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Response of macroinvertebrates to blooms of iron-depositing bacteria

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Abstract

In field measurements and laboratory experiments we assessed the influence of high levels of iron, manganese, and concurrent blooms of iron-depositing bacteria, *Leptothrix ochracea*, on macroinvertebrates. Macroinvertebrate communities in five of six streams were depauerate inside blooms. Reasons for the decreased abundance vary among taxa, with our experiments demonstrating the importance, for one or more species, of (1) direct toxic effects, and,/or smothering, (2) behavioral avoidance of bacterial-coated substrates, and (3) an inability to use bacteria as food. Three mayfly species showed increased mortality when caged inside the blooms, but five trichopterans and one plecopteran did not. Five invertebrates avoided *Leptothrix*-coated substrate in choice trials, while three did not. *Stenonema fuscum* could not ingest *Leptothrix*, and *Neophylax nacatus* had reduced growth feeding on it, but *Heptagenia umbratica* grew equally well on diets of *Leptothrix* or diatoms. This study demonstrates the important role epilithic organisms play in modifying substrates, and how these changes may act to influence benthic abundance and distribution in streams.

Introduction

Iron enriched streams often show lowered macroinvertebrate abundance and diversity, but the causes for this decrease are unresolved. McKnight & Feder (1984) suggested coating of substrates by metal precipitates could be deleterious to stream communities by limiting periphytic colonization and reducing primary productivity. Not only might this limit food for macroinvertebrates, but ferric hydroxide deposition reduces substrate heterogeneity (Osborne *et al.*, 1979) and may smother macroinvertebrates by coating respiratory surfaces (Koryak *et al.*, 1972). Iron oxides may also interfere with ion exchange and osmoregulation.

This study investigates how elevated levels of iron and manganese, and a concomitant bloom of an iron-depositing bacterium, influence the macroinvertebrate community in Unnamed Brook, a small stream in northern Vermont. The bacterium, identified as *Leptothrix ochracea* (Mulder & Deinema, 1981; W. Ghiorse, 1988, pers. comm.), is an aquatic, sheathed bacterium that becomes encrusted with iron and manganese oxides. *L. ochracea* does not obtain energy from the oxidation of these metals, but its presence in aquatic systems is correlated with high levels of iron and manganese (Jones, 1975; Sheldon & Skelly, 1990). *L. ochracea* is common in seeps, pools, and in small patches near the banks of rivers and streams (Ghiorse, 1984, pers. obs.). What makes Unnamed Brook remarkable is the extent to which *L. ochracea* covers available sub-

strate. In the summer this stream harbors a bloom of *L. ochracea* that extends nearly 200 m and blankets substrates with a ocher-colored, gelatinous coating. Diatoms commonly observed upstream of the bloom are absent inside it (Sheldon & Skelly, 1990), and both macroinvertebrate diversity and abundance are greatly reduced.

This study had three objectives: (1) to describe *L. ochracea* bloom dynamics in Unnamed Brook over time, especially as related to changes in Fe and Mn ion concentrations; (2) to quantify patterns in the distribution and abundance of periphytic and benthic organisms; (3) to ascertain the factors responsible for low macroinvertebrate abundance and diversity within *L. ochracea* blooms.

We hypothesized that three factors related to *L. ochracea*'s presence could result in the observed patterns of macroinvertebrate distribution and reduced abundance observed in Unnamed Brook: (1) unsuitable substrate, (2) toxic conditions, and (3) poor food quality. Many authors have cited these factors to account for depleted macroinvertebrate communities found in iron-enriched streams (Parsons, 1968; Warner, 1971; Koryak *et al.*, 1972; Brown, 1977; Greenfield & Ireland, 1978; Letterman & Mitch, 1978; Osborne *et al.*, 1979; Scullion & Edwards, 1980; Matter & Ney, 1981; Sode, 1983; McKnight & Feder, 1984; Rasmussen & Lindegaard, 1988), but only toxicity has been tested (Warnick & Bell, 1969; Brown, 1977). In view of the importance of both substrate and food in determining lotic macroinvertebrate distributions (Minshall, 1984), these parameters must be addressed if macroinvertebrate community structure inside blooms of iron-depositing bacteria is to be understood.

Study site

Unnamed Brook is a small, second order stream located on the northwest face or Morse Mountain in northern Vermont (44° 35′ N, 72° 47′ W). It originates at an elevation of 252 m and drains 83 ha of mixed conifer-hardwood forest before joining the Brewster River 2.0 km to the northwest. Width varies from 0.5 to 3.0 m, and stream depth ranges from less than 0.1 m, to 0.7 m. The substrate is primarily cobble, with sand predominating in pools and runs. Mean annual pH is 6.2 (s.d. = 0.35). Flow ranges from 0.006 to 0.012 m^3 s^{-1}. Ferromanganese flocs from Unnamed Brook contain sheathed, iron-depositing bacteria characteristic of *Leptothrix ochracea* (Mulder & Deinema, 1981; W. Ghiorse, 1988 pers. comm.). Metal oxides and *L. ochracea* have displaced the algal community and cover substrates with an ocher-colored layer 1–4 mm thick (Sheldon & Skelly, 1990).

Twelve sampling stations spaced 50 m apart were established along a 1.2 km stream section containing a bloom of *Leptothrix ochracea* (Fig. 1). Four upstream stations (U 200 to U 50) were clear of the bloom and showed no iron or manganese enrichment. At station 0 where the bloom began, iron and manganese concentrations rose

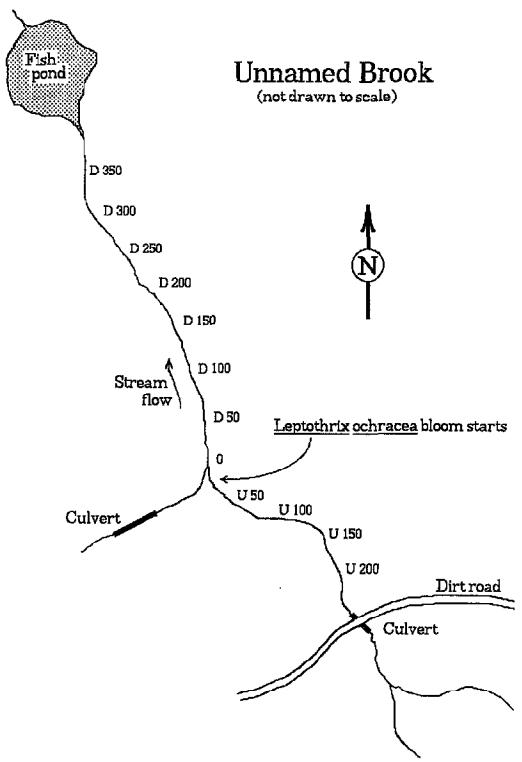


Fig. 1. A map of Unnamed Brook showing the location of the 12 permanent sampling stations and the origin of the *Leptothrix ochracea* bloom. At its height in mid-summer the bloom extended to station D 200.

sharply due to inputs from groundwater. The bloom persisted as long as ionic concentrations remained approximately 0.10 mg l^{-1} or greater. Typically this occurred down to station D 200. Stations D 250 to D 350 were downstream of the bloom and generally free of *L. ochracea*. Iron and manganese concentrations here were lower than stations inside the bloom. For purposes of statistical analysis, the brook was divided into three sections of four stations each: upstream stations U 200 to U 150, bloom stations 0 to D 50, and downstream stations D 200 to D 350.

Materials & methods

Descriptive studies investigated how iron and manganese concentrations, *L. ochracea*, algae, and macroinvertebrates varied in space and time in Unnamed Brook. In addition, macroinvertebrate diversity was measured in five other streams to assess the general response of macroinvertebrates to blooms of iron-depositing bacteria. Three experiments examined how macroinvertebrate substrate preference, mortality from toxic effects, and feeding could account for their low abundance inside *L. ochracea* blooms.

Descriptive work

Water chemistry

We monitored total Fe and Mn in Unnamed Brook throughout the year from June 1989 to August 1990. Water samples were collected either monthly (November 1989 to April 1990) or weekly (all other dates) at each station. Water was collected in acid-washed Nalgene bottles and returned to the lab for analysis. Weekly data were averaged to obtain a mean value for each station over a given month. Total iron and manganese concentrations were determined with a Thermo Jarrell Ash Inductively Coupled Argon Plasma (ICAP) 61 spectrometer. In addition, monthly data on pH, flow volume, and temperature were provided by Smuggler's Notch Ski Resort water treatment facility.

Epilithon assays

In order to document and quantify the transition of the epilithic community from algae to *L. ochracea*, and then back to algae again, we set out artificial substrates in Unnamed Brook, allowed time for epilithic colonization, and then performed assays for chlorophyll *a* and biomass.

Unglazed 15 × 15 cm clay tiles were placed on the stream bed at each station, and removed after 45 d. Epilithon removed from a 14.0 × 7.0 cm section was used to assay biomass. One 7 × 7 cm section was used to assay chlorophyll *a*, and the remaining 7 × 7 cm section was used to assay for ATP (this assay was unsuccessful, probably due to improper storage of samples). Location of these areas were haphazard with respect to the orientation of tiles in the stream. Epilithon was removed from the tile surfaces by scraping with a razor blade, scrubbing with a toothbrush, and rinsing with stream water. Slurries were drawn through pre-weighed glass fiber filters (0.45 μm porosity, 47 mm diameter) with a vacuum pump and dried. Dry weights were determined after drying filters and algae at 80 °C for 24 h. Filters were then heated at 500 °C for three hours to determine ash-free dry weights. Chlorophyll *a* was determined using the spectrophotometric method as outlined in Standard Methods for the Examination of Water and Wastewater (A.P.H.A., 1985). Data were analyzed using a one-way ANOVA with Tukey's multiple comparisons (PROC GLM, SAS Institute, 1985) of upstream, within bloom, and downstream sites.

Macroinvertebrates

Aquatic insects were sampled at each station with 0.09 m^2 Surber bottom sampler having a mesh size of 0.5 mm. Samples were taken monthly throughout the study, except December through February when thick ice prevented access to the stream bed. An effort was made to gather samples from similar cobble substrates, and from areas of similar flow (as judged by eye). Three Surber samples were taken at each station (total area = 0.27 m^2) to depth of 5 cm and combined. (Unnamed Brook showed very low productivity so combining samples was necessary to ensure

representative numbers of macroinvertebrates taken from each station). A 10% formalin solution with phloxine B stain was added to samples before sealing. Invertebrates were counted and identified to the lowest taxonomic level feasible (usually genus, but occasionally to species).

Data from each month were used to calculate H′, the Shannon-Wiener diversity index (Krebs, 1989), for each of the 12 stations. Employing diversity to measure macroinvertebrate responses to perturbation has been criticized (see Godfrey 1978 for discussion), but H′ is commonly used and presented an excellent way to condense monthly Surber sample data. Data were analyzed using a one-way analysis of variance PROC GLM with Tukey's test for comparisons (SAS). Diversity values were compared for the three stream sections for each month of the study.

Surber sampling under-represented *Neophylax* spp., *Glossosoma* spp. and Hydropsychidae, so a visual censuses of these common caddisflies were conducted. (The heavy mineral cases of *Neophylax* and *Glossosoma* caused them to fall to the streambed before being carried into the Surber sampler net, and Hydropsychid refugia sometimes failed to detach from substrates during sampling). These taxa were typically found on or near the tops of stones, were not easily disturbed, and were therefore easy to locate and count. A 50 × 50 cm quadrat sampled three streambed areas at each station. Each placement of the quadrat was within 5 m of a station, and sampled areas of comparable substrate and flow. Individuals were counted only if they could be seen without disturbing substrates. The sampling scheme assumed caddisflies in the bloom behaved no differently than those outside it in terms of their positioning on substrates. This assumption was checked by turning over rocks within the quadrat after a count was made, and looking for individuals of the taxa being censused. Observations indicated counts of individuals from the tops of substrates were representative for a given area. *Neophylax* spp. and *Glossosoma* spp. were censused on August 28, 1989. Hydropsychids were censused on September 10, 1989.

A survey of macroinvertebrates in five other Vermont streams displaying zones of iron deposition and elevated iron and manganese concentrations was conducted to see if the pattern shown in Unnamed Brook was typical. The streams and rivers examined were: the Ompompanoosuc River, Clay Brook, the Fairlee River, the South Branch of the Middlebury River, and a nameless, second order stream located at Smuggler's Notch. In each stream, three sites were examined: one above the point of iron and manganese enrichment, one within the zone of oxide deposition were iron and manganese concentrations were high, and one below the zone of deposition. At each site, three benthic samples and three water samples were taken 50 m apart. Each benthic sample combined the contents of three Surber bottom samplers taken within 3 m of each other. Data from each sample were used to calculate H′, and sites within a stream were compared using a one-way ANOVA. Water samples were collected in Nalgene bottles and total Fe and Mn concentrations were determined on the Thermo Jarrell Ash ICAP 61 spectrometer. An Orion Research digital pH meter (model 701 A) was used to assay pH.

Experimental work

Effect of Leptothrix ochracea *on macroinvertebrate substrate choice*

We conducted choice experiments to see if macroinvertebrates would avoid *L. ochracea*-coated substrates, and if so, to what extent. Each experiment presented insects with a choice between two substrates: those coated with ferromanganese oxides and *L. ochracea* sheaths, and normal stream substrates.

L. ochracea-coated and diatom-colonized cobbles used in these experiments were collected directly from Unnamed Brook. Beech leaves were taken from artificial leaf packs that had been staked into the stream at sites with and without *L. ochracea* and left 45 d. Recirculating stream troughs which simulated riffles, and aquaria which simulated pools, were used to test substrate preference. For each species tested, the apparatus

and substrate chosen were those that best matched the habitat from which the insect was collected.

The insects used in these experiments were collected from Unnamed Brook when possible; otherwise they were taken from streams and rivers near Middlebury, VT. Insects were held at 8 °C in 3.8 l plastic tubs containing stream water and an air stone.

Ten artificial stream troughs measuring 70 × 16 × 6 cm were constructed of Plexiglass. Water was recirculated through each trough by pumps (Milton Roy magnetic drive; 8.9 l min − 1 capacity) driving water through plastic tubing. Water emptied from the troughs into a common reservoir and was drawn back into individual pumps. Nylon screen (1.0 mm) glued to the end of each trough prevented insects from washing out. Water depth in the troughs ranged from 1 to 3 cm, and flow was approximately 20 cm s^{-1}. Cobbles measuring from 5 to 12 cm in diameter were placed in the troughs to simulate stream riffles. A choice was created by placing *L. ochracea*-coated substrate on one side, and diatom colonized substrate on the other side of each trough. Cobbles of each type were matched so the substrate composition was roughly symmetrical between right and left halves. Positions of the substrate types were alternated between sides in adjacent streams to eliminate any right/left bias. To begin a trial, insects were poured from a small cup into the top of each trough.

To test substrate preference of pool-dwelling species, two types of tanks were used. For species that were highly mobile (*Hydatophylax argus*), or evasive (*Heptagenia umbratica*) rectangular Plexiglass tanks measuring 19.5 × 60.5 × 15.5 cm were employed. Cobble coated with *L. ochracea* was placed at one end of each tank, and diatom-colonized substrate was placed on the other. A 8 cm wide strip was left between substrate types. Water depth was 14 cm. *Lepidostoma* sp. were tested in 19 × 10 cm (diameter × depth) circular glass dishes that presented a choice between two small leaf packs. Each pack consisted of four to five leaves placed on opposite sides of the dish, and held in place with a stone. To begin a trial, insects were poured from a small cup into the center of each tank. The orientation of substrates in aquaria and dishes was alternated to avoid directional bias.

All choice experiments were run 24 h at 15 °C

Table 1. Macroinvertebrate survival in enclosures. Data are the proportion of animals surviving at each of four stations. Above, Within, and Below refer to location of stations with respect to the *L. ochracea* bloom. Survivorship differed significantly between stations only for the Ephemeroptera (Chisquare: $p < 0.001$ for each species). Lowest mayfly survivorship was inside the bloom at station D 100 where the bloom was thickest.

Species	Proportion surviving				Days in stream	Date started	Cages per site	Number of inds. per cage
	Above U200	Within 0	Within D100	Below D300				
Trichoptera								
Hydatophylax argus	0.70	0.65	0.58	0.65	50	Jun. 19 '89	15	4
Frenesia difficilis	0.93	0.90	0.97	0.97	42	Aug. 6 '89	5	6
Lepidostoma sp.	0.96	0.96	0.96	0.80	28	Aug. 6 '89	5	5
Molanna sp.	1.00	0.75	0.95	0.85	28	Aug. 6 '89	5	4
Neophylax nacatus	0.97	0.92	0.98	1.00	35	Apr. 20 '90	4	15
Plecoptera:								
Pteronarcys sp.	1.00	0.97	1.00	0.97	50	Aug. 7 '90	6	5
Ephemeroptera:								
Epeorus sp.	0.60	0.58	0.22	0.53	13	May 8 '90	10	5
Stenonema fuscum	0.92	0.81	0.44	1.00	40	May 20 '90	12	4
Heptagenia umbratica	0.79	0.60	0.52	0.90	20	Jun. 14 '90	10	5

and maintained on a twelve hour light/dark regime inside an environment chamber (Environmental Structure Mfg.,Inc.). Data were counts of individuals on each substrate type at the end of 24 h. Individuals not on substrates were not counted. Ten replicates were run for each experiment, and each species was tested three times, except *Neophylax nacatus* which was tested twice. Mann-Whitney U tests (Sokal & Rohlf, 1981) were used to determine substrate preference.

The numbers of individuals per replicate, for each species, were as follows: *Epeorus* sp., 12; *Heptagenia umbratica*, 12; *Hydatophylax argus*, 15; *Isogenoides* sp., 10; *Lepidostoma* sp., 12; *Neophylax consimilis*, 20; *Neophylax nacatus*, 20; *Stenonema fuscum*, 10.

Survival of macroinvertebrates within and outside Leptothrix ochracea *blooms*

We tested nine species from three orders (Trichoptera, Plecoptera, and Ephemeroptera) for extended periods under field conditions to assess the importance of iron, manganese and *L. ochracea* toxicity in lowering insect abundance in Unnamed Brook. Survival of caged insects was compared above, within, and below the *L. ochracea* bloom.

The insects used in these experiments came from Unnamed Brook (*Heptagenia umbratica*, *Neophylax nacatus*, *Frenesia difficilis* Walker, *Lepidostoma* sp., and *Molanna* sp.), the New Haven River (*Stenonema fuscum*, *Epeorus* sp., *Hydatophylax argus*, and *Neophylax consimilis* Betten),

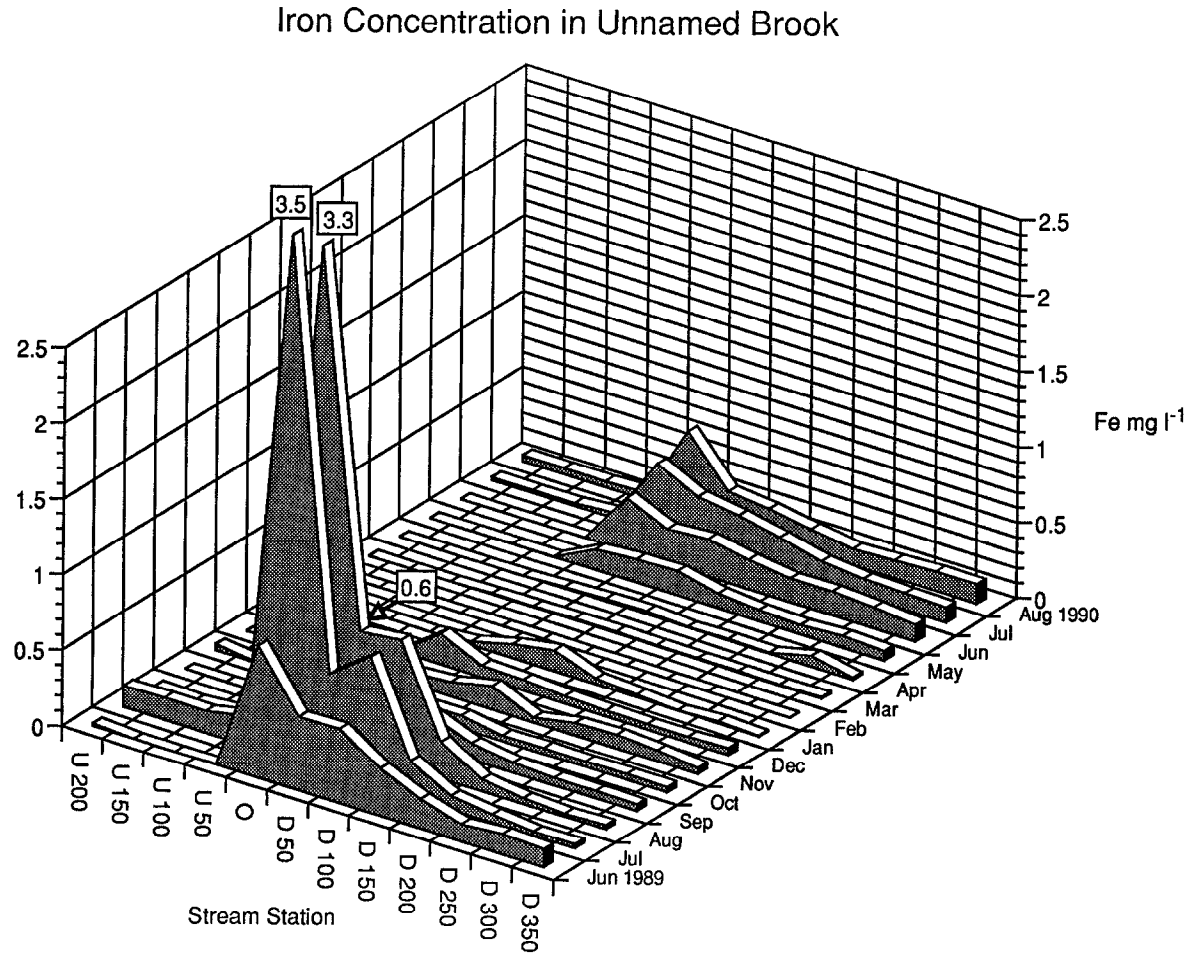


Fig. 2. Monthly concentrations of A. iron and B. manganese at 12 sampling stations in Unnamed Brook from June 1989 to August 1990.

and the South Branch of the Middlebury River (*Pteronarcys* sp.).

Cages were made from 250 ml Nalgene bottles with two 8 × 6 cm holes cut in the sides, and a 3.0 cm diameter circle cut from the bottom. Openings were covered with nylon screen mesh (1.0 mm) attached with hot glue. Depending on species availability, four to six individuals were placed in each bottle along with a small quantity of twigs and leaves from Unnamed Brook to serve as substrate. Cages were held in place by tying them to garden stakes pushed into the streambed.

Sets of bottles were staked out at four sites: above the *L. ochracea* bloom at station U 200; within the bloom at stations 0 and D 100; and below the *L. ochracea* bloom at station D 300. Mortality was checked every week or ten days by directly observing individuals through the mesh, or by removing the cap and shaking them out onto a sieve. An experiment was terminated when larvae began to pupate (Trichoptera), emerge (Ephemeroptera), or until practical considerations dictated. At the end of the experiment the number of individuals alive at each station were compared with a Chi-square test. The duration, starting dates, number of replicates, and number of individuals per cage are given in Table 1.

Insect weight gain on diets with and without Leptothrix ochracea

Feeding experiments were conducted on three scrapers, two that were common in Unnamed

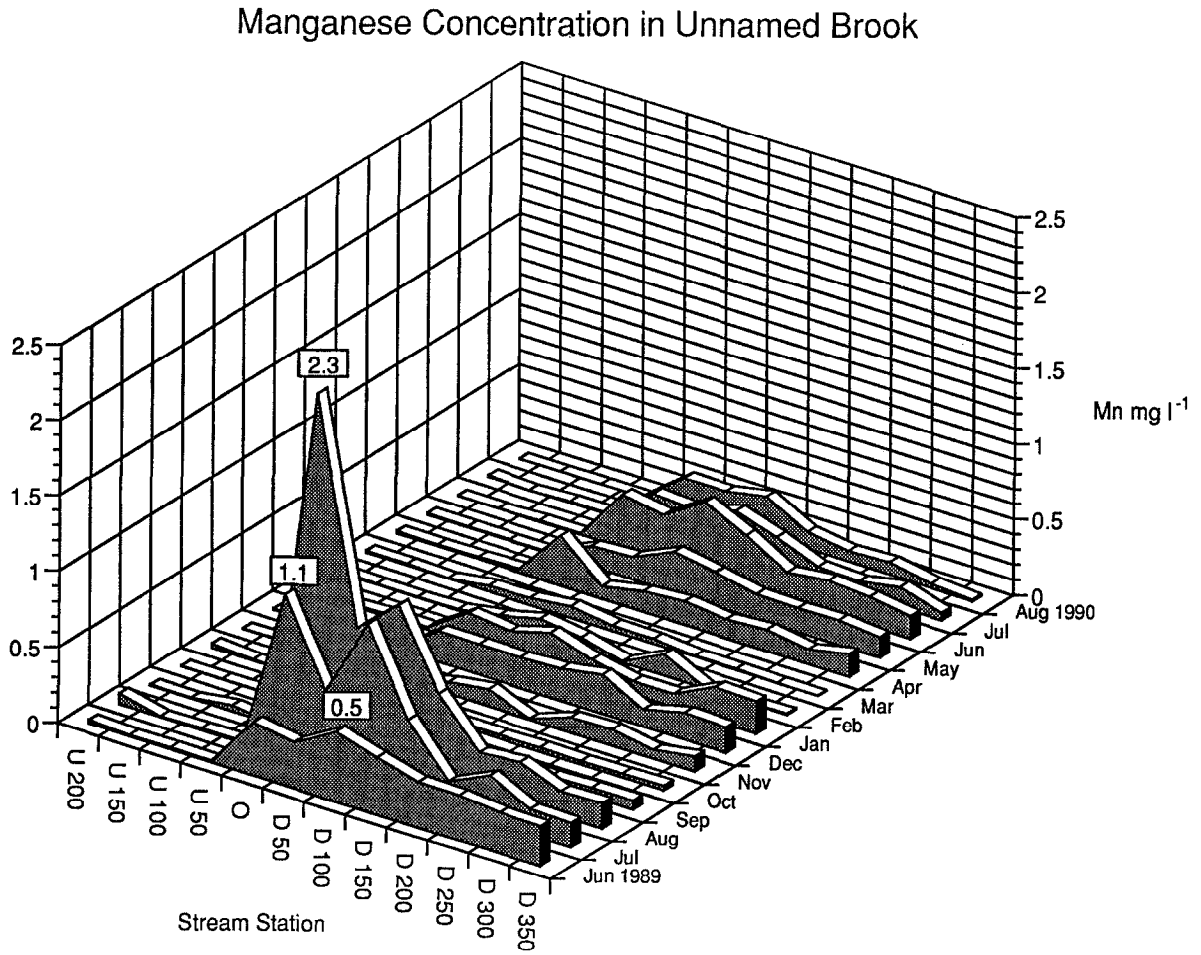


Fig. 2b.

Brook, *Neophylax nacatus*, and *Heptagenia umbratica*, and a third (*Stenonema fuscum*) that was found only rarely, but could be collected in abundant numbers from the New Haven River. All substrate was taken from Unnamed Brook, transported back to the lab in buckets of stream water, and stored at 8 °C until ready for use.

Experiments were run in clear, 4 l plastic tubs. There were three treatments (four tubs per treatment): cobble coated with *L. ochracea*, cobble colonized with diatoms, and cobble scrubbed and washed to remove all organic matter. Each tub contained seven to nine pieces of cobble ranging in size from 7 to 13 cm diameter. An air stone in each tub kept the water aerated. The entire apparatus was housed in a Conviron Model EBVH environment chamber set at 15 °C and kept on a 12:12 light/dark cycle. Similar sized individuals were starved for five days prior to running the experiment. At the end of five days, a subsample of the population was dried (80 °C for 24 h) and weighed to get a starting weight for the population. Experiments were run for ten days with 12 individuals per tub. Individuals were then placed in holding tubs for 24 h to allow their guts to clear before being dried and weighed.

Results

Descriptive studies

Water chemistry

Iron and manganese concentrations in Unnamed Brook varied seasonally, reaching their highest levels in mid-summer, and lowest in February and March (Figs 2, A & B). Concentrations of both ions were higher inside the *L. ochracea* bloom, with peak concentrations of Fe occurring at station 0, and Mn peaking at station 0 or D 100. During the summer of 1989, Fe and Mn concentrations were considerably higher than for the same period in 1990. Summer maxima in 1989 were 3.46 mg l^{-1} and 2.27 mg l^{-1} for Fe and Mn, respectively, compared to only 0.54 mg l^{-1} for Fe and 0.47 mg l^{-1} for Mn in 1990. Nevertheless, the trend of increased mid-summer concentrations within the *L. ochracea* bloom was seen both years.

Iron and manganese concentrations above station 0 were consistently lower than elsewhere in the stream. Concentrations rarely exceeded 0.05 mg l^{-1}. On occasions when they did it was either from digging (e.g. maintenance of a dirt road that crossed Unnamed Brook) or some other disturbance upstream. Below the bloom, iron and manganese concentrations were generally lower than inside the bloom. Iron concentrations tended to gradually trail off after station 0. Manganese showed a similar pattern, but did not always drop off as evenly. In June 1989, for example, manganese peaked at 0.46 mg l^{-1} at D 50, yet the concentration was still 0.27 mg l^{-1} at station D 350. During July and August of 1989 there were three peaks in manganese concentration, two inside the *L. ochracea* bloom, and one below it.

Five other Vermont streams with blooms of iron-depositing bacteria typically showed the highest iron and manganese concentrations inside the blooms (Table 2). The exception was Clay Brook where iron concentrations were higher below the bloom, 0.45 mg l^{-1}, as opposed to 0.19 mg l^{-1} inside the bloom itself.

Epilithon assays

Epilithic biomass in Unnamed Brook was significantly higher inside the *L. ochracea* bloom than above or below (ANOVA: $F = 7.96$, $p = 0.001$; inside > above = below; Tukey's $p < 0.05$). Mean ash-free weights nearly doubled inside the bloom, increasing from 8.58 ± 0.43 mg cm^{-2} above, to 14.9 ± 1.51 mg cm^{-2} within the bloom, and then decreasing to 8.46 ± 1.36 mg cm^{-2} downstream (Fig. 3, A). Most if not all the biomass inside the bloom was *L. ochracea* build-up. Tiles here had a coating of ocher-colored slime that sometimes exceeded 3 mm in thickness. Microscopic examination revealed *L. ochracea* sheaths and oxide precipitates, but very few diatom frustules. Biomass outside the bloom was almost exclusively diatoms with occasional patches of filamentous algae.

Chlorophyll *a* showed the opposite trend (Fig. 3, B). Chlorophyll *a* levels were 0.07 ± 0.01 mg cm^{-2} inside the bloom compared

Table 2. Diversity and water chemistry data from a survey of Vermont streams showing blooms of iron-depositing bacteria. Above, within, and below refer to where the samples were taken in relation to the bloom. Concentrations are in mg l^{-1}.

Stream name (sampling date)	H′ (mean ± S.E.)			pH			[Fe]			[Mn]		
	Above	Within	Below	Above	Within	Below	Above	Within	Below	Above	Within	Below
Smuggler's Notch (May 31, 1990)	0.87 ± 0.06	0.45 ± 0.04	–	6.10	6.38	–	1.10	1.54	–	0.05	0.70	–
Clay Brook (June 25, 1990)	0.54 ± 0.04	0.74 ± 0.01	0.79 ± 0.4	6.56	6.53	6.62	0.01	0.19	0.45	0.03	0.19	0.03
Ompompanusuc River (July 12, 1990)	0.96 ± 0.04	0.49 ± 0.10	0.85 ± 0.05	7.79	7.81	7.68	0.01	0.61	0.23	0.02	0.09	0.04
Fairlee River (July 12, 1990)	0.87 ± 0.06	0.54 ± 0.07	0.72 ± 0.15	7.30	6.76	7.48	0.07	1.84	0.41	0.02	0.13	0.05
South Middlebury River (August 2, 1990)	1.11 ± 0.05	0.83 ± 0.04	0.97 ± 0.02	6.62	6.45	6.55	0.00	0.39	0.03	0.00	0.09	0.00

to mean values of 0.16 ± 0.01 mg cm^{-2} and 0.15 ± 0.03 mg cm^{-2} above and below the bloom. This trend was significant (ANOVA: $F = 9.22$, $p < 0.001$; inside < above = below; Tukey's: $p < 0.05$). Chlorophyll *a*, did not decline at station 0 because tiles there had discrete patches of filamentous algae which kept chlorophyll *a* levels high, but contributed little to biomass. Also interesting is the delayed recovery of producers below the bloom. Although *L. ochracea* and ferromanganese oxides are no longer apparent below station D 200, chlorophyll *a* did not return to above-bloom levels until station D 300.

Macroinvertebrates

Twenty-nine insect genera were common in Unnamed Brook, with larvae from the orders Ephemeroptera, Trichoptera, Plecoptera and Diptera the most abundant. Annelids, nematodes, isopods, gastropods, bivalves and aquatic mites were also found, but insects predominated, both numerically and in biomass.

Macroinvertebrate diversity (H′) was lowest inside the *L. ochracea* bloom during summer (Fig. 4, A & B). When iron and manganese concentrations were high, and the bloom was thickest (e.g. June, August and September of 1989), diversity dropped off precipitously at the upstream edge of the bloom, and then gradually increased as the bloom thinned out. Below the bloom H′ values were often comparable to those found above the bloom. In September 1989, for example, values were 0.99 above the bloom, 0.39 inside the bloom, and 0.90 at station D 200. In 1989, June, August and September all showed H′ values that were significantly lower inside the *L. ochracea* bloom (ANOVA with Tukey's multiple comparisons: $p < 0.05$). The pattern is not as evident in July 1989 because H′ values were low everywhere that month.

Lower diversity inside the bloom was no longer evident ($p > 0.05$) after September 1989, the period when iron and manganese concentrations declined, and the bloom thinned out and receeded. Diversity for the months October 1989 through March 1990 showed no consistent pattern. Unnamed Brook did not exhibit low diversity inside the bloom again until May when Fe and Mn concentrations increased and *L. ochracea* build-up had begun. In June of 1990 the pattern seen the previous summer returned: diversity dropped off at station 0 and remained low throughout the bloom.

The three censused caddisflies (*Glossosoma*

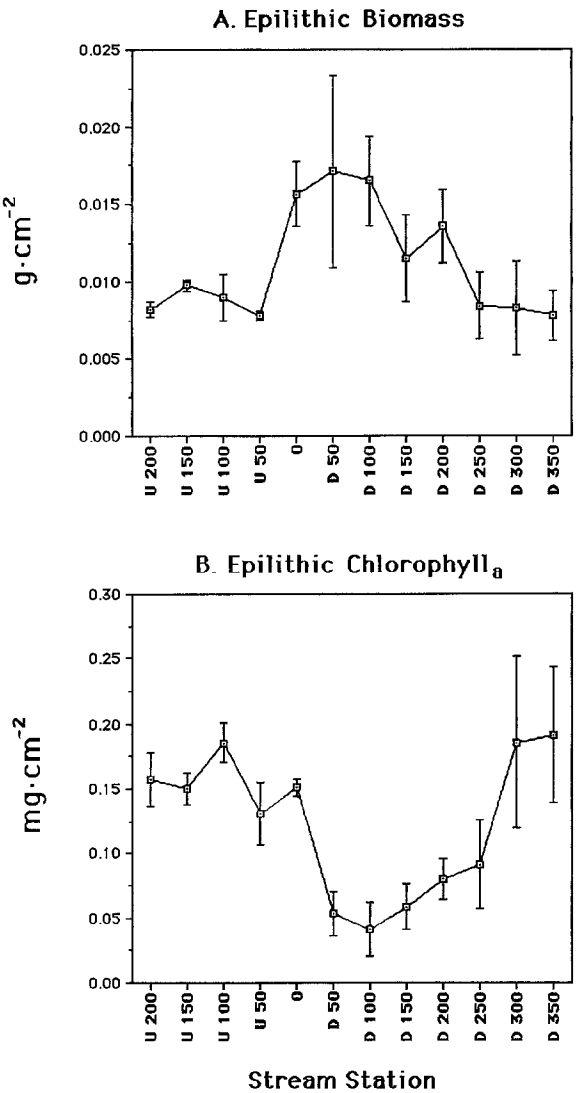


Fig. 3. The nature of the epiliton above, within, and below the bloom. For purposes of statistical analysis, Unnamed Brook was divided into three sections of four stations each: upstream stations U200 to U50 (above), *Leptothrix* bloom stations 0 to D150 (within), and downstream stations D 200 to D 350 (below). (A) Epilithic biomass on clay tiles incubated 45 d in Unnamed Brook. Data points represent the mean ash-free dry weight in g (± 1 S.E.) of epilithon from a 112.5 cm^2 area scraped from three to six tiles. Epilithic biomass was significantly higher inside the bloom than above or below (one-way ANOVA with Tukey's multiple comparisons, $p<0.05$). (B) Epilithic chlorophyll *a* on clay tiles incubated 45 d in Unnamed Brook. Data points represent the mean weight in mg (± 1 S.E.) of chlorophyll *a* from a 56.25 cm^2 area scraped from three to six tiles. Chlorophyll *a* was significantly lower inside the bloom than above or below (one-way ANOVA with Tukey's multiple comparisons, $p<0.05$).

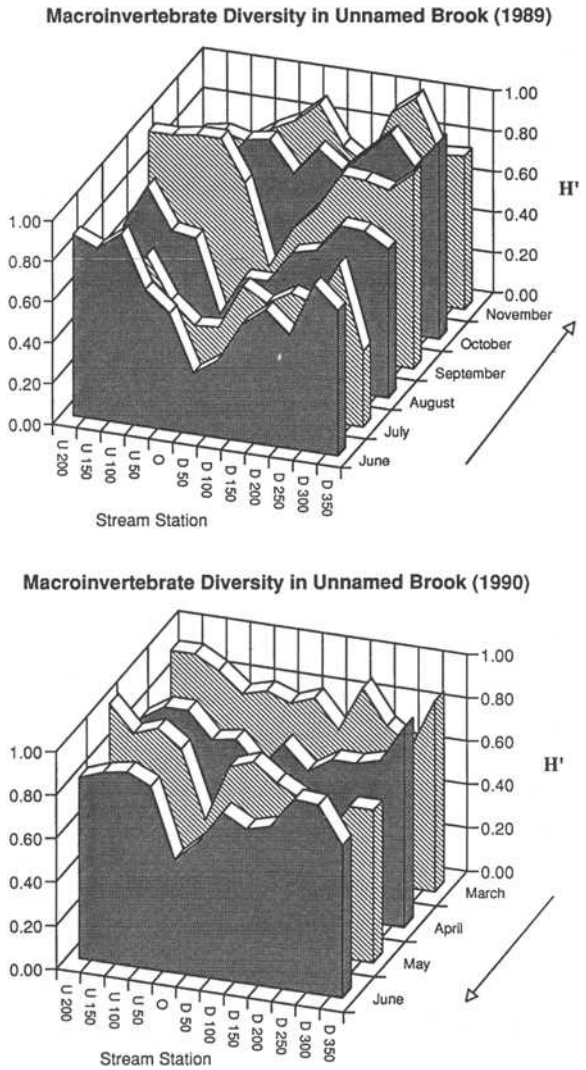


Fig. 4. Monthly macroinvetebrate diversity (H′) at each station in Unnamed Brook: A. June 1989 to November 1989, B. March 1989 to June 1990. Note time axis is reversed in B. to better show development of low diversity inside bloom in May and June.

spp., *Neophylax* spp., and Hydropsychidae) were absent inside the *L. ochracea* bloom, but present above and below it (Fig. 5, A, B & C). No individuals from any of these groups were seen at stations 0, D 50 or D 100, and only few Hydropsychids were found at station D 150.

Four of the five other streams revealed macroinvertebrate diversity patterns similar to Unnamed Brook (Fig. 6). The Ompompanusuc

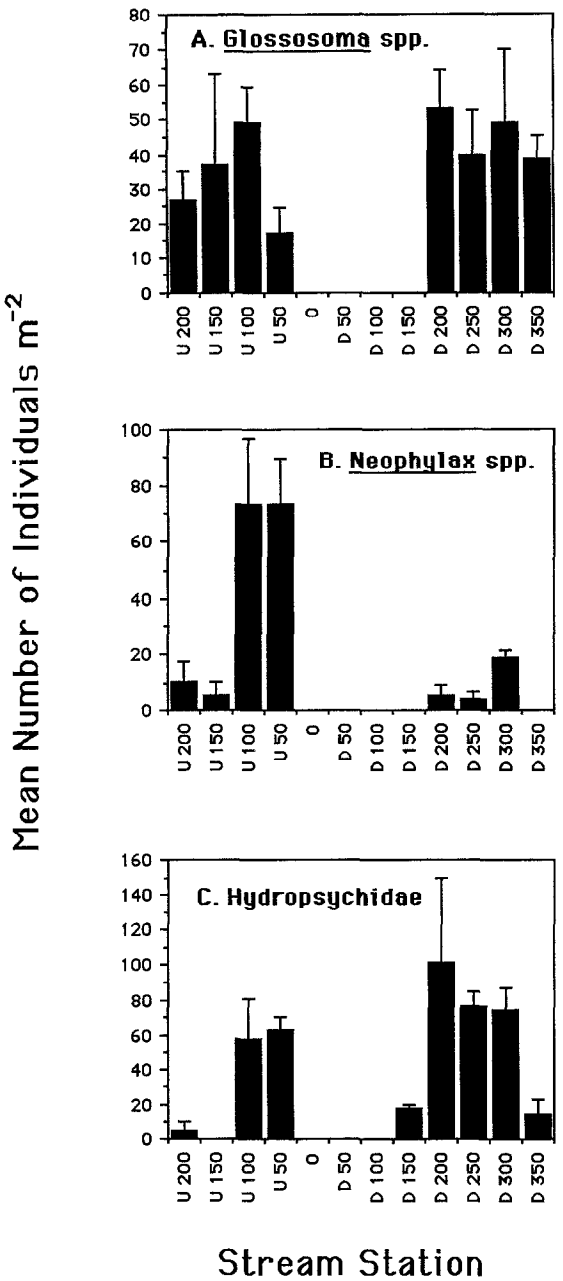


Fig. 5. Results of the Unnamed Brook census for A. *Glossosoma* spp., B. *Neophylax* spp., and C. Hydropsychids. Bars represent the mean number of individuals m^{-2} (± 1 S.E.). When these surveys were conducted the *Leptothrix* bloom extended to station D 200.

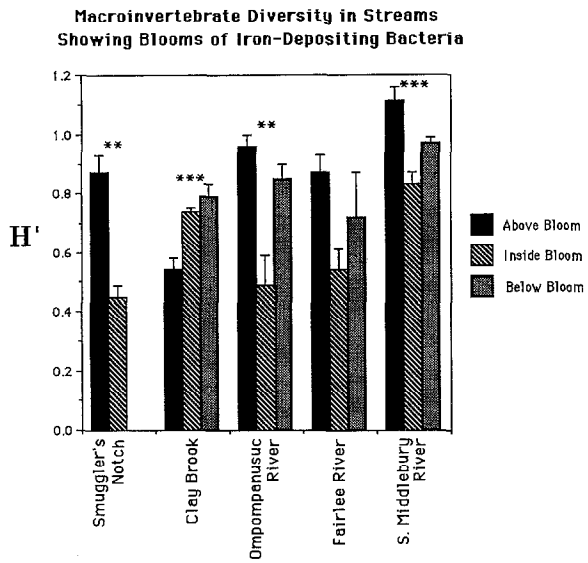


Fig. 6. Results from the macroinvertebrate survey of five Vermont streams showing blooms of iron-depositing bacteria. Bars show mean macroinvertebrate diversity, H' (± 1 S.E.) above, within and below the blooms. The Smuggler's Notch stream does not show a 'below bloom' value because this stream ended in a pond before the bloom disappeared. Levels of significance (ANOVA) are designated as follows: $^{**}p \leq 0.01$; $^{***}p \leq 0.005$.

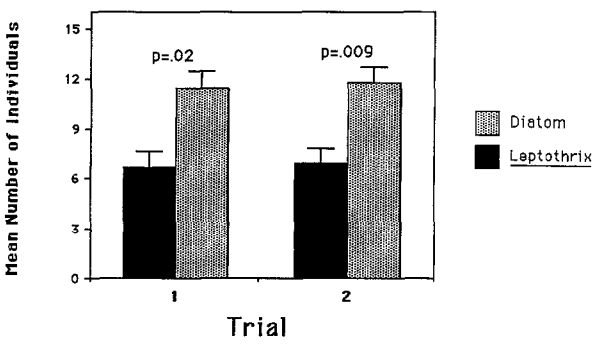
River, the South Branch of the Middlebury River, and the nameless stream at Smuggler's Notch all showed significantly lower macroinvertebrate diversity inside blooms (ANOVA: $p = 0.006$, 0.005, and 0.008, respectively). The Fairlee River also showed lower diversity within the bloom, but this trend was not significant ($p = 0.14$). Clay Brook was unusual in that the lowest diversity was found upstream of the bloom ($p = 0.003$), but this was due to the large numbers of heptageniid mayflies found there.

Experimental studies

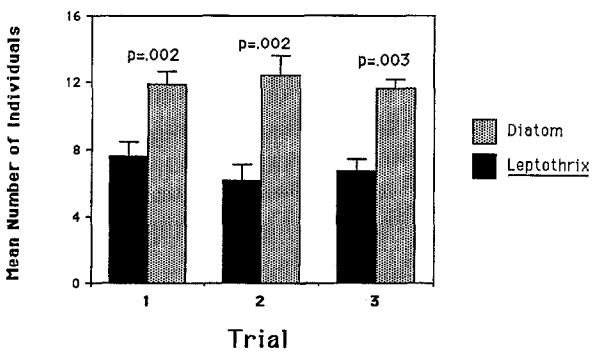
Effect of Leptothrix ochraoea *on substrate choice*

Of the eight species tested, five (*Neophylax nacatus*, *N. consimilis*, *Heptagenia umbratica*, *Lepidostoma* sp., *Epeorus* sp.) showed a preference for *L. ochracea*-free substrates; two (*Hydatophylax argus*, *Stenonema fuscum*) showed no preference; and one (*Isogenoides* sp.) preferred *L. ochracea*-coated substrates (Fig. 7, A–H). All species showed consistent preference, or lack of preference, across three experimental trials (two trials in the case of *N. nacatus*).

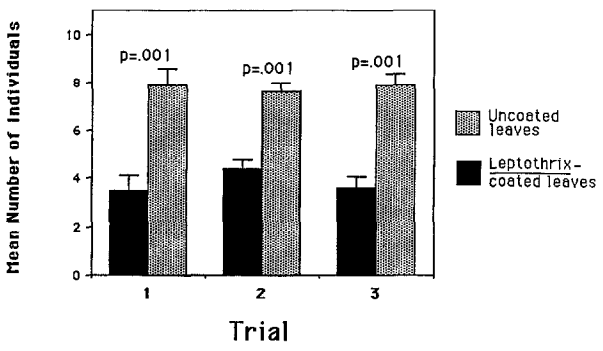
A. Neophylax nacatus Choice Experiment



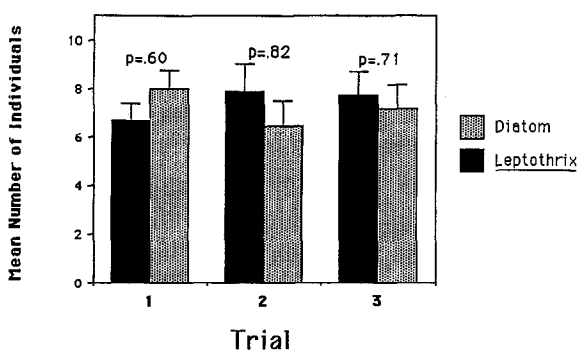
B. Neophylax consimilis Choice Experiment



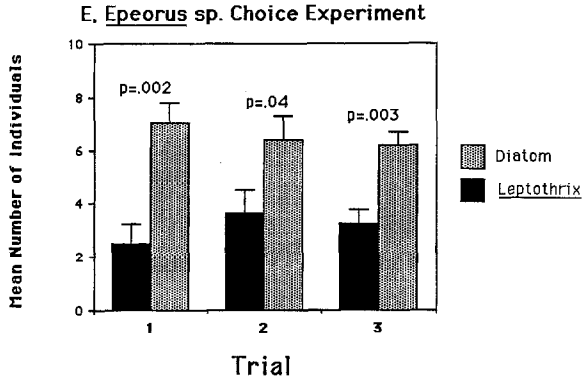
C. Lepidostoma sp. Choice Experiment



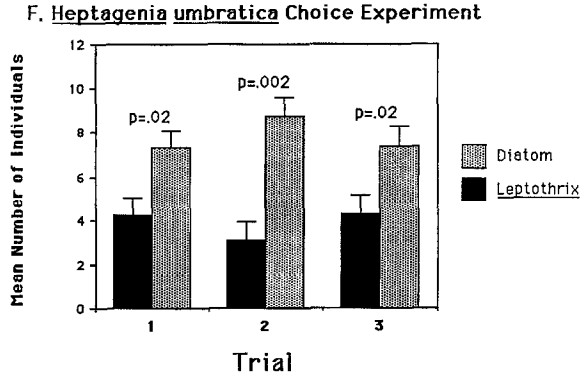
D. Hydatophylax argus Choice Experiment



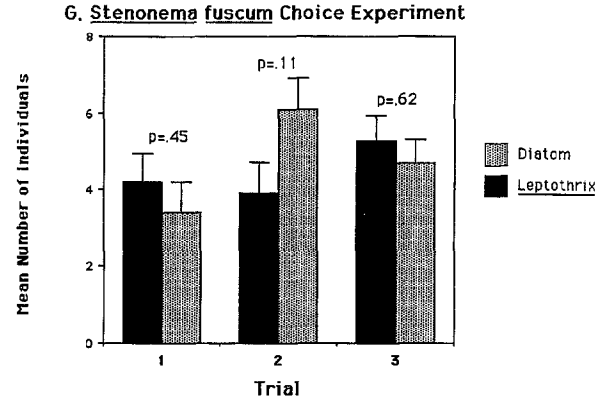
E. Epeorus sp. Choice Experiment



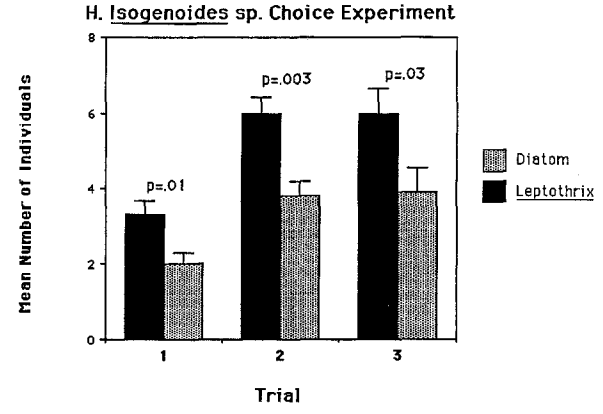
F. Heptagenia umbratica Choice Experiment



G. Stenonema fuscum Choice Experiment



H. Isogenoides sp. Choice Experiment



Survival of macroinvertebrates within and outside Leptothrix ochracea *blooms*

Of the nine species tested, only the three Ephemeroptera suffered greater mortality inside the *L. ochracea* bloom (Chi-square: $p<0.001$, Table 1). For all other species survival was not different inside and outside the bloom. This is particularly remarkable for the four caddisflies tested in 1989, when flocs of *L. ochracea* accumulated to such an extent that cages within the bloom completely filled with gelatinous sheath material and coated their cases.

Insect weight gain on diets with and without Leptothrix ochracea

Heptagenia umbratica, *Neophylax nacatus*, and *Stenonema fuscum* all ingested *L. ochracea* to some degree, but only *H. umbratica* gained weight after ten days of feeding on *L. ochracea*-coated cobbles. Individuals of each species fed diatoms, however, showed significant weight gain whether compared to starved individuals or to the population's starting weights (Tukey's multiple comparisons: $p<0.05$). *Heptagenia umbratica* gained equal weight on *L. ochracea* and diatoms (Fig. 8, A): mean dry weights for both treatments were 2.70 mg.

Discussion

In Unnamed Brook the *Leptothrix ochracea* bloom appears to be maintained by an annual 'pulse' of iron and manganese that occurs from May through September. As these ions increase, *L. ochracea* biomass accumulates, and oxide-laden sheaths blanket substrates. Streams that contain *L. ochracea* usually have iron as the predominant ion, but without manganese these bacteria are less abundant and quickly disappear. Our measurements in six streams indicate that high concentrations of both iron (about 0.19 mg l^{-1}), and manganese (at about 0.09 mg l^{-1}), are necessary for *L. ochracea* blooms to persist. The blooms in Clay Brook and the Fairlee River, for example, ended where manganese dropped off (to 0.03 mg l^{-1} and 0.05 mg l^{-1}, respectively), even though iron concentrations were elevated several hundred meters downstream. Nor does manganese by itself appear capable of sustaining *L. ochracea* blooms. In June, July and August of 1989, for instance, Unnamed Brook displayed manganese concentrations downstream and outside of the bloom that were nearly as high as those inside it.

As the bloom in Unnamed Brook built up in the summer and withdrew in the fall, it created a pattern of disturbance and recovery, one which was reflected in the macroinvertebrate community. Diversity was low inside the bloom during the summer, but in October as the bloom thinned and retreated, diversity increased. Throughout the winter diversity stayed high, and no distinct pattern emerged again until late spring when iron and manganese concentrations increased, *L. ochracea* regained its dominance over the stream bed, and macroinvertebrate numbers declined.

The field data document an inverse relationship between high iron and manganese concentrations and the presence of *L. ochracea*, and invertebrate abundance and diversity. The results of our experiments shed light on three factors behind the low invertebrate diversity within the bloom. We found evidence that different taxa are affected by (1) physical presence of *L. ochracea*, (2) inadequacy of *L. ochracea* as food, and (3) toxic effects of Fe and Mn ions and/or *L. ochracea*.

Avoidance of Leptothrix ochracea*-coated substrates*

Substrate avoidance was an important factor for some species; experiments indicated that *Neophylax nacatus* and *N. consimilis* avoided *L. ochracea*-coated cobble. When viewed in conjunction with

Fig. 7. Results of substrate choice trials. Unless otherwise indicated, the choice was between diatom- and *Leptothrix*-colonized cobble. Bars represent the mean number of individuals found on each type of substrate (± 1 S.E.). P-values over bars are from Mann-Whitney U tests.

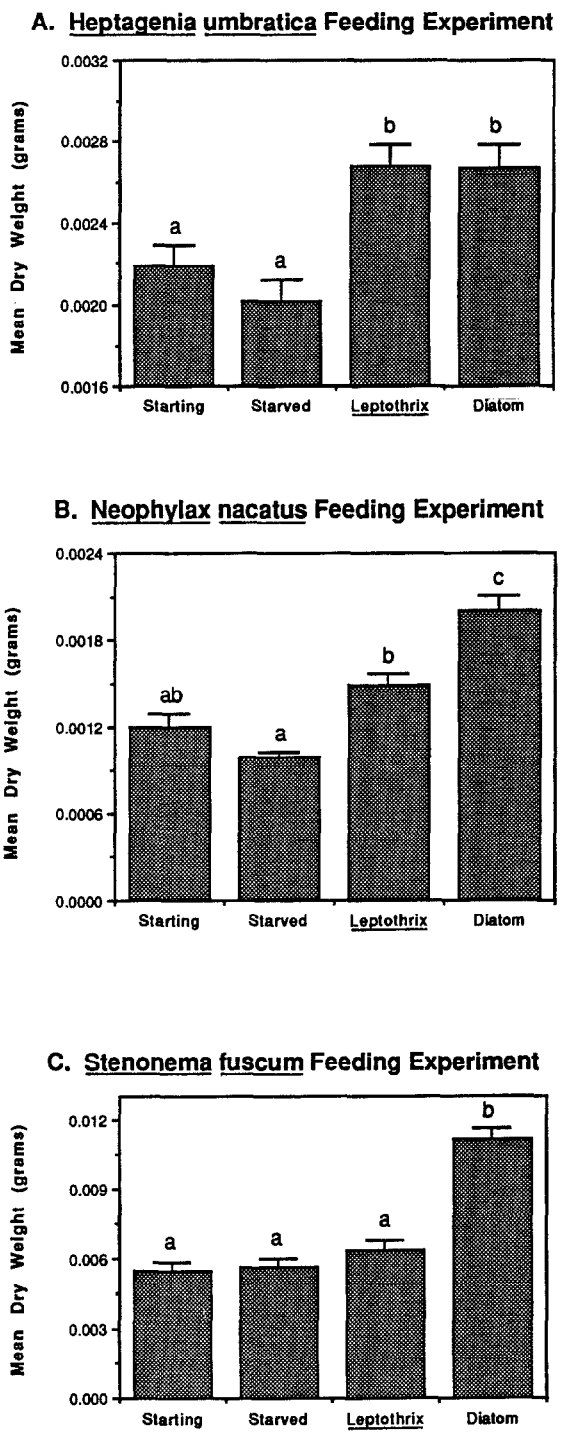


Fig. 8. A–C. Results from feeding trials for *Heptagenia umbratica*, *Neophylax nacatus*, and *Stenonema fuscum*. Treatments are as follows: Starting-weights from subsample of experimental population to establish initial animal weights prior to start of experiment. Starved-weights of animals kept on scrubbed cobble. *Leptothrix*-animals fed on *Leptothrix*-colonized cobble. Diatom-animals fed on diatom-colonized cobble. Each trial was run for ten days. Bars show mean dry weights of animals in grams ($\pm$ 1 S.E.) from each treatment at end of experiment (ANOVA with Tukey's multiple comparisons, $p < 0.05$).

data from the *Neophylax* spp. survey of Unnamed Brook, an interesting pattern emerges. *Neophylax*, a genus that orientates upstream and rarely abandons its case, was found in significantly greater densities (Mann-Whitney U test: $p = 0.04$) above the bloom than below it. The highest *Neophylax* densities for the stream were seem immediately above the bloom at stations U 50 and U 100, where mean densities were 73.32 individuals m^{-2}. Three other species tested also showed a preference for 'clean' substrates: *Epeorus* sp. and *Heptagenia umbratica* preferred diatom-colonized cobble, and *Lepidostoma* sp. preferred *L. ochracea*-free leaves.

The one species that preferred *L. ochracea*-coated substrates was the predacious stonefly *Isogenoides* sp.. Since *Isogenoides* did not utilize epilithon as food, this preference may reflect a tactile response favoring *L. ochracea*. Nevertheless, low prey densities may cause these stoneflies to move through the bloom quickly. Low prey densities would increase foraging time, enhancing the probability of *Isogenoides* entering drift (Hildebrand, 1974), and perhaps exposing it to greater risk of fish predation (Kohler & McPeek, 1989). Both these mechanisms could reduce the abundance of macroinvertebrate predators dependent on the benthos for prey.

Two species, *Hydatophylax argus* and *Stenonema fuscum*, showed no substrate preference. *Hydatophylax argus* may be little affected by *L. ochracea* blooms. Not only did this caddisfly display no substrate preference, it showed no differential mortality between bloom and bloom-free sites. Further, dry weights of survivors from enclosures showed no significant differences between treatments (ANOVA: $p > 0.05$). *Stenonema fuscum* showed no substrate preference, but its absence from *L. ochracea* blooms can be predicted from the results of survivorship and feeding experiments. *S. fuscum* showed greater mor-

tality inside the bloom, and feeding experiments revealed *S. fuscum* was unable to utilize *L. ochracea* as food. Caged *S. fuscum* individuals inside the bloom may have succumbed to toxic factors, the effects of starvation, or both. In order to survive, *S. fuscum* would have to move out of *L. ochracea* blooms.

Inadequacy of Leptothrix ochracea *as food*

Feeding experiments revealed *L. ochracea* could be ingested by *Heptagenia umbratica* and *Neophylax nacatus*, but only *H. umbratica* gained significant weight feeding on it. *Stenonema fuscum* ingested only minute quantities and gained no weight at all. Contrary results for the two mayflies (*H. umbratica* and *S. fuscum*) were surprising. Both have similar mouthpart arrangements (Needham *et al.*, 1972), show similar modes of feeding (Merritt & Cummins, 1984), and feed on the same foods (T. Wellnitz, pers. obs.). Despite these similarities, important differences may exist between feeding appendages or in the way food is collected from substrates. After ten days the labial palps of *S. fuscum* from *L. ochracea* treatments were caked with *L. ochracea* sheath material, and in some individuals the mouthparts appeared clogged. When gut contents were examined only trace amounts of *L. ochracea* were found. For the most part, the guts of *S. fuscum* fed *L. ochracea* were empty. *Heptagenia umbratica*, on the other hand, showed little mouthpart-caking and individuals fed *L. ochracea* possessed guts as full as those fed diatoms.

Unlike *Stenonema fuscum*, the caddisfly *Neophylax nacatus* had no difficulty ingesting *L. ochracea*, but the lack of significant weight gain may indicate *L. ochracea* was an inferior food for this species. Indeed, calorimetric analysis showed *L. ochracea* from Unnamed Brook contained only 6.2×10^3 joules g^{-1} ash-free dry weight as compared to 18.9×10^3 joules g^{-1} for diatom-dominated periphyton (R. Tyzbir, 1989 pers. comm.). Increased ingestion rates would be necessary to compensate for this lower food quality. Though they lost weight, *Neophylax nacatus* fed *L. ochracea* did have fuller guts than those fed diatoms.

Toxic effects within the Leptothrix ochracea *bloom*

The caging of larvae within and outside blooms did not distinguish between the effects of *L. ochracea*, and the effects of high iron and manganese concentrations. Nevertheless, toxic conditions constitute an important factor in lowering mayfly abundances inside blooms. Mayfly survivorship was significantly lower inside the Unnamed Brook bloom, and may have been caused by metal toxicity, suffocation, or some other factor. Studies have shown some mayflies cannot tolerate iron concentrations above 0.30 mg l^{-1} (Warnick & Bell, 1969; Rasmussen & Lindegaard, 1988), and Ephemeroptera may be especially vulnerable to the coating of exposed respiratory surfaces. In fact, not only did *L. ochracea* sheaths encrust the mayfly's tracheal gills, but these accumulations appeared to hamper gill motion as well (T. Wellnitz, pers. obs.).

In contrast, Trichoptera showed no differential survivorship between sites inside and outside the bloom. Caddisflies appear to be protected from *L. ochracea* build-up by their cases. *Neophylax* spp., while having cuticles stained by ferromanganese oxides, did not have the heavy *L. ochracea* accumulations their cases did. And unlike the gill-beating motions used by mayflies, caddisfly ventilation did not appear adversely affected by *L. ochracea*. We noted *Hydatophylax argus* from enclosures filled with *L. ochracea* were able to flush *Leptothrix* flocs from the interior of their cases by employing the same abdominal undulations used to ventilate (T. Wellnitz, pers. obs.).

The one stonefly tested, *Pteronarcys* sp., survived well at all stream sites despite *L. ochracea* build up on its body and ventral gills. *Pteronarcys* may be protected from metal toxicity by its large size alone. Smock (1983) found the adsorption by aquatic insects of iron and, to a lesser extent, manganese was a function of body size; with increasing body size there was an exponential de-

crease of metal concentrations in tissues. *Pteronarcys* was the largest insect tested, with mean weights more than five times greater than *Stenonema fuscum*, the largest mayfly. *Pteronarcys*' large size and small surface to volume ratio should have made this insect more vulnerable to smothering, but this did not appear to be the case. More puzzling still, the ventral gills of this stonefly appeared to collect *L. ochracea* as it moved across coated substrates. Ventilating movements ('push-ups') appeared more frequent in individuals inside blooms as compared to outside, but this was not quantified.

Synthesis

All three factors – substrate avoidance, food quality, and toxicity – have a role in lowering macroinvertebrate abundance and diversity in *L. ochracea* blooms. How these factors vary in their importance from species to species is demonstrated by *Stenonema fuscum*, *Neophylax nacatus*, and *Heptagenia umbratica*. Table 3 summarizes the results of the choice, feeding, and survivorship experiments run on these species. It reveals not only the different responses, but also suggests how factors may act serially or in concert to lower abundance. Both *Heptagenia umbratica* and *Neophylax nacatus* avoided *L. ochracea*-coated substrates. If they did not, they would in all likelihood perish: *Heptagenia umbratica* from toxic factors, and *N. nacatus* from starvation. *Stenonema fuscum*, on the other hand, did not avoid *L. ochracea* despite the fact this mayfly could not ingest the bacteria. If *S. fuscum* did not succumb to starvation, toxic factors would kill them. (It should be noted that *S. fuscum* was rare in Unnamed Brook, requiring subjects used in our experiments to be taken from the New Haven River, a stream without blooms of iron-depositing bacteria. The possibility exists that 'naive' *S. fuscum* may have responded differently to *L. ochracea*-coated substrates than individuals previously exposed). Each of the three species displayed vulnerability to a unique set of factors, and no one factor appeared more important than the others for lowering diversity inside the bloom. It is also conceivable that other, untested factors may be acting. Peterson & Peterson (1983), for instance, showed that dissolved metals can alter behavior, and it would be worthwhile to evaluate what role Fe and Mn concentrations have on movement and feeding.

Table 3. Summary of feeding, substrate choice, and survivorship experiments conducted on three macroinvertebrates. A plus designates a factor that could reduce a species' abundance inside *L. ochracea* blooms. A zero indicates the factor had no effect on the species.

	Poor food	Avoidance	Toxicity
Heptagenia umbratica	0	+	+
Neophylax nacatus	+	+	0
Stenonema fuscum	+	0	+

This study clearly demonstrates the importance of substrate modification by *Leptothrix ochracea* and concomitant oxide deposition in lowering macroinvertebrate diversity in streams showing *L. ochracea* blooms. More generally, it has shown the need to monitor disturbances throughout the year to understand their dynamics, and has demonstrated how natural communities can respond in a complex fashion to environmental perturbations.

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References

American Public Health Association, 1986. Standard Methods for the Examination of Water and Wastewater, 16th edn. American Public Health Association, Washington D.C., 1268 pp.

Brown, B. E., 1977. Effects of mine drainage on the river Hayle, Cornwall. A.) Factors affecting concentration of Cu, Zn, and Fe in water, sediments and dominant invertebrate fauna. Hydrobiologia 52: 221–233.

Ghiorse, W. C., 1984. Biology of iron- and manganese-depositing bacteria. Ann. Rev. Microbiol. 38: 515–560.

Godfrey, P. J., 1978. Diversity as a measure of benthic macroinvertebrate response to water pollution. Hydrobiologia 57: 111–122.

Greenfield, J. P. & M. P. Ireland, 1978. A survey of the macrofauna of a coal-waste polluted Lancashire fluvial stream. Envir. Pollut. 16: 105–122.

Hildrew, A. G. & C. R. Townsend, 1976. The distribution of two predators and their prey in an iron-rich stream. J. anim. Ecol. 45: 41–57.

Hildebrand, S. G., 1974. The relation of drift to benthos density and food level in an artificial stream. Limnol. Oceangr. 19: 951–957.

Jones, J. G., 1975. Some observations on the occurrence of the iron bacterium *Leptothrix ochracea* in fresh water, including reference to large experimental enclosures. J. appl. Bact. 39: 63–72.

Kohler, S. L. & M. A. McPeek, 1989. Predation risk and the foraging behavior of competing stream insects. Ecology 70: 1811–1825.

Koryak, M., M. A. Shapiro & J. L. Sykora, 1972. Riffle zoobenthos in streams receiving acid mine drainage. Wat. Res. 6: 1239–1247.

Krebs, C. J., 1989. Ecological Methodology. Harper & Row Publisher, New York, 654 pp.

Letterman, R. D. & W. J. Mitch, 1978. Impact of mine drainage on a mountain stream in Pennsylvania. Envir. Pollut. 17: 53–73.

Matter, W. J. & J. J. Ney, 1981. The impact of surface mine reclamation on headwater streams in Southwest Virginia. Hydrobiologia 78: 63–71.

McKnight, D. M. & G. L. Feder, 1984. The ecological effect of acid conditions and precipitation of hydrous metal oxides in a Rocky Mountain stream. Hydrobiologia 199: 129–138.

Merritt, R. W. & K. W. Cummins, 1984. An Introduction to the Aquatic Insects of North America. Second Edition. Kendal/Hunt Publishing Company, Dubuque, IA, 722 pp.

Mulder, E. GZ. & M. H. Deinema, 1981. The sheathed bacteria. In: Starr, M. P., H. Stroph, H. G. Truper, A. Balows, H. C. Schlegel (eds), The Prokaryotes: A Handbook on the Habitats, Isolation, and Identification of Bacteria. Volume 1. Springer-Verlag, Berlin: 425–440.

Needham, J. G., J. R. Traver & Y. Hsu, 1972. The biology of Mayflies. E. W. Classey Ltd. Hampton, Middlesex, England, 759 pp.

Osborne, L. L., R. W. Davies & K. J. Linton, 1979. Effects of limestone strip mining on benthos macroinvertebrate communities. Wat. Res. 13: 1285–1290.

Parsons, J. D., 1968. The effects of acid strip-mine effluents on the ecology of a stream. Arch Hydrobiol. 66: 25–50.

Petersen, L. B. M. & R. C. Petersen, Jr., 1983. Anomalies in hydropsychid capture nets from polluted streams. Freshwat. Biol. 13: 119–128.

Rasmussen, K. & C. Lindegaard, 1988. Effects of iron compounds on macroinvertebrate communities in a Danish lowland river system. Wat. Res. 22: 101–108.

SAS Institute, 1985 sas Institute, Cary, N.C.

Scullion, J. & R. W. Edwards, 1980. The effects of coal industry pollutants on the macroinvertebrate fauna of a small river in the South Wales coalfield. Freshwat. Biol. 10: 141–162.

Sheldon, S. P. & D. K. Skelly, 1990. Differential colonation and growth of algae and ferromanganese-depositing bacteria in a mountain stream. J. Freshwat. Ecol. 5: 475–485.

Sokal, R. R. & F. J. Rohlf, 1981. Biometry. The principles and practice of statistics in biological research. Freshwat. Biol. 13: 301–311.

Smock, L. A., 1983. Relationships between metal concentration and organism size in aquatic insects. Freshwat. Biol. 13: 313–321.

Sode, A., 1983. Effect of ferric hydroxide on algae and oxygen consumption by sediment in a Danish stream. Arch. Hydrobiol. Suppl. 65: 134–162.

Warner, R. W., 1971. Distribution of biota in a stream polluted by acid mine drainage. Ohio J. Sci. 71: 202–215.

Warnick, S. L. & H. L. Bell, 1969. The acute toxicity of some heavy metals to different species of aquatic insects. J. Wat. Pollut. Cont. Fed. 41: 280–284.