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Bacterial growth on stream insects: potential for use in bioassessment

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Abstract. Growth of filamentous bacteria (*Sphaerotilus* sp., *Leptothrix* sp.) on aquatic insects was evaluated for its usefulness as a bioindicator of detrimental nutrient levels in streams. Field measurements of insect abundance, nutrient concentrations, and incidence/degree of bacterial growth on insects upstream and downstream of livestock pastures were made in 2 Virginia, USA streams. Laboratory studies were conducted to determine the effect of bacterial growth on insect survival. Elevated concentrations of dissolved nutrients (0.13–0.35 mg/L orthophosphate, 1.29-2.13 mg/L nitrate) downstream of pastures were associated with growth of filamentous bacteria, which colonized the gills and body surface of aquatic insects. Significantly lower densities of insects (up to 66% less) occurred at downstream sites. In laboratory studies, 100% mortality of heavily infested mayflies (>25% of body covered, including gills) occurred within 30 d, whereas >85% of individuals without bacterial growth survived and grew normally. The pattern of mortality in the laboratory closely paralleled the differences in density observed in the field. Bacterial growth on aquatic insects appears to be a reliable bioindicator of nutrient enrichment, and the degree of infestation associated with reduced insect survival can be quickly detected in the field or laboratory using a hand lens (10–15× magnification). This bioindicator shows promise as a significant addition to EPA Rapid Bioassessment Protocols because simple visual assessment of benthic samples may be sufficient to identify a cause for impaired macroinvertebrate communities. Bacterial growth should be useful for detecting nutrient impacts in streams as well as evaluating the success of management practices to control nutrients from point or non-point sources.

Key words: bioindicator, stream pollution, eutrophication, benthic macroinvertebrates, aquatic bacteria, nitrogen, phosphorus, Ephemeroptera.

The nutrient status of streams can markedly influence the growth of their microflora and microfauna, and directly or indirectly affect many other characteristics of the biota. In nutrient-poor systems, increases in nutrients are associated with increased production of aufwuchs and macroinvertebrates (Lorch and Ottow 1986, Krueger and Waters 1983). In some situations, such enrichment may be considered beneficial if the increase in primary and secondary production is translated into larger populations of game fishes valued by society (Rasmussen 1986, Lenat and Crawford 1994). However, there is a well-known threshold for biological stimulation beyond which elevated nutrients have detrimental effects. For example, adding excessive organic nutrients can cause hyper-eutrophication (Hynes 1969), a condition in which the biological community collapses and few species remain (Hynes 1960, Curtis 1969).

One of the notable effects of adding nutrients to streams is increased growth of aquatic bacteria, particularly filamentous genera such as *Sphaerotilus* and *Leptothrix*. These bacteria may be stimulated to bloom with modest increases in dissolved nutrients (e.g., 0.5 mg/L phosphate or 0.8 mg/L nitrate; Phaup and Gannon 1967, Lemly 1982). Once in the bloom stage, they may form large colonies on a variety of stream substrata, including aquatic insects.

Water quality in southern Appalachian streams can be seriously degraded by organic nutrients leached from animal wastes if livestock are allowed to graze in the riparian zone (Lemly 1982). It is well known to scientists and fisheries professionals that maintenance of an ungrazed, vegetated buffer strip or riparian zone is important in reducing nutrient and sediment inputs to streams (USEPA 1977, Young et al. 1980, Schlosser and Karr 1981), but these practices are not uniformly followed by livestock growers in the mountain regions of Virginia and elsewhere (e.g., Yow 1996). Recovery and enhancement of trout-stream habitat in the southeastern USA is a major effort of state fish and game agencies as well as the US Forest Ser-

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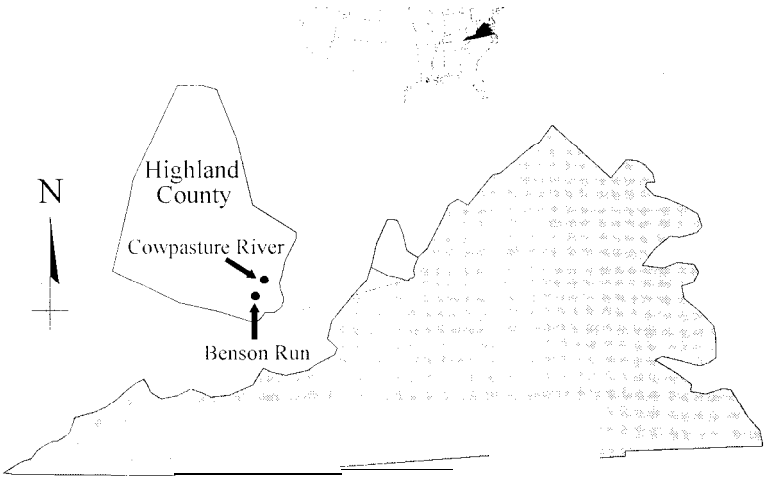

FIG. 1. Location of the study streams in the Appalachian Mountain region of Virginia.

vice. These efforts can be undermined by poor livestock management practices, particularly in locations where trout streams traverse private grazing land and then re-enter state or national forests.

One of the basic tools that agency fishery biologists use to evaluate stream conditions is the EPA Rapid Bioassessment Protocol for macroinvertebrates (Plafkin et al. 1989). Although this method reveals impaired biological communities, it does not identify cause-effect linkages, i.e., whether the impairment is caused by chemical pollutants, sedimentation, nutrient enrichment, or other perturbations. In addition to this basic screening tool, biologists need assessment methods that are specific to certain types of pollution or other disturbances. This paper describes research to evaluate a potential bioindicator of detrimental nutrient enrichment in streams based on bacterial growth on aquatic insects.

Methods

Field surveys

A series of field samples was taken from 2 streams to determine the possible influence of livestock grazing on the relative abundance of aquatic insects by making upstream–downstream comparisons. Insects were collected from 2nd-order (Benson Run, lat 38°15′31″N, long 79°29′51″W) and 3rd-order (Cowpasture River, lat 38°16′10″N, long 79°29′14″W) streams in the Appalachian Mountains of Virginia (Fig. 1). These are low-gradient (<5%), moderate-elevation (700–800 m asl) streams that support brook trout (*Salvelinus fontinalis*) and other cold-water fishes typical for this mountain region. Three quantitative samples were collected in each of 6 riffles of each stream in March, June, and September 1995 (3 samples / riffle / date) with a portable invertebrate box sampler (PIBS, Ellis-Rutter Associates, Punta Gorda, Florida). Each sample consisted of 2 PIBS collections that were pooled, 1 from the middle of the stream and 1 equidistant from the center of the stream to the margin of the wetted channel. Three of the riffles were downstream (within 300 m) of cattle pastures (7–15 ha, 18–35 animals per pasture) where active grazing and deposition of animal waste was occurring immediately adjacent to the streams. The remaining 3 riffles were upstream (within 300 m) of cattle pastures or associated nutrient inputs. Riffles selected for sampling had similar substratum texture (pebble size), water depth, wetted channel width, and current velocity. Insects were preserved in 70% ethanol and returned to the laboratory for

processing. Ephemeroptera, Plecoptera, and Trichoptera were identified to family, enumerated, and examined for bacterial growth using a dissection microscope (10-200X magnification). Some individuals of each family were prepared and viewed with scanning electron microscopy (SEM) using a Philips Model 501 instrument.

Filamentous bacteria were identified to genus (400–1000× magnification using a compound microscope with phase-contrast optics and supplemental fiber optic light sources) with identification keys that use external morphological features of the sheaths (e.g., Buchanan and Gibbons 1974). When present in mature stages, which was the case for bacteria examined in this study, sheath-forming bacteria are easy to identify using simple characteristics such as the presence or absence of iron or manganese oxide crusts on sheaths and the presence or absence of swollen tips on sheaths. Even preserved material is simple to identify, and there is seldom a need for culturing or staining.

The extent of bacterial growth on individual insects was quantified using a block-grid recording technique. An outline sketch of a generalized mayfly, caddisfly, or stonefly (an enlargement of a line-drawing from a taxonomic key) was copied onto quad-ruled engineering paper (25 squares/cm²; each insect ~240 mm long, 1 insect per page) and used as a data sheet for recording bacterial growth. An insect was viewed under the microscope, and bacterial growth was recorded by shading the corresponding body part on the sketch with a highlighter pen. A dorsal view and a ventral view were sketched for each insect. The highlighted squares in both views were counted and compared to the total number of squares within the outline of the insect to calculate the % of the body covered by bacteria. At least 100 individuals per family from each site on each date were assessed for prevalence and degree of infestation (a single exception involved only 83 Pteronarcidae in 1 sample).

Three types of statistical analyses were conducted on insect data: 1) Differences among insect orders in % infestation/insect population and % coverage/individual by bacteria were tested for statistical significance using G-tests (Sokal and Rohlf 1981). Data for each stream were considered separately, but within a reach (e.g., downstream) data for the individual PIBS samples were combined across all 3 sampling dates before analysis. 2) The density of insects (number /m²) was calculated and upstream–downstream comparisons (by insect order and family) were made using 2-factor ANOVA (location X date) on log-transformed densities. 3) Product-moment correlation (Sokal and Rohlf 1981) was used to examine associations between % reduction in downstream density and % of downstream individuals heavily infested by bacteria.

On each collection date concentrations of dissolved nutrients (total nitrate and orthophosphate in 0.45-µm-filtered samples) were measured (5 replicates) at locations where insects were sampled, using methods approved by USEPA for in-situ analysis (cadmium reduction method for nitrate, ascorbic acid method for orthophosphate, USEPA 1992). Upstream-downstream comparisons of nutrient levels were made for each month (March, June, September) using t-tests. The insect surveys and nutrient measurements were done when stream flows were moderate (defined visually by comparing stage on sampling dates to active channel width) to avoid possible influences of high flows on nutrient measurements (e.g., spurious introduction of animal waste).

Laboratory tests

In June and September 1995, live mayflies (*Epeorus* sp.) from the downstream reach of Cowpasture River were placed into aerated, polypropylene jars and transported (in an ice-water bath at 15°C to prevent thermal stress on the insects) to Virginia Polytechnic Institute and State University for survival studies. Three Plexiglas® aquaria with recirculating, aerated, and temperature-controlled water supplies were used to conduct these experiments (Fig. 2). Each aquarium held five 1.5-L chambers (containing several 3-5 cm Cowpasture River cobbles) into which insects were placed. The sides and bottoms of the chambers supported holes large enough to allow water to circulate freely but small enough to prevent insects from escaping. Chambers were submerged to a depth of 10 cm. Conditions in the aquaria mimicked a riffle downstream of a small plunge pool, i.e., the inlet flow was diffused through 4 holes and cascaded in free-fall for 25 cm before reaching the water in the aquarium, which generated considerable turbulence and bubbles throughout the

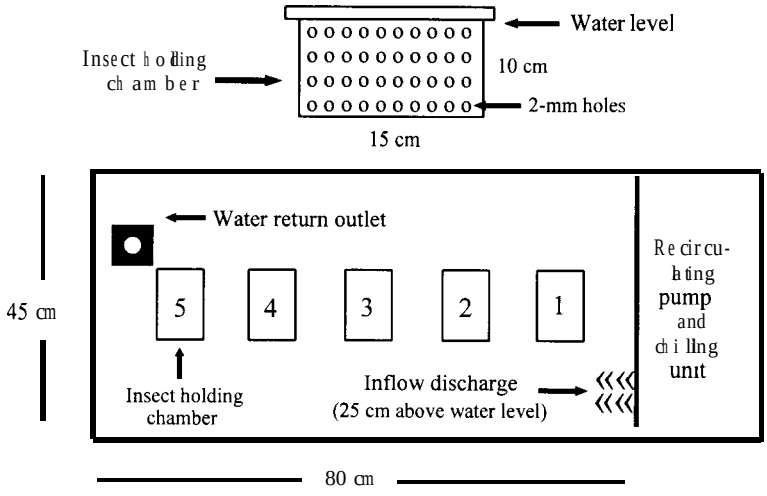

FIG. 2. Schematic top view of aquarium containing 5 chambers used to hold mayflies in the survival experiments and a side view of a single chamber. A total of 3 aquaria and 15 chambers was used.

aquarium. The water circulation rate in aquaria was ~960 L/h (manufacturer's operational specifications for the recirculating pump), yielding a mid-tank current velocity of 10–15 cm/s, based on timing of air bubbles traveling through the aquaria.

Epeorus was selected for study because: 1) they are common in both streams, 2) they are scrapers that can feed on easy-to-grow biofilms, making them amenable to long-term laboratory study, and 3) field collections showed that they were heavily colonized by bacteria. Mid-instar life stages (6-8 mm length, excluding caudal cerci) were used. Temperature and pH were checked daily and adjusted when necessary to maintain the same values as the stream water (15±1°C; pH 6.5±0.2, mean ± maximum deviation). A 12h:12h light:dark regime was maintained throughout each study, which lasted ~30 d. During experiments, mayflies fed on algae and associated microorganisms that grew as a biofilm on the stones in the experimental chambers. Dogwood leaves (conditioned by incubating dried leaves in the chambers for 30 d prior to introducing the insects) were placed among the cobbles to supplement mayfly diets and stimulate the growth of the biofilm. Dogwood leaves were used because they condition quickly, and this species is a common understory tree in the riparian zone of the study streams. Insects were recovered and enumerated at the end of each study and % mortality was determined. Surviving insects were examined for bacterial growth under a dissection microscope.

Two 30-d mayfly survival studies were conducted; the 1st from 12 June to 14 July 1995 and the 2nd from 25 September to 27 October 1995. Prior to beginning each experiment, mayflies were examined under a dissection microscope and divided into 2 groups based on the presence or absence of bacterial infestation: 1) individuals with heavy bacterial growth (>25% of total body covered, including gills), and 2) individuals with no visible bacterial growth. Gross visual estimates, rather than the quantitative black-grid procedure used for preserved insects, were used to determine the degree of bacterial infestation. Although the visual method was somewhat less precise, it allowed insects to be processed quickly to reduce possible handling stress. Moreover, the experience gained from quantifying bacterial growth on hundreds of insects from previously collected samples made it easy to identify and select individual mayflies with >25% body coverage. Each testing chamber received 5 individuals (June study) or 7 individuals (September study) from 1 of the 2 groups, and each chamber was randomly assigned to 1 of the 3 aquaria (Sokal and Rohlf 1981, Table 1).

TABLE 1. Experimental design for survivals studies with *Epeorus* sp. Treatments: X = infected, 0 = non-infected, — = empty chamber. June experiment: 5 individuals/chamber; September experiment: 7 individuals/chamber.

Study 1 (12 June-14 July 1995)

	Chamber				
	1	2	3	4	5
Aquarium #					
1	x	0	x	0	x
2	x	0	0	x	0
3	0	x	x	0	—

Study 2 (25 September-27 October 1995)

	Chamber				
	1	2	3	4	5
Aquarium #					
1	0	x	x	0	x
2	0	x	0	x	0
3	x	0	—	0	x

Results

Field surveys

Nutrient concentrations in both streams were significantly lower in locations upstream of the cattle pastures than at downstream sites (Table 2). The density of insects, especially Ephemeroptera, in the study streams was significantly lower (up to 66% less) in downstream reaches where bacterial growth occurred (Table 3). All of the field-collected mayfly nymphs that supported heavy bacterial growth were early to mid-instars. However, mayfly nymphs without bacterial growth were represented by all life stages, including mature individuals ready to emerge. This pattern differed for Plecoptera; a few mature nymphs (12%) supported heavy growths of bacteria. All taxa of insects from reaches downstream of cattle pastures exhibited growths of filamentous bacteria; no bacteria were detected on insects at upstream sites. Prevalence and degree of infestation was highest in Ephemeroptera (Table 4), and the degree of infestation was greatest in Ephemerellidae and Heptageneiidae (Fig. 3). Individual Ephemerellidae and Heptageneiidae were often nearly covered by bacterial colonies (coverage of infested individuals = 12-89% and 22–94%, respectively). In each stream, there was a significant positive association between % of downstream individuals heavily infested and % reduction in downstream density of insects (Fig. 3). Prevalence of infestation among insect orders was similar in both streams but the degree of bacterial growth on Ephemeroptera and Trichoptera was significantly greater in Cow pasture River (Table 4). There was no apparent seasonal

TABLE 2. Mean concentrations of dissolved nutrients (±1 SE) in 2 Virginia, USA streams during 1995, n = 5. Upstream denotes reference sites where nutrient concentrations were uninfluenced by cattle pastures. Downstream denotes sites where grazing and deposition of animal waste was occurring adjacent to the stream. t-probabilities (upstream-downstream comparison): *** = $p < 0.001$.

	Total orthophosphate (mg/L)			Total nitrate (mg/L)		
Stream	Upstream	Downstream	t-prob.	Upstream	Downstream	t-prob.
Benson Run						
March	0.02	0.13	***	0.13	1.41	***
	(0.009)	(0.016)		(0.019)	(0.078)	
June	0.05	0.35	***	0.11	1.55	***
	(0.016)	(0.009)		(0.020)	(0.090)	
September	0.05	0.24	***	0.15	1.29	***
	(0.011)	(0.033)		(0.010)	(0.024)	
Cow pasture River						
March	0.03	0.29	***	0.19	1.66	***
	(0.014)	(0.022)		(0.018)	(0.049)	
June	0.05	0.25	***	0.15	2.13	***
	(0.025)	(0.010)		(0.021)	(0.013)	
September	0.02	0.21	***	0.15	1.78	***
	(0.029)	(0.014)		(0.044)	(0.066)	

TABLE 3. Comparison of insect densities (mean no. of individuals/m² ± 1 SE) upstream (up) and downstream (down) of cattle pastures in 2 Virginia, USA streams during March, June, and September 1995. ANOVA results list F-values for analyses of log-transformed data (df = 1, 17 for locations; 2, 17 for dates and location × date). Significant ANOVAs ($p < 0.05$) are bold-faced. EPT = Ephemeroptera, Plecoptera, and Trichoptera.

	March		June		September		Anova results		
Taxon	Up	Down	Up	Down	Up	Down	Location	Date	Location × date
Benson Run									
Total EPT	891 (40.7)	503 (33.9)	1020 (25.2)	698 (32.2)	941 (29.0)	559 (19.7)	**58.77**	**0.224**	**0.782**
Ephemeroptera	359 (19.3)	184 (11.0)	472 (36.0)	329 (21.5)	407 (18.7)	232 (24.3)	**82.93**	**0.475**	**0.143**
Plecoptera	290 (21.2)	172 (14.5)	311 (20.0)	198 (32.1)	330 (23.6)	183 (18.2)	**53.22**	**0.296**	**0.131**
Trichoptera	242 (26.6)	147 (21.0)	237 (36.9)	171 (12.2)	204 (18.4)	144 (19.6)	**28.47**	**0.110**	**0.472**
Cow pasture River									
Total EPT	1288 (31.0)	660 (19.7)	1415 (42.2)	863 (20.3)	1072 (36.9)	703 (24.5)	**96.33**	**6.68**	**0.494**
Ephemeroptera	572 (39.7)	248 (27.3)	615 (56.2)	312 (18.7)	503 (34.5)	318 (16.9)	**126.76**	**0.116**	**0.735**
Plecoptera	367 (24.2)	175 (12.6)	419 (18.4)	282 (23.5)	339 (30.2)	247 (16.7)	**56.77**	**0.127**	**0.843**
Trichoptera	349 (44.3)	237 (26.9)	381 (30.0)	269 (12.6)	230 (19.3)	138 (11.7)	**19.85**	**5.19**	**0.112**

difference in infestation over the March-September sampling period in either stream.

Description of the infestation

Taxonomic identification revealed that bacteria assemblages were composed of both *Sphaerotilus* sp. and *Leptothrix* sp. Several growth stages of bacteria were apparent, ranging from early colonists to mature forms with extensive sheath networks (Fig. 4). Qualitative observations revealed that bacterial growth was typically heaviest on insect gills (i.e., the longest bacterial sheaths), but bacterial colonies with similar sheath density occurred on all insect body surfaces. Under low magnification, the bodies of bacteria-infested insects appeared fuzzy or supported a light-colored film (Fig. 5). Bacterial growth on heavily infested (>25% covered) individuals was easily detected with a hand lens when insects were immersed in water or preservative. Caudal cerci of Ephemeroptera and Plecoptera proved to be particularly good for rapid screening of bacterial growth in the field using this method (Fig. 6).

Laboratory experiments

In both experiments, *Epeorus* sp. that supported heavy bacterial growth suffered 100% mortality within the 30-d experimental run. In contrast, mean survivorship among uninfested

TABLE 4. Prevalence (% of individuals infested) and degree (% of body covered/individual) of bacterial growth on insects collected from sites downstream of cattle pastures in 2 Virginia, USA streams during March, June, and September 1995 (based on examination of 1033-1519 individuals per order). No bacteria infestation was detected at upstream sites. Percentages followed by different letters were significantly different at $p < 0.05$, as determined by G-tests.

	Benson Run		Cow pasture River	
Taxon	% infested	Mean % coverage	% infested	Mean % coverage
Ephemeroptera	61 (a)	46 (a)	56 (a)	59 (d)
Plecoptera	43 (b)	28 (b)	45 (b)	24 (b)
Trichoptera	39 (b)	14 (c)	44 (b)	23 (b)

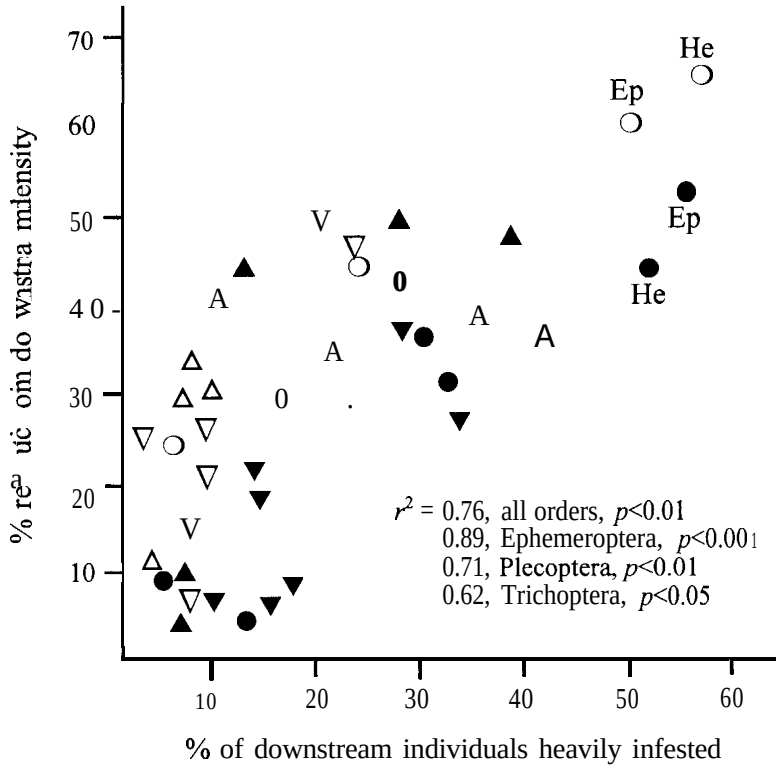

FIG. 3. Association (product-moment correlation; n=6 for Ephemeroptera, 7 for Plecoptera and Trichoptera) between severity of bacterial infestation on aquatic insects and degree of reduction in insect density downstream of cattle pastures in 2 Virginia, USA streams sampled during March, June, and September 1995. Each symbol represents a mean of 3 monthly measures for a different insect family; filled symbols indicate data for Cow-pasture River (●=Ephemeroptera, ▲=Plecoptera, ▼=Trichoptera) and open symbols indicate data for Benson Run (O=Ephemeroptera, △=Plecoptera, ▽=Trichoptera). He=Heptageniidae, Ep=Ephemerellidae.

mayflies was 86.7% (±3.3 SE, n=3) in the June experiment and 90.6% (±3.5 SE, n=3) in the September study. Surviving individuals appeared healthy and some had grown to the point of developing wing pads. None of the surviving mayflies had been colonized by bacteria, indicating that there was no chamber-to-chamber transfer or growth of bacteria.

Discussion

Effects on insect survival

Occurrence of high standing crops of aquatic bacteria below point sources of organic nutrient enrichment in stream systems, as documented here, is not uncommon. Hynes (1960) described this condition in relation to outfalls from sewage treatment plants nearly 40 y ago. He also presented photographs of insects colonized by sewage fungus that appear quite similar to the condition shown in Figs. 5 and 6 of this paper. However, colonization of insects by nearly monotypic growths of filamentous bacteria to the extent seen in this study has not been well documented. Moreover, the possibility of using this phenomenon as a bioassessment tool has not previously been investigated. My data indicate that such growths influence insect survival and, thus, have important biological consequences. I suggest that the occurrence of epizoic bacterial colonization can be a useful, quick indicator of point- or non-point-source nutrient enrichment.

Results of this investigation suggest a cause-effect linkage between nutrient levels, bacterial growth, and insect mortality. Nutrients were significantly and consistently higher in stream reaches affected by animal wastes from grazing

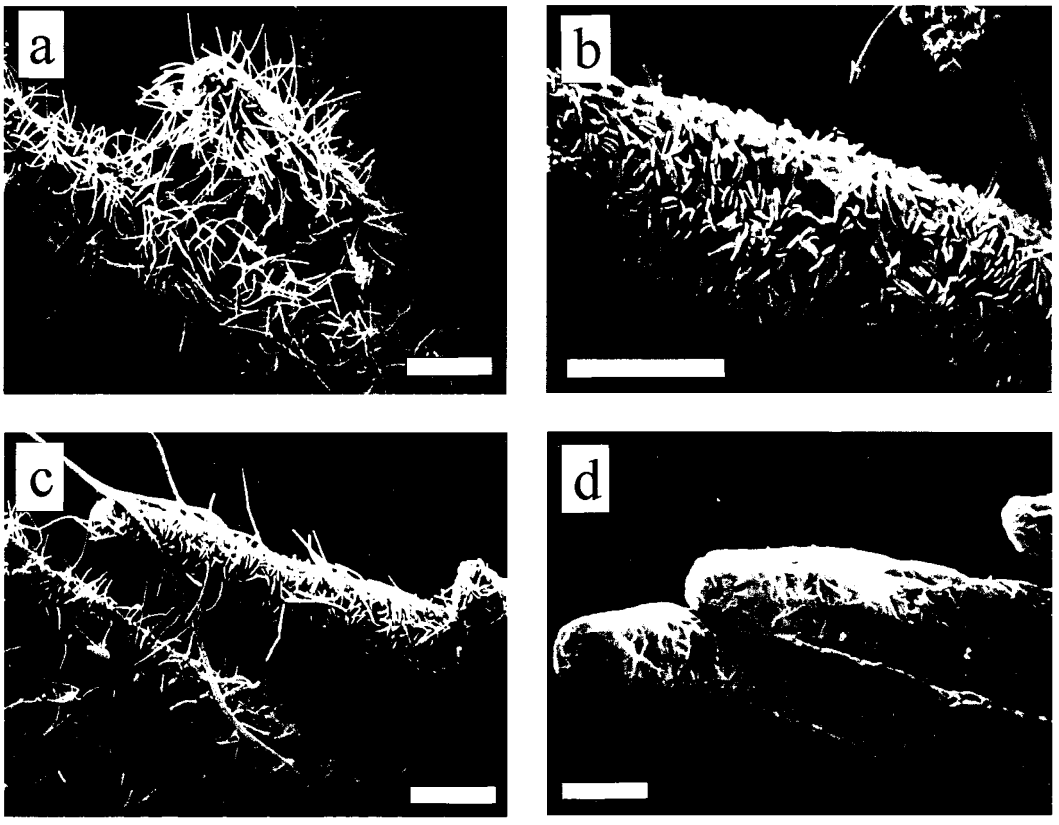

FIG. 4. Scanning electron micrographs of gills of *Epeorus* sp. illustrating early (b) and advanced (a, c) stages of bacterial colonization by *Sphaerotilus* sp. and *Leptothrix* sp. Plate d shows normal, uncolonized gills. Infestation of the degree shown in plates a and c was associated with 100% mortality in laboratory survival studies. Scale bars = 200 μm.

livestock, which were the only locations where bacterial growth on insects occurred. Results of the survival studies suggest that mortality associated with the bacterial growth can have a major influence on stream insect populations. For example, mayflies from the field samples were heavily colonized by bacteria, (e.g., up to 58% of Heptageneiidae). In the laboratory experiments all of the heavily infested mayflies died, whereas >85% of those without bacterial growth survived and appeared to be healthy. The density of mayflies in the study streams was significantly lower in reaches where bacterial growth occurred (Table 3), and there was a significant positive association between the degree of bacterial infestation and the extent to which insect populations were depressed (Fig. 3). Moreover, none of the field-collected nymphs that supported heavy bacterial growth were mature. However, nymphs without bacterial growth were represented by all life stages, including mature individuals with well-developed wing pads, which suggests that heavily colonized individuals were not surviving to emergence.

Although bacterial growth sufficient to alter benthic insect communities is easy to detect visually, initial development of the condition may be rather insidious because only modest increases in nutrients are necessary. Results of this study (Table 2) as well as earlier work (Lemly 1982) indicate that nutrient-poor streams that receive inputs sufficient to shift nutrient status on even a small scale (i.e., local increases of 0.05–0.1 mg/L phosphate, 0.5–0.8 mg/L nitrate) are vulnerable. Thus, it is not necessary for a stream to be grossly polluted for bacterial growth to develop on resident benthic insects.

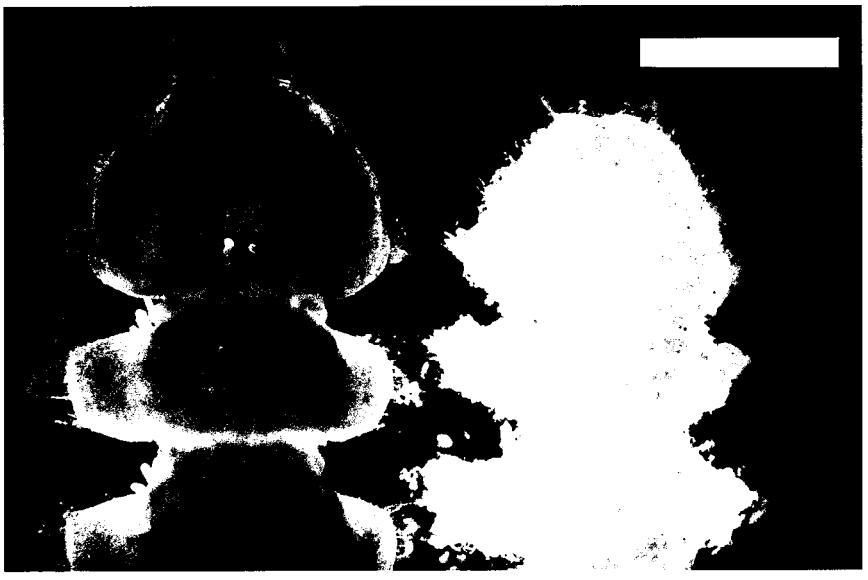

FIG. 5. Appearance of bacterial growth on *Blepharicera* sp. collected from Cow pasture River, viewed at 15X magnification (immersed in 80% ethanol). The individual on the left (from an upstream reference site) is free of filamentous bacteria, whereas the individual on the right (from a nutrient-enriched site) supports heavy growth of *Sphaerotilus sp.* and *Leptothrix sp.* Scale bar = 15 mm.

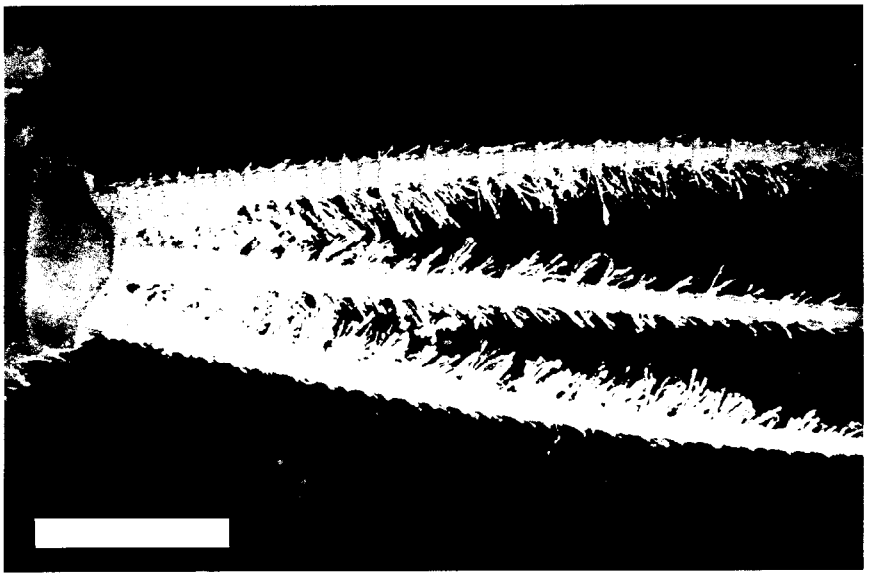

FIG. 6. Characteristic appearance of heavy bacterial growth (>25% body covered) on caudal cerci of a mayfly viewed at 15× magnification (immersed in 80% ethanol). Bacterial sheaths can be seen extending outward from the cerci. Infestation of this magnitude was associated with 100% mortality of mayflies in laboratory studies. The condition can be diagnosed in the field using a hand lens with 10–15× magnification. Scale bar = 3 mm.

Use in bioassessment

Practical application of bacterial infestation as a bioindicator of nutrient enrichment in a field setting requires only a hand lens with 10–15× magnification. Qualitative samples of insects can be viewed on-site, allowing a screening-level field assessment to be conducted within minutes.

Preservation of insects in ethanol or formalin, or manipulation of insects with collection equipment such as brushes and forceps do not dislodge the bacteria. Consequently, severity of infestation can be confirmed in the laboratory without loss of data. Archived samples collected as part of a long-term monitoring program or other research purposes can also be evaluated. Immersing individual insects into water or preservative suspends bacterial filaments attached to the lateral edges of the body for easy recognition, particularly on the caudal filaments of heavily infested Ephemeroptera and Plecoptera (Fig. 6). Individuals whose bodies are >25% covered by bacteria (i.e., the level associated with 100% mortality in my laboratory experiments) can be rapidly detected in the field or laboratory.

The method can be easily applied to fresh or preserved benthic samples collected using the EPA Rapid Bioassessment Protocol (RBP, Plafkin et al. 1989), which is widely used by fishery biologists in the southern Appalachian region and elsewhere in the USA. The RBP was developed for application to streams and rivers, and focuses on numerical relationships between Ephemeroptera, Plecoptera, and Trichoptera to assess whether a benthic macroinvertebrate community is healthy or impaired. These 3 orders of insects are also among the best to use in detecting growths of filamentous bacteria. Positive diagnosis of bacterial growth can strengthen RBP analyses by identifying a probable cause for impaired macroinvertebrate communities, and it can help to focus subsequent investigations because nutrient enrichment is indicated as a major contributing factor. The simplicity and speed of the method allow it to be incorporated into the EPA RBP with little additional effort by those conducting stream surveys.

My data suggest that bacterial growth on insects can be a practical tool for identifying the existence of non-point-source nutrient inputs into stream systems, as well as evaluating the severity of biological impacts from known sources. Such information provides a 1st step in the implementation of remediation programs to initiate stream recovery. Many land uses can contribute to elevated nutrients in streams. However, organic and inorganic nutrients associated with agriculture probably pose the greatest threat to coldwater resources in the Appalachian Mountain region. For example, livestock wastes contain high concentrations of nitrogen, phosphorus, and ammonia, which are easily dissolved in rainwater and washed into streams from pastures and feedlots (Hrubant et al. 1978, Hrubant and Detroy 1980). In-stream nutrient and bacterial concentrations can change dramatically in response to such runoff (Stephenson and Street 1978, Lemly 1982, this study), ultimately resulting in detrimental effects to benthic organisms that serve as a food resource for important sport fish such as brook trout. Water-quality problems associated with grazing livestock are pervasive in the southeastern USA (e.g., Yow 1996). Allowing cattle to graze in the riparian zone likely played a major role in the biological effects observed in this study.

The US Forest Service and other federal and state agencies have developed and implemented best management practices (BMPs) for forestry and agricultural activities. Although BMPs have been widely implemented, they are seldom monitored to determine their long-term success. Recent reviews by the Forest Service have identified the need to evaluate the effectiveness of BMPs in meeting water-quality goals and objectives (Dissmeyer 1994). Results of my studies have direct application to these efforts. Assessing bacterial growth on insects should be a useful technique for evaluating the effectiveness of BMPs in controlling nutrients associated with forestry, agriculture, and other land uses.

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