SBS-4202 APP

USING BACTERIAL GROWTH ON INSECTS TO ASSESS NUTRIENT IMPACTS IN STREAMS

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Abstract. A combination field and laboratory study was conducted to evaluate the ability of a recently developed bioindicator to detect detrimental nutrient conditions in streams. The method utilizes bacterial growth on aquatic insects to determine nutrient impacts. Field investigations indicated that elevated concentrations of nitrate and phosphate were associated with growth of filamentous bacteria on insect body surfaces, and that there was a significant reduction in the density of major insect taxa in the nutrient-enriched stream reaches. Laboratory investigations confirmed a strong linkage between bacterial growth and reduced survival of insects. Survival was examined for insects with bacterial infestation ranging from 0% to greater than 50% coverage of the body surface. A threshold for catastrophic mortality occurred at about 25% body coverage; there were few survivors above that amount. Based on these findings, the diagnostic endpoint for the bioindicator is 25% body coverage by bacterial growth, a level that signifies major impacts and is also easy to detect visually. This study provides additional evidence that the insect-bacteria bioindicator is a reliable tool for assessing nutrient impacts on stream macroinvertebrate communities. The bioindicator should prove useful for identifying nutrient-impacted sites as well as monitoring the success of management actions to improve water quality.

Keywords: aquatic bacteria, benthic macroinvertebrates, bioindicator, eutrophication, nitrogen, phosphorus, stream pollution

1. Introduction

Nutrient enrichment of streams is a long-standing problem that continues to have substantial local and regional consequences. For example, water quality of streams in the southern Appalachian Mountains of the U.S. can be seriously degraded by organic nutrients leached from animal wastes if cattle or other livestock are allowed to graze in the riparian zone (Lemly, 1982). Local efforts to recover native brook trout (*Salvelinus fontinalis*) and improve habitat for cold-water fishes are often undermined by poor livestock management practices (Yow, 1996). At a regional scale, the cumulative effects of nutrient-enriched streams has resulted in eutrophication of important Atlantic Coast estuaries such as Chesapeake Bay in Virginia and the Albemarle-Pamlico system in North Carolina. Recent outbreaks of a toxic estuarine dinoflagellate (*Pfiesteria piscicida*), which caused massive fish kills and

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affected human health, have been attributed to nutrient enrichment (Glasgow *et al.*, 1995).

Natural resource managers need to be able to precisely evaluate nutrient enrichment for two reasons: 1) to measure the extent and severity of detrimental effects on aquatic life, and 2) to monitor the success of efforts to reduce nutrient impacts – i.e., to determine if best management practices result in measurable improvements. With regard to streams, one of the basic tools that investigators in the U.S. use to evaluate biological conditions is the EPA Rapid Bioassessment Protocol (RBP) for macroinvertebrates (Plafkin *et al.*, 1989). Although this method reveals impaired benthic communities, it has an important weakness – it does not identify cause-effect linkages, i.e., whether the impairment is due to chemical pollutants, sedimentation, nutrient enrichment, or other perturbations.

Recognizing the inherent weakness of RBP for nutrient assessment, Lemly (1998) devised a method by which growth of filamentous bacteria (*Sphaerotilus* sp., *Leptothrix* sp.) on aquatic insects can be used as a bioindicator of detrimental nutrient levels in streams. He found that simple visual assessment of benthic samples using a hand lens (10–15× magnification) is sufficient to identify sites where nutrient impacts are likely to be occurring. Laboratory studies determined that bacterial growth reduced insect survival, providing evidence of a cause-effect linkage between bacterial growth and impaired insect communities found in the field.

Although Lemly's insect-bacteria assessment method shows promise as a significant addition to the RBP, his findings were limited to one watershed. Additional field testing is necessary to validate the method and determine its potential for broad application in stream nutrient impact assessment. This paper presents the results of a combination field and laboratory study conducted to provide part of that information.

2. Methods

2.1. Field surveys

A series of benthic samples was taken to determine the possible influence of livestock grazing on the relative abundance of aquatic insects by making upstream-downstream comparisons. Insects were collected from a 2nd-order stream (Craig Creek) located in the Appalachian Mountains of Virginia (Figure 1). Craig Creek is a low gradient (<5%), moderate-elevation (750 m above sea level) stream that supports brook trout (*Salvelinus fontinalis*) and other coldwater fishes typical for this mountain region. Three quantitative samples were collected in each of 6 riffles of the stream in March, June, and September 1996, and again in March, June, and September 1997 (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

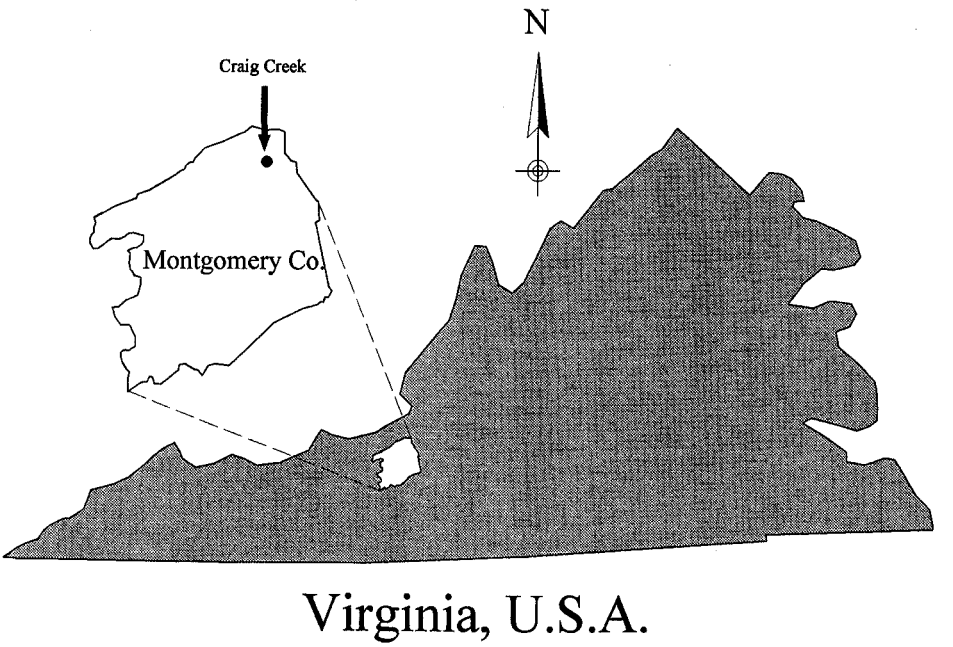

Figure 1. Location of Craig Creek in the Appalachian Mountain region of Virginia.

from the center of the stream to the margin of the wetted channel. Three of the riffles were downstream (within 300 m) of a cattle pasture (10 ha, 26 animals) where active grazing and deposition of animal waste was occurring immediately adjacent to the stream. The remaining 3 riffles were upstream (within 300 m) of the cattle pasture. 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 (10–200× magnification). Some individuals of each order 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 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 cm^{-2}; 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 percent of the body covered by bacteria. At least 100 individuals per family from each site on each date were examined to determine prevalence and degree of infestation.

Three types of statistical analyses were conducted on insect data: 1) Differences among insect orders in percent infestation/insect population and percent coverage/individual by bacteria were tested for statistical significance using *G*-tests (Sokal and Rohlf, 1981). Data for each stream reach 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 m^{-2}) was calculated and upstream-downstream comparisons (by insect order and family) were made using 2-factor ANOVA (location × date) on log-transformed densities. 3) Product-moment correlation (Sokal and Rohlf, 1981) was used to examine associations between percent reduction in downstream density and percent 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).

2.2. LABORATORY TESTS

Four experiments were conducted to determine effects of bacterial growth on insect survival. In August 1996, and June, July, and August 1997, live mayflies (*Epeorus* sp.) from the downstream reach of Craig Creek 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 Tech University for survival studies. Three Plexiglas® aquaria with recirculating, aerated, and temperature-controlled water supplies were used to conduct these experiments (Figure 2). Each aquarium held

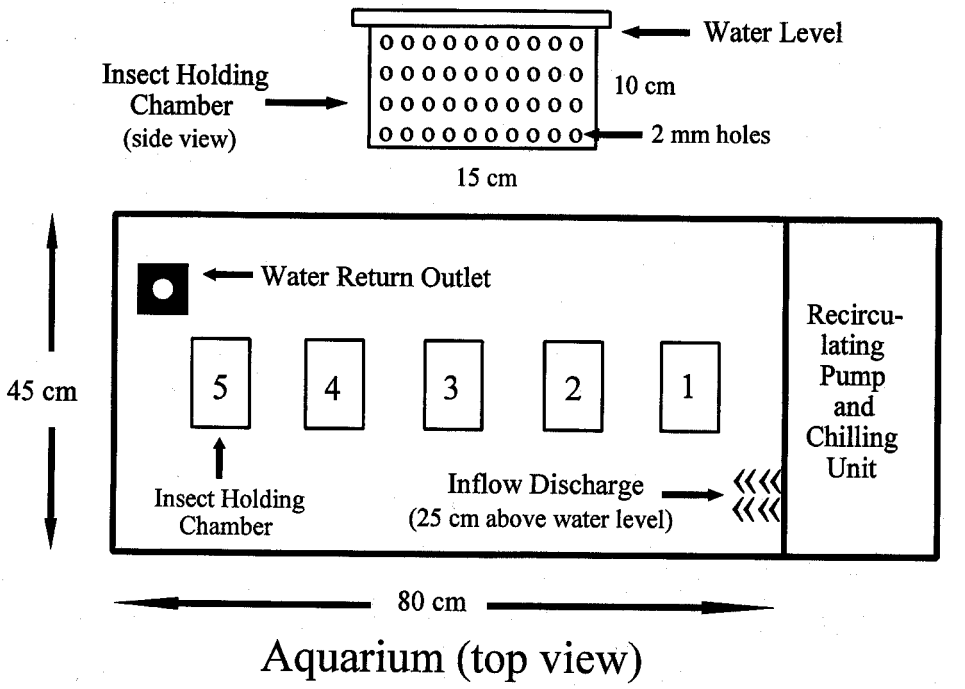

Figure 2. Schematic top view of aquarium containing 5 chambers used to hold mayflies in the survival experiments and a side view of a single chamber. A total of 3 aquaria and 15 chambers were used.

five 1.5-L chambers (containing several 3–5 cm Craig Creek cobbles) into which insects were placed. The sides and bottoms of the chambers were drilled with 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 aquarium. The water circulation rate in aquaria was ~960 L h^{-1} (manufacturer's operational specifications for the recirculating pump), yielding a mid-tank current velocity of 10–15 cm s^{-1}, based on timing of air bubbles traveling through the aquaria.

Epeorus sp. was selected for study because: 1) they are common in Craig Creek, 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 12 h:12 h light:dark regime was maintained throughout each experiment, which lasted ~30-d. During experiments, may-

flies fed on algae and associated microorganisms that grew as a biofilm on the stones in the experimental chambers. Dogwood (*Cornus florida*) leaves were placed among the cobbles to supplement mayfly diets and stimulate the growth of the biofilm. Leaves were conditioned by incubating them in the chambers for 30-d prior to introducing the insects. Dogwood leaves were used because they condition quickly, and this species is a common understory tree in the riparian zone of the study stream. Insects were recovered and enumerated at the end of each experiment and percent mortality was determined. Surviving insects were examined for bacterial growth under a dissection microscope.

Four 30-d mayfly survival experiments were conducted; 28 August to 30 September 1996, 2 June to 2 July 1997, 16 July to 15 August 1997, and 26 August to 26 September 1997. Previous survival studies (Lemly, 1998) had divided mayflies into two test groups; individuals without bacterial growth, and individuals with >25% body coverage. In those experiments, all mayflies with >25% body coverage failed to survive. However, it was not clear what degree of bacterial growth constituted a 'lethal' level. Based on those findings, it was decided that the new experiments should examine whether a threshold for survival existed. This question was investigated by establishing test groups that represented bacterial coverage ranging from 0 to >50%. The four survival experiments tested the following levels of infestation: Experiment 1) two groups – 0 and >50% body coverage; Experiment 2) three groups – 0%, 10–25%, and 25–50% coverage; Experiment 3) three groups – 10–20%, 20–30%, and 30–40% coverage; Experiment 4) three groups – <10%, 10–20%, and 20-30% coverage.

Prior to beginning each survival experiment, mayflies were examined under a dissection microscope and divided into the desired test groups based on the degree of bacterial infestation. Gross visual estimates, rather than the quantitative block-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 possible to sort mayflies into the proper test groups. Each testing chamber received 10 individuals (August 1996 study) or 7 individuals (1997 studies) from one of the groups, and each chamber was randomly assigned to 1 of the 3 aquaria (Sokal and Rohlf, 1981).

3. Results

3.1. Field surveys

Nutrient concentrations in Craig Creek were significantly lower upstream of the cattle pasture than at downstream sites (Table I). The density of insects, especially Ephemeroptera, was significantly lower (up to 72% less) in the downstream reach

TABLE I

Mean concentrations of dissolved nutrients (±1 SE) in Craig Creek Virginia, U.S.A. during 1996–1997, n = 5. Upstream denotes reference sites where nutrient concentrations were uninfluenced by cattle. Downstream denotes sites where grazing and deposition of animal waste was occurring adjacent to the stream. t-probabilities (upstream-downstream comparison): *** = $p<0.01$

Year	Month	Total orthophosphate (mg/L)			Total nitrate (mg/L)		
		upstream	downstream	t-prob.	upstream	downstream	t-prob.
1996	March	0.06 (0.008)	0.21 (0.013)	***	0.10 (0.012)	1.66 (0.044)	***
	June	0.05 (0.011)	0.39 (0.011)	***	0.15 (0.019)	1.95 (0.049)	***
	September	0.04 (0.012)	0.44 (0.020)	***	0.15 (0.010)	1.73 (0.021)	***
1997	March	0.04 (0.010)	0.35 (0.026)	***	0.14 (0.017)	1.89 (0.033)	***
	June	0.04 (0.015)	0.32 (0.007)	***	0.16 (0.021)	1.95 (0.017)	***
	September	0.02 (0.020)	0.31 (0.012)	***	0.13 (0.030)	2.38 (0.031)	***

where bacterial growth occurred (Table II, Figure 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 (3.7%) supported heavy growths of bacteria. All taxa of insects from sites downstream of the cattle pasture exhibited growths of filamentous bacteria; no bacteria were detected on insects at upstream sites. Prevalence and degree of infestation was highest in Ephemeroptera (Table III), and the degree of infestation was greatest in Ephemerellidae and Heptageneiidae (Figure 3). Individual Ephemerellidae and Heptageneiidae were often nearly covered by bacterial colonies (coverage of infested individuals = 5–92 and 15–94%, respectively). There was a significant positive association between % of downstream individuals heavily infested and percent reduction in downstream density of insects (Figure 3). There was no apparent seasonal difference in infestation over the March-September sampling period in either year.

TABLE II

Comparison of insect densities (mean no. of individuals $m^{-2} \pm 1$ SE) upstream (up) and downstream (down) of cattle pastures in Craig Creek Virginia, U.S.A. during March, June, and September 1996-97. 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

Year	Taxon	March		June		September		ANOVA results		
		up	down	up	down	up	down	location	date	location × date
1996	Total EPT	911 (30.7)	483 (23.9)	1035 (22.2)	668 (20.0)	981 (23.0)	522 (18.7)	**55.22**	0.212	0.690
	Ephemeroptera	379 (18.3)	124 (10.2)	502 (31.1)	229 (18.5)	417 (18.7)	202 (20.3)	**142.90**	0.525	0.136
	Plecoptera	321 (19.2)	152 (12.7)	311 (18.4)	178 (22.1)	330 (21.6)	143 (15.3)	**43.61**	0.2939	0.107
	Trichoptera	260 (20.6)	140 (13.0)	257 (26.9)	155 (14.2)	224 (10.4)	135 (12.3)	**35.23**	0.144	0.392
1997	Total EPT	1148 (22.6)	550 (14.7)	1325 (32.6)	663 (12.3)	1172 (30.2)	509 (20.5)	**101.15**	0.396	0.240
	Ephemeroptera	635 (39.7)	140 (27.3)	615 (56.2)	260 (18.7)	543 (34.5)	208 (16.9)	**214.39**	0.121	0.402
	Plecoptera	329 (14.0)	166 (10.6)	344 (12.3)	202 (20.4)	367 (10.9)	217 (15.7)	**50.11**	0.345	0.261
	Trichoptera	360 (24.1)	193 (15.9)	351 (20.1)	255 (21.6)	371 (12.7)	142 (9.7)	**41.26**	0.195	0.113

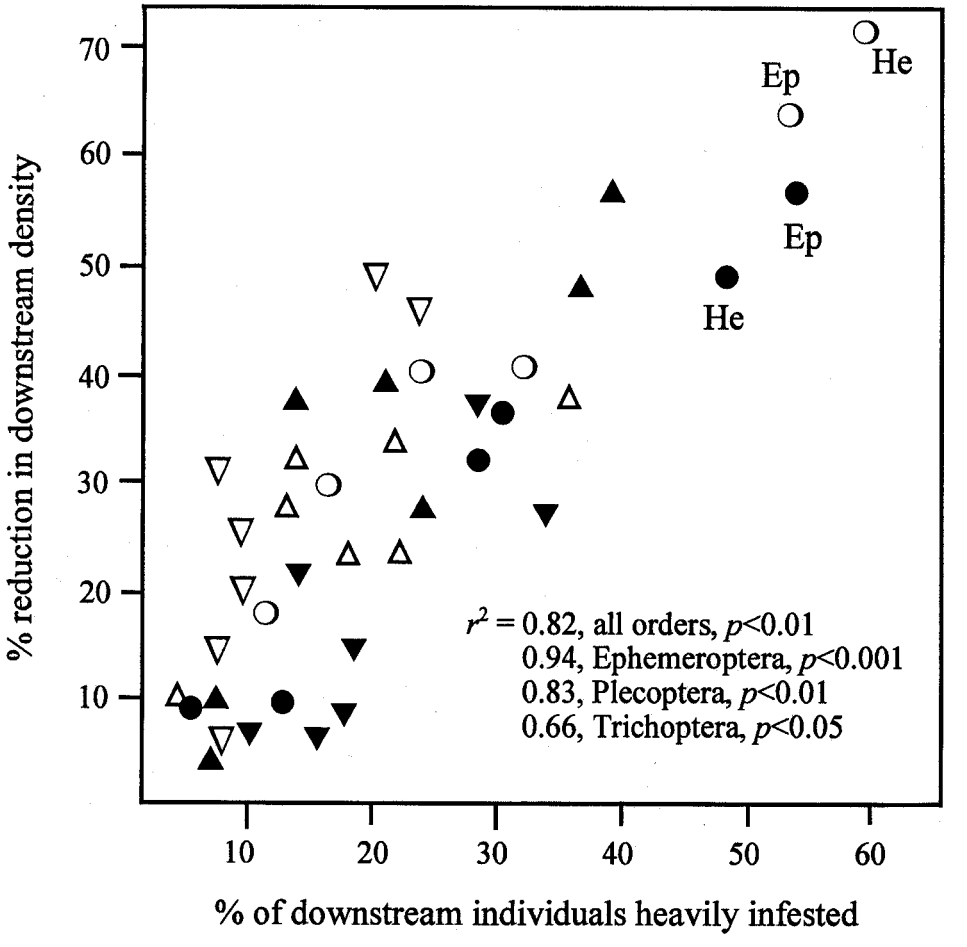

Figure 3. Association (product-moment correlation; $n = 6$ for Ephemeroptera, 7 for Plecoptera and Trichoptera) between severity of bacterial infestation on aquatic insects and degree of reduction in insect density downstream of cattle pastures in Craig Creek Virginia, U.S.A. during March, June, and September 1996–1997. Each symbol represents a mean of 3 monthly measures for a different insect family; filled symbols indicate data for 1996 (● = Ephemeroptera, ▲ = Plecoptera, ▼ = Trichoptera) and open symbols indicate data for 1997 (○ = Ephemeroptera, △ = Plecoptera, ▽ = Trichoptera). He = Heptageneiidae, Ep = Ephemerellidae.

3.2. Characteristics of Bacterial Infestation

Taxonomic identification determined that bacterial assemblages were composed of both *Sphaerotilus* sp. and *Leptothrix* sp. Qualitative observations indicated that bacterial growth was especially luxuriant on insect gills (i.e., the longest bacterial sheaths), but bacterial colonies with similar sheath density occurred on all insect body surfaces. Scanning electron microscopy revealed the extent to which these filamentous bacteria were able to colonize individual gill filaments (Figure 4). Un-

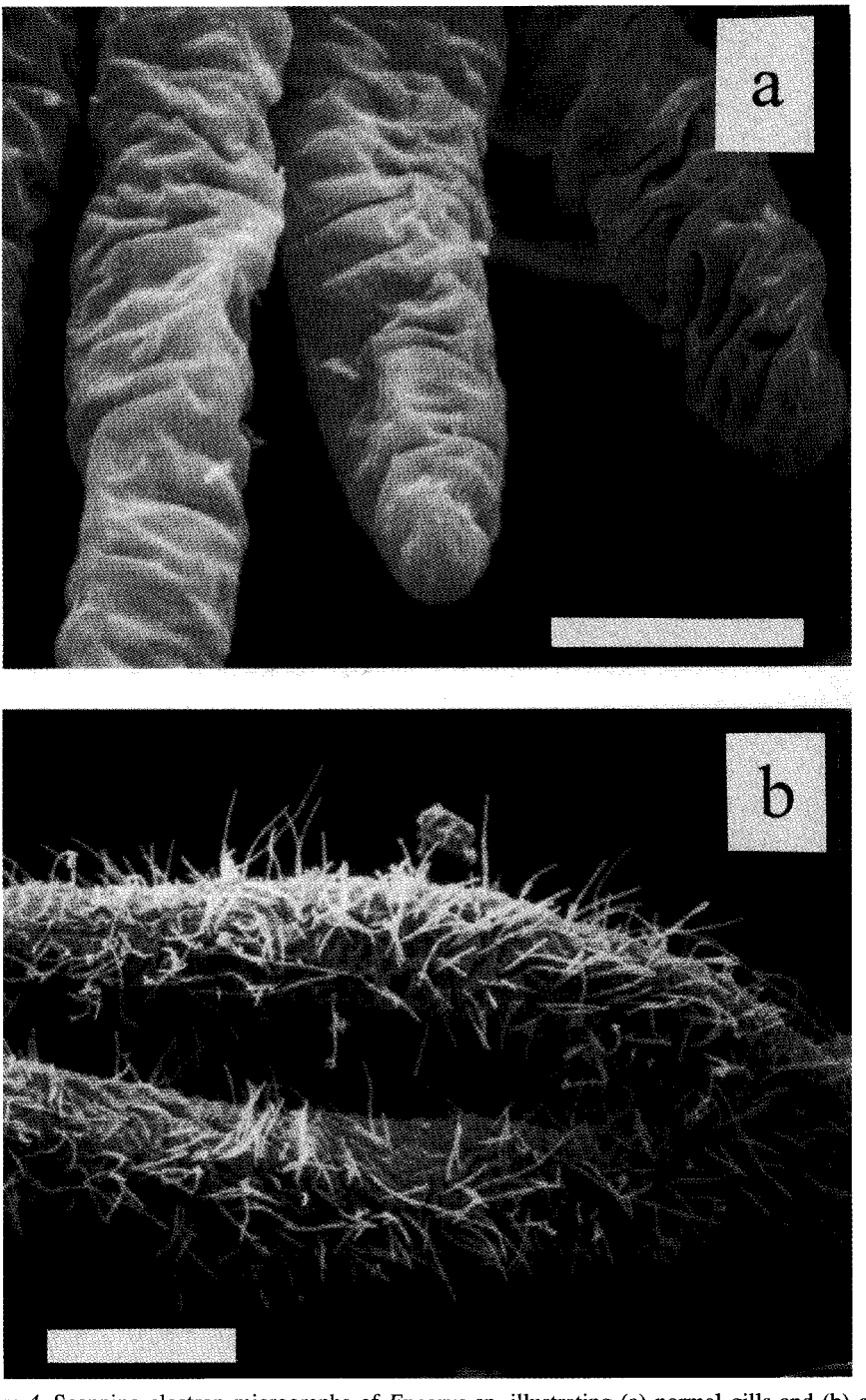

Figure 4. Scanning electron micrographs of *Epeorus* sp. illustrating (a) normal gills and (b) gills heavily infested (>25% covered) with the filamentous bacteria *Sphaerotilus* sp. and *Leptothrix* sp. Infestation of the degree shown in plate b was associated with 100% mortality in laboratory survival studies. Scale bars = 250 μm.

TABLE III

Prevalence (% of individuals infested) and degree (% of body covered/individual) of bacterial growth on insects collected from sites downstream of cattle pastures in Craig Creek, Virginia U.S.A. during March, June, and September 1996–1997 (based on examination of 915–1263 individuals per order). No bacterial infestation was detected at upstream sites. Percentages followed by different letters were significantly different at $p<0.05$, as determined by G-tests

Taxon	1996		1997	
	% Infested	Mean % coverage	% Infested	Mean % coverage
Ephemeroptera	68 (a)	49 (a)	72 (a)	57 (d)
Plecoptera	49 (b)	25 (b)	41 (b)	29 (b)
Trichoptera	37 (b)	15 (c)	44 (b)	20 (b)

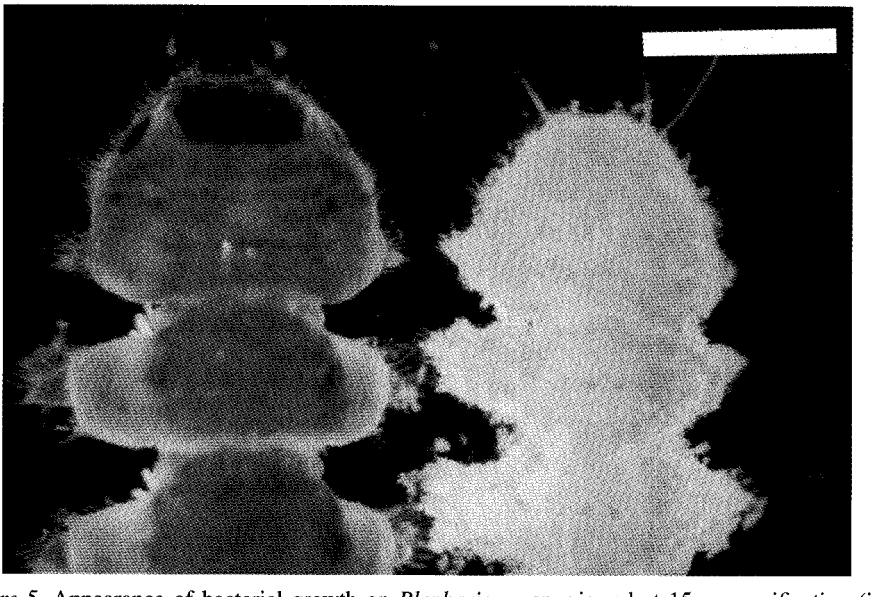

Figure 5. Appearance of bacterial growth on *Blepharicera* sp. viewed at 15× magnification (immersed in 80% ethanol). The individual on the left is free of filamentous bacteria, whereas the individual on the right supports heavy growth (>25% body coverage) of *Spaherotilus* sp. and *Leptothrix* sp. Infestation of this magnitude is diagnostic of nutrient impacts on stream insect populations. Scale bar = 1.5 mm.

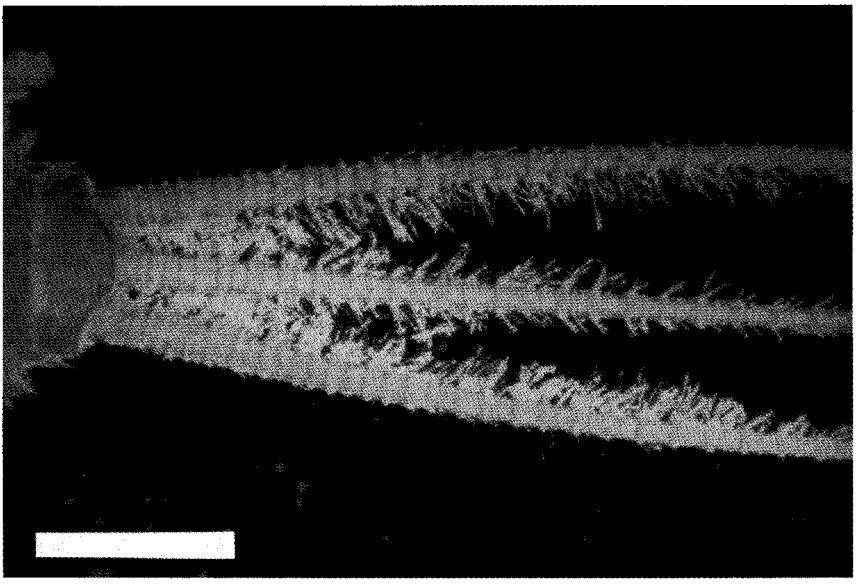

Figure 6. Characteristic appearance of heavy bacterial growth (>25% body coverage) on caudal cerci of a mayfly viewed at 15× magnification (immersed in 80% ethanol). Bacterial sheaths can be seen extending outward from the cerci. Infestation of this magnitude is a reliable indicator of nutrient impacts on benthic macroinvertebrate communities. The condition can be easily diagnosed in the field using a hand lens with 10–15× magnification. Scale bar = 3 mm.

der low magnification, the bodies of infested insects appeared fuzzy, supporting a light-colored film (Figure 5). Bacterial growth on heavily infested (>25% covered) individuals was easily detected with a hand lens (10–15×) when insects were immersed in water or preservative. Caudal cerci of Ephemeroptera and Plecoptera proved to be particularly good for rapidly screening individuals to assess the degree of bacterial growth, both in the lab and field (Figure 6).

3.3. Laboratory Experiments

In all four experiments, *Epeorus* sp. that supported heavy bacterial growth (>25% body coverage) suffered nearly 100% mortality within the 30-d experimental run. In contrast, mean survivorship among uninfested mayflies was 83.7% (±2.1 SE, n = 3) in the 1996 experiment and 92.1% (±3.0 SE, n = 9) in the 1997 tests. 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. A plot of the relationship between severity of bacterial infestation and survival of *Eporus* sp. revealed that a threshold for catastrophic mortality existed at about the 25% level of body coverage (Figure 7). Almost all individuals with >25% body coverage died, but many of those with 10–25% coverage survived and appeared to be healthy and essentially unaffected.

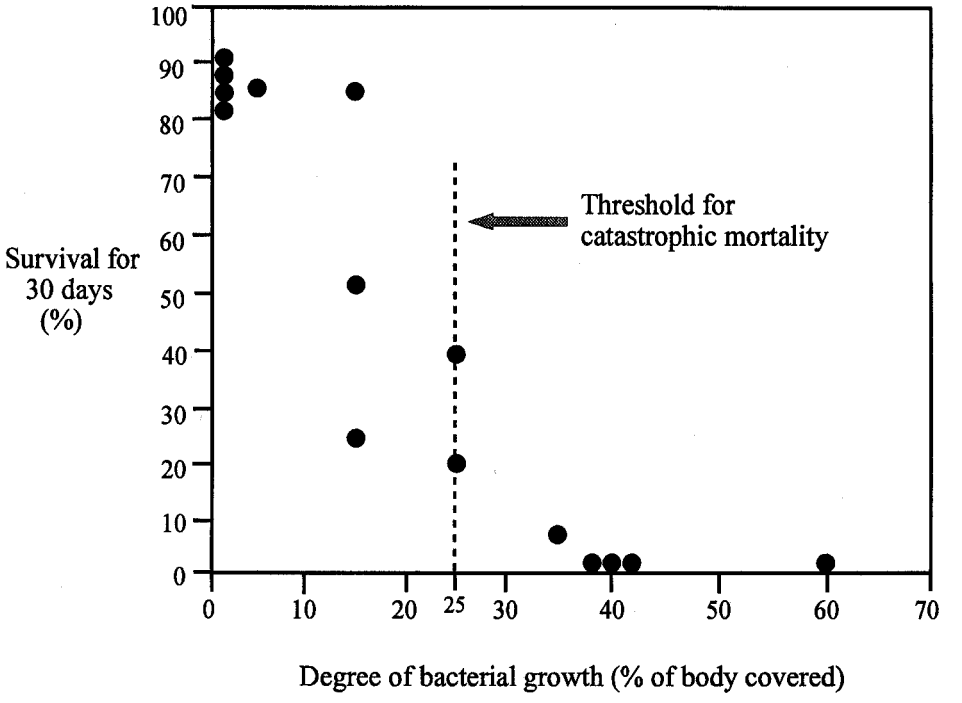

Figure 7. Relationship between degree of bacterial infestation and survival of *Epeorus* sp. for 30-d in the laboratory. Each dot represents 35–70 individuals. A threshold for catastrophic mortality exists at about the 25% level of body coverage. Beyond this level of infestation, very few individuals survive.

4. Discussion

4.1. Diagnostic capability

The findings of this study mirror those of Lemly (1998), who concluded that the occurrence of epizoic bacterial colonization of aquatic insects can be a useful, quick indicator of detrimental point- or non-point-source nutrient enrichment. The present study supports that conclusion. A major new finding was that a mortality threshold exists. The degree of bacterial growth associated with the mortality threshold can be used as a diagnostic endpoint. When mortality data from the laboratory experiments is examined in combination with relationships between insect density and bacterial infestation in the field, 25% body coverage emerges as the diagnostic endpoint for the bioindicator. Survival of insects with 10–25% coverage can be good but above 25%, survival is unlikely. Thus, the metric used to signify harmful impacts of nutrients on stream insect populations is 25% body coverage by filamentous bacteria.

As with Lemly's earlier findings, results of this study seem to indicate a cause-effect linkage between nutrient levels, bacterial growth, and insect mortality. However, since the laboratory studies utilized field-infested mayflies, it is not possible

to know if the bacteria alone were responsible for death, or a combination of bacteria and other stressors to which the insects were exposed in the field (e.g., sediment or organic loading). Nevertheless, the evidence for cause-effect is strong. Nutrients were significantly and consistently higher in stream reaches affected by animal wastes from grazing livestock, which were the only locations where bacterial growth on insects occurred. Results of the survival studies, in combination with evidence from the field surveys, indicate 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 61% of Heptageneiidae, Figure 3). In the laboratory experiments <10% of the heavily infested mayflies survived, whereas >80% of those without bacterial growth survived and appeared to be healthy. The density of mayflies in the study stream was significantly lower in reaches where bacterial growth occurred (Table II), and there was a significant positive association between the degree of bacterial infestation and the extent to which insect populations were depressed (Figure 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.

4.2. Reliability and simplicity

This is now the second study to experimentally confirm that bacterial infestation of insects has practical application as a bioindicator of detrimental nutrient enrichment in a field setting. Importantly, detection of the diagnostic endpoint (insects with ≥25% body coverage) is easily accomplished with a hand lens or low-magnification dissection scope (10–15×). Intensive quantitative benthic sampling is not necessary; qualitative kick samples of insects are adequate and they 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 (Figure 6). Individuals whose bodies are >25% covered by bacteria (i.e., the indicator level for impact assessment) can be rapidly detected in the field or laboratory.

4.3. Application to Rapid Bioassessment

The bioindicator can be easily applied to fresh or preserved benthic samples collected using the EPA Rapid Bioassessment Protocol (RBP; Plafkin *et al.*, 1989), which is used by fishery biologists throughout the U.S.A. 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.

5. Conclusions

This study provides additional evidence that the insect-bacteria bioindicator is valid for application to nutrient assessment in streams. Bacterial growth on insects is a practical tool for identifying the existence of detrimental non-point-source nutrient inputs, as well as evaluating the severity of biological impacts from known sources. Rapid field or laboratory screening of benthic samples is possible. A discovery of insects whose bodies are $\geq 25\%$ covered by filamentous bacteria is all that is necessary to reliably diagnose harmful impacts of nutrients on stream macroinvertebrate communities. The information provided by this bioindicator will be useful for detecting nutrient problems as well as monitoring the success of management actions to improve water quality. Additional investigations are needed to evaluate the possible application of the method to other ecosystems (lakes, wetlands) and nutrient sources (chemical fertilizers, industrial and municipal wastewater).

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