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A Field Verification of the Use of the Autotrophic Index in Monitoring Stress Effects

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Most studies on stress effects are single species lethality tests conducted in laboratories. Such tests are limited in their environmental application because, except for gross generalization, they cannot be used to predict stress effects in more complex ecosystems. In situ testing of stress effects has traditionally examined structurally based taxonomic and nontaxonomic changes such as species diversity and biomass accumulation. However, field studies usually are difficult to replicate, are time consuming and expensive, may require skilled taxonomists, and may not describe functional responses in the ecosystem.

We have modified and improved a nontaxonomic method for measuring stress effects in streams using simple, state-of-the-art techniques. Our approach was to analyze changes in autotrophic and heterotrophic portions of the stream microbial community by measuring ATP and chlorophyll a, and to relate these changes to structural and functional changes in the stream macroinvertebrate community.

Our first hypothesis is that a balance exists in microbial communities between autotrophic and heterotrophic components. Stress causes a shift in this balance by favoring the growth of tolerant species. Similar hypotheses have been proposed by a number of researchers (e.g., WEBER 1973, RODGERS 1977). However, most attempts to verify these hypotheses in the field have failed. The unsuccessful attempts were probably caused by sampling difficulties due to inadequate sample sizes, variations between substrates and microhabitats, and inability to repeat experiments. In addition, most researchers did not use a community as diverse as the microbial community. The intricate food webs and trophic relationships in the microbial community reflect complex interactions similar, although on a smaller scale, to those seen in the larger stream ecosystem.

Our second hypothesis is that changes in autotrophy and heterotrophy in the microbial community could be related to structural, and more specifically, functional changes in the macroinvertebrate community.

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MATERIALS AND METHODS

Data were collected from Cedar Run, a small stream receiving chlorinated sewage effluent near its headwaters, and from Wilson Creek, a relatively clean stream with high macroinvertebrate diversity. Both streams are located about 2 km southeast of Blacksburg, Virginia (Montgomery County). Sampling stations were selected in riffle zones on Cedar Run and Wilson Creek as shown in Figure 1. Stations 1, 6, and 7 represent upstream and downstream reference stations, and Stations 2-5 represent stressed stations at increasing distances downstream from the outfall.

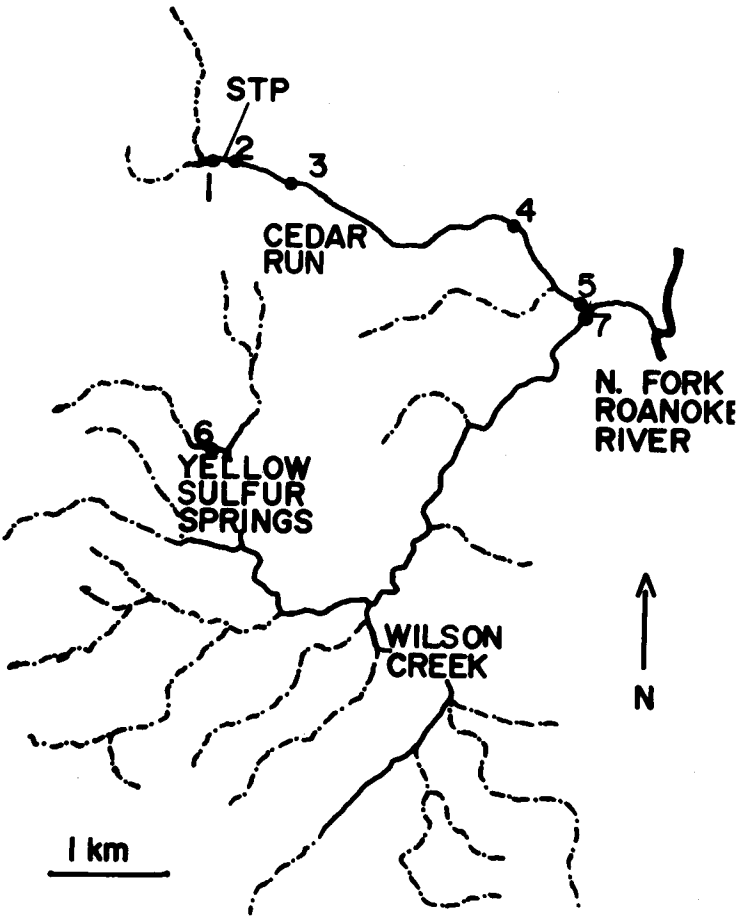

Figure 1. Cedar Run and Wilson Creek station locations.

Uniformly sized polyurethane foam substrates (5 x 6.5 x 8 cm) were used to sample the microbial community as described by CAIRNS et al. (1979). Preliminary experiments on Cedar Run showed that these substrates colonized rapidly and contained approximately the same number and kinds of organisms as did natural organic and sediment substrates. Polyurethane foam substrates provided a uniform substrate and were used rather than other natural or artificial substrates because they produced a large volume of extract. This volume allowed several samples to be taken from the same sponge community, permitted statistical analysis, and improved replicability.

Substrates were allowed to colonize in the stream for 3, 7, and 10 days. They were collected in ZIPLOC® bags and returned to the laboratory on ice within 3 h of collection. The microbial community was squeezed from the sponges over clean beakers.

ATP was extracted by injecting 1.0 mL of the microbial community into 19 mL of boiling tris buffer (Tris-(hydroxymethyl)aminomethane, 0.02 M, pH 7.75), heating for 15 min, freezing at -20°C, and assaying within 7 days. Assays were done using standard bioluminescence techniques with FLE-50 firefly enzyme (Sigma Chemical Co.) on a Labline #9140 ATP Photometer (AMERICAN PUBLIC HEALTH ASSOCIATION [APHA] 1976).

Chlorophyll a levels were measured by injecting 1.0 mL of the microbial community into 9 mL of 100% spectrograde acetone, resulting in a 90% acetone extraction medium, and extracting for 24 h at 4°C in the dark (HOLM-HANSEN 1978). Chlorophyll a determinations were made using a calibrated Turner Designs Fluorometer. To determine our efficiency of extraction in acetone, preliminary studies were conducted comparing acetone and DMSO (dimethylsulfoxide) extractions (SHOAF & LIUM 1976). The extraction in acetone equaled or exceeded that in DMSO for fall and winter communities because of the large diatom component in the microbial community.

Macroinvertebrates were sampled at the same time at each station using an Ellis-Rutter Portable Invertebrate Box Sampler or a Surber Sampler, depending on the stream depth. Macroinvertebrates were identified to genus and species when possible and were classified into functional groups based on the system outlined by MERRITT & CUMMINS (1978). Opportunistic species such as Physa gyrina were classified based on their functional position at each station (e.g., as collector-gatherers on detritus at Station 2 rather than periphyton grazers).

RESULTS AND DISCUSSION

Sponge colonization reached a peak within 7 days with no appreciable biomass accumulation after that period. Data for this paper are from 7-day samples, although 3 and 10-day samples showed similar trends. Only results from October 1979 are presented in this discussion. These data are representative of experiments completed between July 1979 and January 1980. Seasonal variation is discussed when appropriate.

ATP levels were consistently low at all reference stations (ranging from 10 to 50 µg/L for October data) and increased significantly (α=0.05) at downstream stations (ranging from 80 µg/L at Station 2 to 700 µg/L at Station 4). Chlorophyll *a* levels were consistently high at reference stations (from 0.3 to 1.5 mg/L) and lower immediately below the outfall (0.1 mg/L at Station 2). The low levels of chlorophyll *a* were maintained for some distance downstream before increasing to levels as high or higher than the reference stations (e.g., 1.7 mg/L at Station 4; 2.3 mg/L at Station 5). Seasonal blooms of green algae at Station 3 caused the largest variation in chlorophyll *a* concentrations.

An Autotrophy Index (AI) similar to the one proposed in Standard Methods for the Examination of Water and Wastewater (APHA 1976) was used to test whether the balance between autotrophy and heterotrophy was upset in Cedar Run; however, the index was adapted to use ATP-based biomass estimates rather than ash-free dry weight (Equations 1 and 2):

$$AI = \frac{\hat{B} \text{ total (mg/L)}}{\text{Chlorophyll } a \text{ (mg/L)}} \qquad \text{[Eq. 1]}$$

where

$$\hat{B}_{total} = \frac{\text{ATP (ng/L)}}{2{,}400} = \text{Est. total living biomass} \qquad \text{[Eq. 2]}$$

ATP has frequently been used to estimate total living biomass, although its use has been restricted predominately to lentic habitats (e.g., HOLM-HANSEN & BOOTH 1966). Because a considerable portion of the organic matter in streams is nonliving and often of allochthonous origin, ATP-based biomass estimates should provide a better approximation of the active microbial community than traditional gravimetric measurements, i.e., ash-free dry weight.

The October chlorophyll *a* and ATP data were converted to AIs and plotted against the macroinvertebrate diversity

(Shannon-Weaver $\bar{d}$) for species collected during the same sampling period (Figure 2). Initial examination of the AIs alone led to the conclusion that the balance between autotrophy and heterotrophy in the microbial community was considerably altered by the sewage effluent. The reference stations consistently had low AIs, usually below 50, which indicated a large autotrophic component in the community. Immediately below the outfall, the AI was significantly higher, which indicated a disproportionate increase in the heterotrophic components of the microbial community. High AIs were observed downstream, and they decreased partially at Station 5. Compared to reference station AIs, downstream AIs were significantly higher.

The pattern of macroinvertebrate diversity ($\bar{d}$), a taxonomically based structural indicator of stress, indicates an inverse relationship with the microbial AIs (Figure 2). Higher $\bar{d}$ values were assoicated with low AIs at reference stations. Immediately below the outfall, the $\bar{d}$ fell as the AI increased, while apparent recovery of the diversity was observed farther downstream. Similar results could be obtained by comparing the microbial AIs with the number of macroinvertebrate taxa from each station.

There is some indication that the apparent recovery of macroinvertebrates based on diversity is misleading. If macroinvertebrates are classified into functional groups based on feeding strategy, they still appear to be stressed at downstream stations. The proportions of each major functional group for October 1979 is depicted in

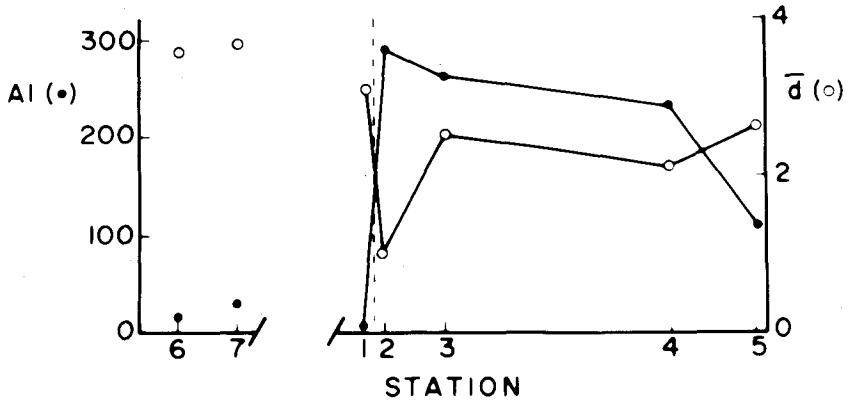

Figure 2. Microbial autotrophy index vs macroinvertebrate diversity (October 1979).

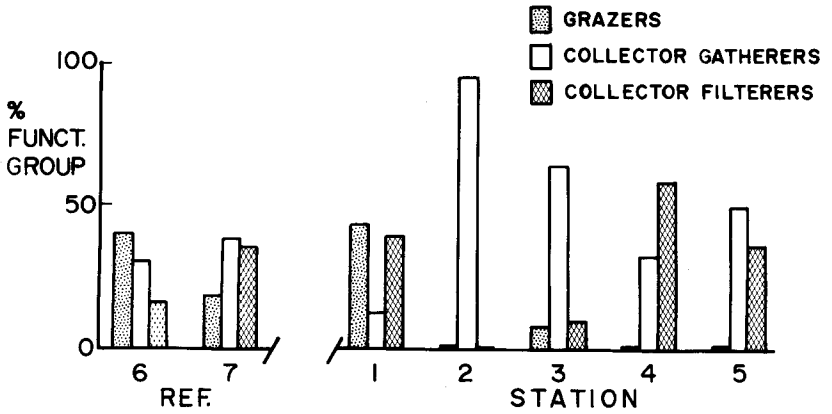

Figure 3. Proportion of macroinvertebrate functional groups (October 1979).

Figure 3. Grazers, filterers, and collector-gatherers were well represented at all reference stations. Shredders were not well represented in this particular stream system. Immediately below the outfall, the more tolerant collector-gatherers occupied a much greater proportion of the community; collector-filterers increased in importance farther downstream. Grazers never recovered their importance in downstream communities even though they were well represented in the downstream control Station 7.

These changes in the proportions of macroinvertebrate functional groups at downstream stations indicate that the groups have not recovered. This pattern corresponds with the autotrophy index which also indicates nonrecovery at downstream stations. These data suggest that although the stream appears to recover structurally, as evidenced by the increase in downstream diversity, it is still stressed functionally.

The relationship between microbial trophic states and macroinvertebrate functional groups can be demonstrated most dramatically by examining a plot between the AI and the proportion of grazers in the macroinvertebrate communities. Figure 4 shows the results of this comparison for the October data using the log of the AI to normalize the AI variance for parametric statistical tests. A negative correlation ($r = -0.94$, $\alpha = 0.01$) existed between the log AIs and the proportion of grazers.

In conclusion, stress does upset the balance between autotrophy and heterotrophy in stream microbial communities.

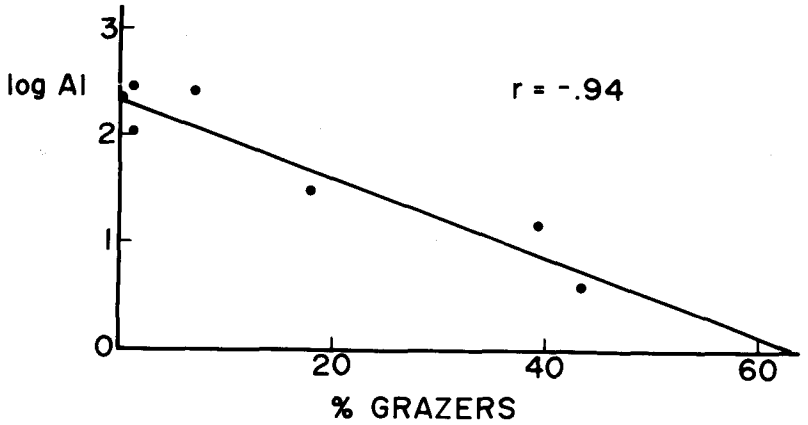

Figure 4. Log microbial autotrophy index vs percent grazers (October 1979).

This imbalance was shown by low AIs at all reference stations and increased AIs below the sewage outfall. Furthermore, changes in the AI were inversely related to changes in the structure of the macroinvertebrate community, as represented by the macroinvertebrate diversity and by an even greater relationship between the macroinvertebrate functional groups and the microbial trophic state. Finally, because the nontaxonomic measurements of ATP and chlorophyll a can be generated quickly (usually within one week) and because the data are easily replicated and show definite relationships to traditional taxonomic responses to stress, nontaxonomic monitoring of the microbial community could be a useful tool in field analysis of stress effects.

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