Interactions among nutrients, algae and invertebrates in a New Zealand mountain stream

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SUMMARY 1. Colonization of nutrient-diffusing substrata by periphyton and invertebrates was investigated at forested and open sites in a small, mountain stream and a spring in the South Island of New Zealand.

2. Substrata had colonization surfaces made from 100 μm mesh plankton netting that enabled algal assemblages to be removed intact for scanning electron microscopy. They also allowed small volumes of solvent to be used for the extraction of photosynthetic pigments.

3. At all sites, periphyton assemblages were dominated by species of *Achnanthes*, *Cocconeis* and *Gomphonema*, and except in the forest in winter, periphyton biomass was always greater on enriched (N+P added) than control substrata.

4. Invertebrates colonizing diffusion substrata were principally larval Chironomidae (Orthocladiinae). No larvae were present in winter, but in three spring and summer trials mean larval densities were higher on nutrient-enriched than control substrata at all sites.

5. The inclusion of an insecticide Malathion in diffusion substrata, reduced insect colonization at open and forested sites. After 28 days, no concurrent increases in algal pigment concentration were observed on nutrient-enriched or control substrata at the forested site. However, pigment concentrations were higher on substrata incorporating Malathion at the open site suggesting that algal standing crop was depressed by the activities of grazers.

Introduction

Epilithic algal assemblages in streams are influenced by a suite of environmental factors including water temperature, light, physical abrasion, nutrient availability and grazing (Rounick & Gregory, 1981). The relative importance of these factors differs seasonally and between streams, depending on climate, flow regime,

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phenology of riparian vegetation, and the life histories and abundance of grazing herbivores.

Nutrient limitation of epilithic algae has been demonstrated experimentally in a number of North American river studies (see Grimm & Fisher, 1986; Hershey & Hiltner, 1988, and references therein), and Winterbourn, Collier & Graesser (1988) found that periphyton growth was potentially nutrient-limited in some forested streams on the west coast of New Zealand's South Island. Their preliminary studies with nutrient-diffusing substrata indicated nitrogen

limitation in acid brownwater streams and phosphorus limitation in clear glacial-valley streams. Similarly, Biggs & Close (1989) found that on the east coast of the South Island several gravel-bed rivers with sources in the mountains, foothills and lowlands appeared to be phosphorus limited, especially during periods of low flow in summer.

Nutrient-diffusing substrata in the form of porous clay flowerpots (Fairchild & Lowe, 1984; Fairchild, Lowe & Richardson, 1985; Lowe, Golladay & Webster, 1986) or saucers (Hill & Knight, 1988a) have been used to investigate responses of periphyton to nutrient additions in lakes and streams, whereas petri dishes containing a sand–agar–nutrient mixture were deployed successfully in streams by Pringle & Bowers (1984), Pringle *et al.* (1986) and Pringle (1987). In the present study, agar–nutrient solutions were poured into plastic cups that were capped by removable plankton netting to form the diffusion surface on which periphyton developed.

My objective was to examine the effects of small-scale nutrient additions on periphyton assemblages at shaded and open sites in a small sub-alpine stream at different seasons. The distribution and abundance of invertebrates among periphyton patches (i.e. individual diffusion substrata) was also examined, and in two trials an insecticide was incorporated into some diffusion substrata to assess the impact of grazing herbivores on periphyton biomass.

Methods

The stream environment. The study was conducted in Middle Bush Stream, Cass, on the eastern slopes of the Southern Alps, South Island, New Zealand. The stream originates in springs at about 850 m a.s.l. and drains a small catchment (approximately 4 ha) that includes bare scree slopes, subalpine scrub, tussock grassland and a stand of mountain beech (*Nothofagus solandri* var. *cliffortioides* Hook f. Poole). Details of the stream environment and biota are given in papers summarized by Winterbourn (1987).

Field experiments were carried out at four sites, two within the beech forest (Site 1 on the mainstream and Site 2 on a major tributary), and two about 100 m (Site 3) and 200 m (Site 4) downstream of Sites 1 and 2 where riparian vegetation was a mixture of tussock and scrub dominated by matagouri (*Discaria toumatou* Raoul). Channel width at all sites was 0.8–1.0 m and bed materials were predominantly cobbles and coarse gravels. Although the stream channel is subject to frequent scouring and redistribution of bed materials, flow conditions at the four sites were similar (5–10 l s^{-1}), and on sampling days surface current velocities were in the range 10–30 cm s^{-1}. Illuminance recorded immediately above the water surface on three early afternoons during February–April 1988 ranged from 147 to 355 lux at forested sites and 5270–10,750 lux at open sites.

Physicochemical analyses of water samples on three occasions during the present study showed that stream water was moderately hard (30–36 g m^{-3} CaCO$_3$), slightly alkaline (pH 7.6–8.0) and had conductivity ranging from 75 to 99 μS cm^{-1} at 25°C. On all dates, concentrations of nitrate-N and reactive phosphate-P in water samples were <40 mg m^{-3} and <4 mg m^{-3}, respectively. Water temperatures recorded with calibrated maximum–minimum thermometers

TABLE 1. Programme of field experiments, nutrient treatments, and water temperatures at forested and open sites in Middle Bush Stream, 1987–88. NA=not applicable.

Experiment	Months	Duration of experiment (days)	Sites	Treatments*	Water temperature (°C) Forest	Open
1	March–April 88	42	1, 2, Spring	C, N+P	6–10	10
2	Aug.–Sept. 87	32	1, 2	C, N, P, N+P, N+P+M	6–13	NA
3	Oct.–Nov. 87	25	1, 2, 3, 4	C, N+P	6–11	6–13
4	Jan.–Feb. 88	31	1, 2, 3	C, N+P	8–14	8–18
5	June–July 88	30	1, 2, 3, 4	C, N+P	3–7	1–9
6	Oct.–Nov. 88	28	1, 3	C, N+P, Malathion	5–12	5–13
7	Nov. 88	13	3	C, N+P, Malathion	NA	6–12

* C=no-nutrient controls; N=NaNO$_3$ (0.66 M); P=KH$_2$PO$_4$ (0.51 M); N+P=NaNO$_3$+KH$_2$PO$_4$ (Expt 1, 0.66 M+0.06 M; Expt 2, 0.33 M+0.03 M; Expts 3–7, 1.5 M+0.09 M; M=Micronutrients (as in Pringle *et al.*, 1986).

during individual field experiments are shown in Table 1.

An unshaded, constant temperature (10°C) spring was also used as a field site in Experiment 1. It was about 200 m northwest of Middle Bush Stream and had almost identical water chemistry.

Bioassay technique. Diffusion substrata were constructed from 60 ml plastic cups filled with 2% agar–nutrient solutions. The upper surface of the agar was covered with fine plankton netting (100 μm mesh) secured tautly to the cup by a snap-on, plastic ring. The netting (area 12.6 cm^2) formed the colonization surface through which nutrients diffused. Sets of twelve cups were pressed tightly into alternate cells of square (0.1 m^2), plastic trays (modular floor gratings) and anchored in the stream with heavy stones placed along the sides of each tray so as not to obstruct flow patterns.

Nutrient treatments and initial concentrations used in the seven field experiments are shown in Table 1. Six replicates of each treatment were used routinely in all experiments. Individual substrata (cups) were placed in random order within trays in Experiment 2 but in all other trials treatment cups were alternated. In Experiments 6 and 7 the insecticide Malathion was added to a second set of control and nutrient-enriched substrata that were placed immediately downstream of the untreated (no insecticide) substrata. The dosage selected (2 g l^{-1} in the form of a 50% emulsion; i.e. 0.006 M Malathion) has successfully induced migration of insects from diffusion surfaces in other experiments (Gibeau, Moller & Miller, 1988; G. G. Gibeau, pers. comm.).

Substrata were submerged in the stream with their upper colonizing surfaces within 5 cm of the air–water interface (baseflow conditions) for 13–42 days (Table 1). Upon removal, periphyton assemblages were examined with a scanning electron microscope (SEM) and photosynthetic pigments were extracted in acetone to provide an indicator of algal biomass. In Experiments 3–7 all animals associated with each substratum were removed from netting and containers following acetone extraction of pigments, identified and counted. The body lengths of all chironomid larvae present at the end of Experiments 6 and 7 were measured.

Laboratory analyses. A segment of netting ($\approx$100 mm^2) from one randomly selected cup per treatment per site was removed for examination of algal assemblages. Segments were preserved in 3% glutaraldehyde and subsequently prepared for SEM viewing as described by Rounick & Winterbourn (1983). Representative algal assemblages were photographed at magnifications up to 10 K and from these identifications were made. In Experiment 4 (February 1988), diatom counts were made from enlarged prints of five 0.22 mm^2 fields per segment (randomly selected with the SEM out of focus).

Periphyton pigment concentrations were obtained after removing netting surfaces from all cups and transferring them to vials containing 5 ml of 90% acetone. Concentrations of chlorophyll *a* and phaeophytin were estimated after extraction at 5°C for up to 24 h in the dark using the method of Moss (1967a, b). Chlorophyll *a* and phaeophytin values were combined to provide a measure of 'total pigment'. Pigment concentration was preferred to organic carbon or dry weight values as an indicator of algal biomass since epilithic communities included variable amounts of dead non-algal organic matter.

At the end of Experiment 6 (November 1988), algal pigment concentration on stones taken from the stream bed at Sites 1 and 3 was also measured for comparison with diffusion substrata. Ten stones (60–85 mm maximum linear dimension) from each site were immersed in 90% acetone to extract pigments and concentrations were then determined as described above. Results are expressed per cm^2 of vertically projected (planar) stone surface as described by Hershey & Hiltner (1988).

Results

Except in November 1988, all field experiments were carried out at times when (fortuitously) no heavy spates occurred. Implanted substrata were not disturbed or damaged in any trial, but small and variable amounts of fine particulate material settled unevenly on diffusion surfaces in most months, especially at Site 2. No results were obtained for Site 4 in February 1988 as surface flow ceased during the course of the experiment.

Experiment 1

To test the effectiveness of the diffusion substrata in releasing nutrients over an extended
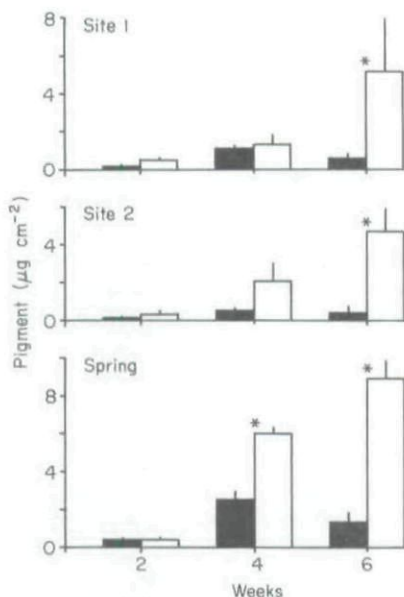
FIG. 1. Mean pigment concentration (+1 SE) on control (closed bars) and nutrient-enriched (open bars) substrata after 2, 4 and 6 weeks at three sites. *Treatments significantly different ($P<0.05$).
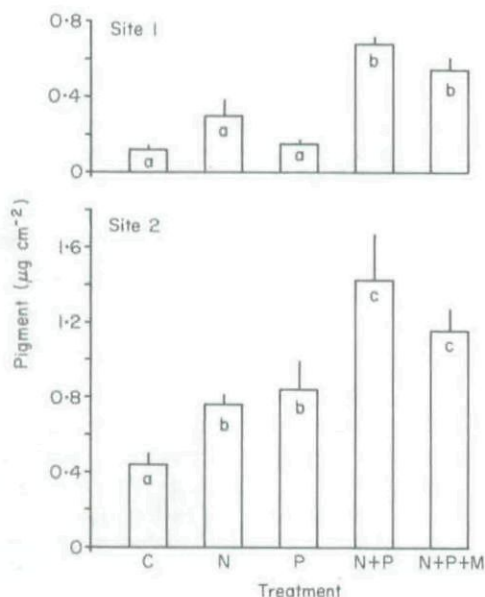

FIG. 2. Mean pigment concentration (+1 SE) after 32 days on diffusion substrata providing five different nutrient treatments. C=no-nutrient control; N=nitrogen added; P=phosphorus added; N+P=nitrogen and phosphorus added; N+P+M=nitrogen, phosphorus and micronutrients added (see Table 1 for details). Treatments designated by the same letter are not significantly different (U-test; $P>0.05$).

period, control (agar only) and nutrient-enriched (N+P) substrata were removed from Sites 1 and 2, and the spring site after 2, 4 and 6 weeks. Pigment concentrations were higher on nutrient-enriched than control substrata on all sampling days (Fig. 1), the differences being significant at all sites in week 6 and in the spring in week 4 (Mann-Whitney U-tests, $P<0.05$). Pigment concentrations peaked on control substrata after 4 weeks. However, they continued to rise on nutrient-enriched substrata indicating that nutrients were diffusing from the agar throughout the 6 weeks of the experiment.

Experiment 2

A 32-day experiment at the two forested sites in August–September 1987 incorporated five different nutrient treatments and indicated that algal communities were limited by both nitrogen and phosphorus at both sites (Fig. 2). Thus, pigment concentration was highest when both N and P were added together, and no further increase was observed when N+P treatments were supplemented with micronutrients.

Experiments 3–6

Total pigment concentrations on diffusion substrata at the end of four subsequent trials incorporating control and N+P treatments only are shown in Fig. 3. Except at the forested sites in mid-winter (July), algal pigment concentration was always greater on nutrient-enriched than control substrata, significantly so in ten of eleven paired site comparisons (U-tests, $P<0.05$).

On average, pigment concentration was 8.3 times higher on nutrient-enriched than control substrata at forested sites and 17.7 times greater on the enriched substrata at open sites (Table 2). The presence of very low pigment concentrations on both types of substratum in winter at the forested sites, suggests that algal growth was primarily light-limited there at that time.

Pigment concentrations on stream bed stones taken on 9 November 1988 were 0.27 ± 0.04 μg cm^{-2} ($\bar{X}+SE$; $n=10$) at Site 1 and 0.87 ± 0.15 μg cm^{-2} at Site 3. They were therefore much more similar to those attained on control than nutrient-enriched substrata (Fig. 3).
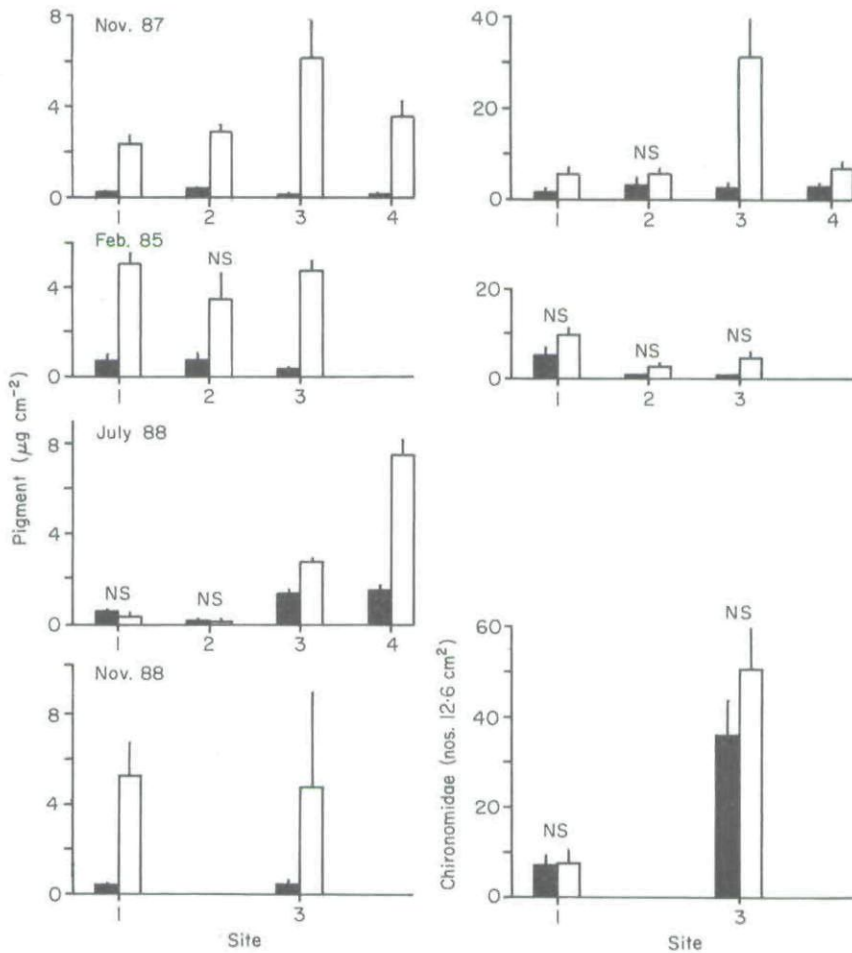
FIG. 3. Mean (+1 SE) pigment concentrations (left) and larval chironomid densities (right) on control (closed bars) and nutrient-enriched (open bars) diffusion substrata in 4 months following colonization periods of approximately 4 weeks. Note: no results were obtained for Site 4 in February when surface flow ceased. No chironomids were present in July. All within-site pairs are significantly different (*U*-tests, $P < 0.05$) unless indicated by NS.

Algal assemblages

Periphyton assemblages that developed on diffusion substrata at all sites consisted almost exclusively of diatoms (Fig. 4a, b). The same complement of common species occurred on control and nutrient-enriched substrata at all four sites although *Diatoma hiemale* var. *mesodon* (Ehr.) Grun. was not found at Site 1 and was seen only once at Site 2. In contrast, its filaments

TABLE 2. Ratios between mean pigment concentration on nutrient-enriched and control substrata (N+P:C) at open and forested sites at the end of four field experiments.

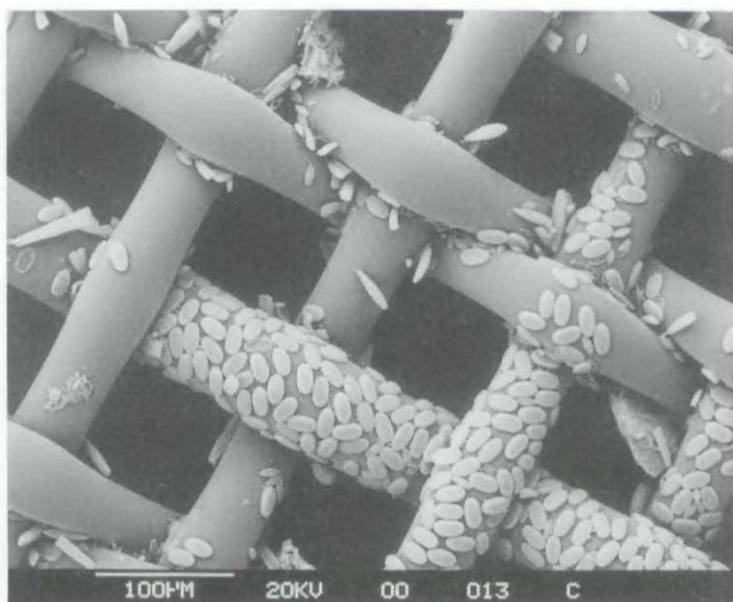| Experiment	Season	N+P:C ratios	
		Forested	Open
3	Spring	13.3	39.3
4	Summer	6.3	16.2
5	Winter	0.7	3.7
6	Spring	13.0	11.4

FIG. 4(a)

FIG. 4(b)

FIG. 4. Scanning electron micrographs of diatom assemblages and a chironomid tube on diffusion substrate netting. (a) Diatom assemblage dominated by *Gomaphonema, Cocconeis* and *Achnanthes* species on nutrient-enriched substratum, Site 1, February 1988; (b) a patchy distributed algal assemblage dominated by *Cocconeis*, Site 2, February 1988; (c) tube of an orthoclad larva, Site 3, November 1987. In all figures pores in the netting are 100 μm wide.
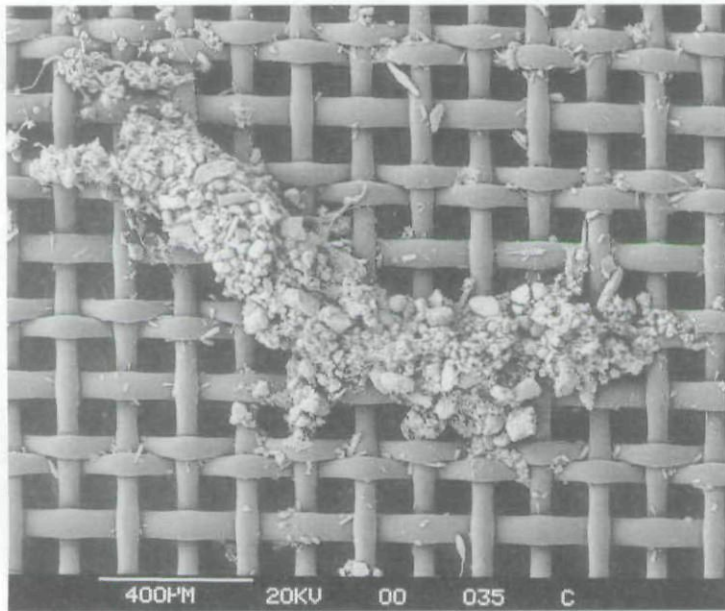
FIG. 4(c)

formed a prominent, if patchy, overstorey at the open sites except in February. Patches of *Synedra ulna* (Nitz.) Ehr. were also prominent at Site 4 in July, and because of their large size probably made an important contribution to the high pigment values on nutrient-enriched substrata there. At the forested sites, and beneath the *Diatoma* overstorey at open sites, the periphyton was dominated by a mixed assemblage of prostrate species (*Achnanthes lanceolata* (Breb.) Grun., *A. minutissima* Kutz and *Cocceneis placentula* Ehr.), erect species (*Fragilaria vaucheriae* (Kutz.) Peters. and a small *Synedra* species), and stalked species of *Gomphonema* and *Gomphoneis*.

The three prostrate species and *Gomphonema subclavatum* (Grun.) were numerically dominant on diffusion substrata from Sites 1, 2 and 3 in February (Table 3) but their small scale distributions, especially those of *Cocconeis*, were highly aggregated. The cell counts confirm that diatom densities were highest on nutrient-enriched substrata and that *A. lanceolata* in particular showed a pronounced positive response to enrichment at all three sites.

Invertebrate colonization

At all sites the most abundant colonists were larvae of two undescribed species of Chiro-

TABLE 3. Numbers of diatoms on segments of plankton netting taken from control and nutrient-enriched (N+P) substrata at the end of Experiment 4 (February 1988). Counts are means (and 1 SD) per SEM field (0.22 mm²), $n = 5$.

	Site 1		Site 2		Site 3	
Taxa	Control	N+P	Control	N+P	Control	N+P
Cocconeis placentula Ehr.	4 (3)	96 (114)	140 (94)	9 (7)	51 (20)	34 (38)
Achnanthes lanceolata (Bréb.) Grun	5 (2)	21 (26)	26 (17)	384 (129)	0	235 (95)
Achnanthes minutissima Kütz	4 (2)	280 (71)	18 (28)	19 (9)	23 (9)	38 (18)
Gomphonema subclavatum (Grun.)	1 (1)	138 (66)	8 (6)	34 (20)	13 (4)	7 (3)
Gomphonema constrictum Ehr.	0	0	2 (1)	7 (7)	0	0
Synedra sp.	<1	0	<1	<1	0	4 (3)
Fragilaria vaucheriae (Kütz.) Peters.	<1	0	1 (1)	0	0	0
Total diatoms	15 (6)	535 (94)	195 (129)	453 (131)	87 (30)	318 (112)

nomidae (both Orthocladiinae). In November 1987 and February 1988 they made up over 90% of all animals found, but in November 1988 larvae of *Maoridiamesa harrisi* Pagast (Diamesinae) were also common. Other Chironomidae seen in small numbers were *Polypedilum canum* Freeman, *Tanytarsus vespertinus* Hutton and a species of Macropelopiini. Larvae of the blackfly, *Austrosimulium australense* (Schiner), were also present in November of both years, and occasional stoneflies (*Zelandobius furcillatus* Tillyard, *Cristaperla fimbria* Winterbourn), mayflies (*Deleatidium* sp.), empidids and ceratopogonids were found. Non-insect colonists taken in small numbers were unidentified mites, copepods, nematodes and the planarian, *Neppia montana* (Nurse). In many streams elsewhere, larval caddisflies and gastropod molluscs are amongst the most important invertebrate grazers. However, molluscs do not occur in Middle Bush Stream and the caddisfly species present are all predatory or detritivorous species (Winterbourn, 1982).

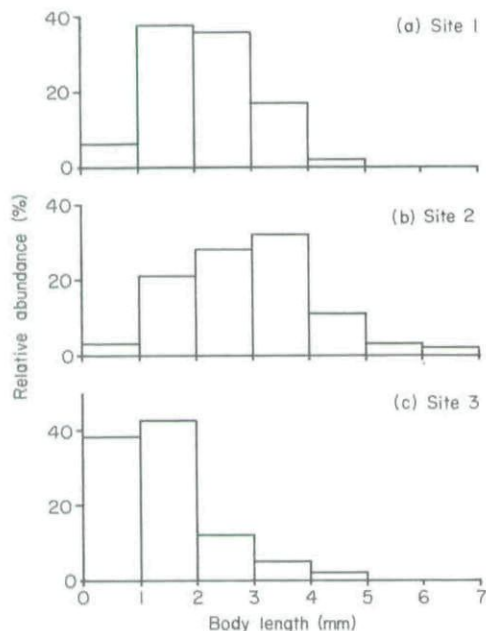Most chironomid larvae on diffusion substrata

FIG. 5. Size frequency distributions of larval Chironomidae from all diffusion substrata combined (control, nutrient-enriched, with and without Malathion) at the end of Experiment 6 (a and b, duration 28 days) and Experiment 7 (c, 13 days).

occupied tubes constructed from fine inorganic and detrital particles (Fig. 4c). Few diatoms grew on the surfaces of chironomid tubes at any site but the guts of larvae cleared with lactophenol and examined microscopically contained a mixture of diatom species in addition to fine detrital particles and silt. In July, no chironomids were present but in the other months mean densities of larvae (all species combined) were higher on nutrient-enriched than control substrata (Fig. 3).

The faunas present on Malathion-treated and untreated diffusion substrata after a 28-day colonization period (November 1988) were similar at Sites 1 and 3 (78–90% Chironomidae, 4–14% *A. australense*), and chironomid size class distributions were not significantly different on insecticide-treated and untreated substrata within sites (Chi-squared=7.4 and 6.9, $P>0.05$; Fig. 5). However, larval densities were higher on both control and nutrient-enriched substrata without insecticides than on their Malathion-treated counterparts. These differences in density were significant at Site 1 (*U*-test; $P<0.05$) but not at Site 3 where intersample variation was high (coefficients of variation 49–55%). The addition of Malathion to control and nutrient-enriched diffusion substrata at Site 1 resulted in no significant difference in algal pigment concentration after 28 days, but at Site 3 pigment concentration was significantly higher (*U*-test, $P<0.05$) on controls treated with Malathion (Fig. 6).

Although inconclusive, the results obtained in this experiment suggested that Malathion had been partially successful in limiting colonization of substrata by insects. To test this further, another trial was run at Site 3 where colonization by chironomids had been higher. However, it was limited to 13 days to maximize the rate of release of insecticide (see Fairchild *et al.*, 1985). Upon retrieval of substrata, mean chironomid densities were found to be about 5 times higher on those without insecticide (Fig. 6) and blackfly larvae were present only on the untreated surfaces. Nevertheless, algal pigment concentration was unaffected by the presence or absence of insecticide (Fig. 6), and there was no evidence that the mainly very small midge larvae (Fig. 5), or other transient grazers had influenced the build up of periphyton. Furthermore, these findings and those obtained at Site 1 in the previous experiment indicated that the very low concen-
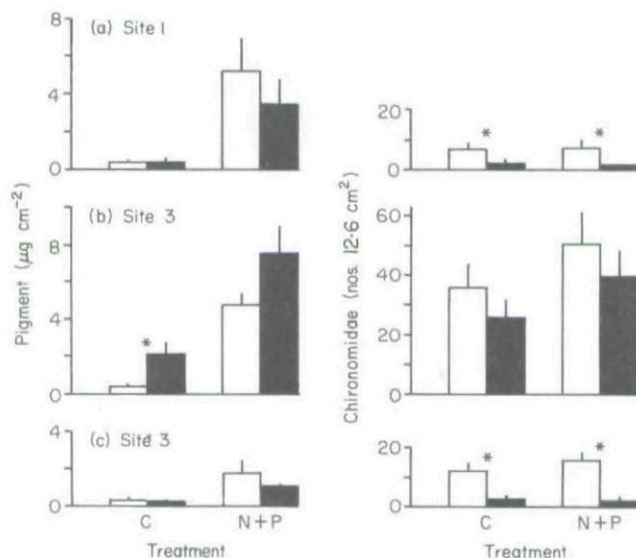
FIG. 6. Mean (+1 SE) pigment concentration and densities of larval Chironomidae on nutrient-enriched (N+P) and control (C) diffusion substrata with (closed bars) and without (open bars) Malathion added. a and b, after 28 days (Experiment 6); c, after 13 days (Experiment 7). *Treatments significantly different ($P<0.05$).

tration of phosphorus in Malathion had not stimulated algal growth.

Discussion

Colonization experiments with nutrient-diffusing substrata indicated that algal assemblages were nutrient limited at forested and open sites in Middle Bush Stream. An exception was at the shaded sites in winter when low light availability rather than nutrients may have been the critical limiting factor. Both nitrogen and phosphorus appeared to limit algal growth and on the three occasions that the concentration of nitrate-N in stream water was measured, it was less than the threshold of 0.055 g m^{-3} below which Grimm & Fisher (1986) found that nitrogen additions stimulated periphyton growth. Low concentrations of nitrate-N, and soluble P have also been reported from other spring waters in the Cass Basin (Stout, 1972), and the results of diffusion substrate experiments at six open and forested stream sites indicate that nutrient limitation of algal growth may be common in this region (Winterbourn & Fegley, 1989).

Non-quantitative examinations of algal assemblages on plankton netting indicated that most diatom taxa responded positively to com-

bined additions of nitrogen and phosphorus, and the counts made at the end of the February experiment suggested that the response of *Achnanthes lanceolata* was particularly strong. This is consistent with the findings of Jones (1978), Fairchild *et al.* (1985), Lowe *et al.* (1986) and Hill & Knight (1988a) who obtained positive growth responses by *A. lanceolata, A. minutissima* and *Cocconeis placentula* to combined nitrogen and phosphorus additions. However, my findings contrast with those of Keithan, Lowe & De Yoe (1988) who reported that because common diatom taxa varied in their responses to nutrient additions, chlorophyll *a* concentrations among nutrient treatments at a single site were not significantly different.

The algal assemblages that developed on diffusion substrata in Middle Bush Stream consisted predominantly of diatoms and were similar taxonomically to those on stones in riffles (Winterbourn & Fegley, 1989). However, the latter included more filamentous green algae and Cyanophyta whose paucity on the netting may have been a consequence of its texture or the relatively short time available for colonization. Epilithic communities taxonomically similar to those on the artificial substrata have been reported from other New Zealand rivers and streams by Biggs & Price (1987) and Biggs

(1988), and from forested and open streams elsewhere (e.g. Hansmann & Phinney, 1973; Rushforth, Squires & Cushing, 1986). Several species of *Achnanthes* and *Cocconeis* in particular, are regarded as typical early colonists that multiply rapidly (Korte & Blinn, 1983) and have broad ecological niches (Steinman & Lamberti, 1988). They are also adnate forms that appear to be little affected by invertebrate grazing (Pringle, 1985; Robinson & Rushforth, 1987; Hill & Knight, 1988b), although in Middle Bush Stream their high relative abundance is probably more a reflection of their colonizing ability and relative immunity to frequent physical disturbance (Robinson & Rushforth, 1987).

Although chironomid larvae were sometimes abundant on the netting overlying diffusion substrata, they were not found on the upper surfaces of stones collected in their vicinity. In contrast, McAuliffe (1983) found that larvae of *Rheotanytarsus* and *Eukiefferiella* species were prevalent on flat smooth surfaces of stones in a North American stream, and in an earlier study in Middle Bush, orthoclad and other chironomid larvae were abundant colonists of woody substrata on which fine particulate matter had settled (Winterbourn, 1982). Since algal pigment concentration on control diffusion substrata colonized by midges was similar to that on stones at the same site (November 1988), it is possible that the pitted netting surface with its greater particle-trapping ability provided more favourable conditions for chironomid larvae. In particular, larvae require fine particulate organic matter and silt for tube making. Very few diatoms were incorporated into tubes on diffusion substrata in strong contrast to the findings of Hershey *et al.* (1988) and Pringle (1985) who concluded that larvae within tubes could act as sources of nutrients to which certain diatoms were attracted.

Although chironomid larvae frequently occur in association with algae, the relationship has been quantified infrequently. In an earlier New Zealand study, Towns (1981) found that densities of all chironomid species were higher outside than beneath a canopy erected over a section of New Zealand stream, and concluded that this was a response to the presence of living algae. On a much larger spatial scale, Hershey *et al.* (1988) examined the density response of *Orthocladius rivulorum* Kieffer to stream fertilization (and a consequent increase in epilithic

algal biomass) and found it was not consistent from year to year. On a far smaller scale, Fairchild & Lowe (1984) found maximum numbers of early instar *Corynoneura* larvae on phosphate-diffusion flowerpots, apparently because of the greater algal biomass there.

McLachlan, Brennan & Wotton (1978) reported that the amount and size distribution of food particles strongly affected the spatial and temporal distributions of chironomid larvae in a spate-prone northern England stream, and because larval numbers were significantly correlated with the weight of epilithic material in 25 cm² stone samples, they concluded that the chironomid community was food limited. In contrast, Ogilvie & Clifford (1986) considered that food supply was not limiting in Dead Horse Creek, Alberta, where numbers of a grazing caddisfly, *Oligophlebodes zelti* (Nimmo), were also significantly correlated with periphyton biomass. Instead, they suggested that behaviours promoting such a distribution pattern had evolved in response to the heterogeneous distribution of periphyton rather than to its short supply *per se*. Such an interpretation may best explain the aggregated distribution of midge larvae on diffusion substrata in Middle Bush Stream.

The final 13-day experiment showed that colonization of substrata was principally by very small larvae whose size class distribution contrasted with that observed at the end of the prior 28-day trial when larvae of many sizes were present. Principal modes of colonization are inferred to be drift and oviposition, the latter being indicated by the presence of a few egg masses attached to diffusion substrata. The criteria used by females and first instar larvae to select oviposition and tube building sites, respectively, therefore appear to be critical in determining subsequent larval distribution patterns. The nature of these criteria is unclear although perception of periphyton patches is implicated, and/or larvae may respond positively to the presence of other individuals and settle in their close vicinity. While aquatic invertebrates may be attracted to substrate patches supporting a high biomass of periphyton, it is also well known that their feeding activities can reduce standing crops (Jacoby, 1985; Lamberti, Feminella & Resh, 1987; Hill & Knight, 1988b) and, in some instances, result in an increase in the rate of primary production (Lamberti & Resh, 1983).

By incorporating insecticides (Malathion and Lindane) into nutrient-diffusing substrata in the Kuparuk River, Alaska, Gibeau *et al.* (1988) successfully reduced invertebrate colonization (and by inference feeding) and observed increases in both algal production and biomass accrual.

Similar experiments in Middle Bush Stream also indicated that invertebrate colonization was reduced by insecticide treatment, but an increase in algal pigment concentration over that present on no-insecticide controls was found only at the open site below the forest. Although of a preliminary nature only, these results suggests that grazing insects can affect periphyton standing crops in Middle Bush Stream as can snails that inhabit springbrooks in this geographic region (Winterbourn & Fegley, 1989).

Finally, it seems worthwhile to comment on the advantages and limitations of the particular diffusion substrate technique used in this work to complement the more general evaluation of Pringle (1987). My principal innovation was to use removable plankton netting as the diffusion surface on which the periphytic community developed. Unlike porous clay flowerpots (Fairchild & Lowe, 1984) or the agar–sand dishes introduced by Pringle & Bowers (1984), they enable periphyton to be removed intact for viewing or perhaps for subsequent measurement of primary production (e.g. with ^{14}C), or for use in experiments with grazers.

Like any artificial substratum, however, netting is unlikely to provide a perfect analogue for natural stream channel materials. Nevertheless, in this and parallel studies (Winterbourn & Fegley, 1989), diatom assemblages were similar on stones and netting. Less ideally though, some algae colonized the agar surface and the underside of netting whose relatively coarse pore size permitted the passage of diatoms as well as light. In other studies (unpublished), I have used glass-fibre filters rather than plankton netting as a removable surface, but they are less robust and subject to tearing when damp. However, both filters and netting can be cut and folded, which increases their versatility, and, if pigments are to be measured, only small volumes of extractant are needed. A removable diffusion surface also enables intact communities to be moved among stream sites where they can be subjected sequentially to different environmental conditions

and/or combinations of diffusing substances. Through manipulations of this kind it should be possible to gain further insights into periphyton community dynamics.

Acknowledgments

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