.5 mm, etc. This method produces a very rapid estimate of the number in each circle. A table of dry mg biomass values has been prepared based on original data and extensive published values (137 references, [4]). Here is an example: Scraper mayflies. Heptageniidae plus Ephemerellidae (*Drunella):* mg per individual coefficients a = 0.0072, b = 2.659. Estimated mg dry biomass per individual n: 5 mm (median 2.5 mm) = 0.08, 10 mm (median 5 mm) = 1.53, etc.

Much more data on dry biomass from fresh animals to establish regressions is needed. Because only a small portion of stream macroinvertebrates have been established. Lots of unfinished business work needs to be done.

Clearly, there are many more than three areas for exciting research in stream ecology. So have at it!

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