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ALBERTA OIL SANDS ENVIRONMENTAL RESEARCH PROGRAM

Interim Report on Ecological Studies on the Lower Trophic Levels of Muskeg Rivers Within the Alberta Oil Sands Environmental Research Program Study Area

Project AF 2.0.2

May 1979

Sponsored jointly by

Alberta ENVIRONMENT

Environment Canada Environnement Canada

15th Floor, Oxbridge Place
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Edmonton, Alberta, Canada
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ALBERTA OIL SANDS ENVIRONMENTAL RESEARCH PROGRAM

RESEARCH REPORTS

These research reports describe the results of investigations funded under the Alberta Oil Sands Environmental Research Program, which was established by agreement between the Governments of Alberta and Canada in February 1975 (amended September 1977). This 10-year program is designed to direct and co-ordinate research projects concerned with the environmental effects of development of the Athabasca Oil Sands in Alberta.

A list of research reports published to date is included at the end of this report.

Enquiries pertaining to the Canada-Alberta Agreement or other reports in the series should be directed to:

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Interim Report on Ecological Studies on the Lower Trophic Levels of Muskeg Rivers Within the Alberta Oil Sands Environmental Research Program Study Area

Project AF 2.0.2

AOSERP Report 58

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The Hon. John W. (Jack) Cookson
Minister of the Environment
222 Legislative Building
Edmonton, Alberta

and

The Hon. John Fraser
Minister of the Environment
Environment Canada
Ottawa, Ontario

Sirs:

Enclosed is the report "Interim Report on Ecological Studies on the Lower Trophic Levels of Muskeg Rivers Within the Alberta Oil Sands Environmental Research Program Study Area".

This report was prepared for the Alberta Oil Sands Environmental Research Program, through its Aquatic Fauna Technical Research Committee (now the Water System), under the Canada-Alberta Agreement of February 1975 (amended September 1977).

Respectfully,

W. Solodzuk, P.Eng.
Chairman, Steering Committee, AOSERP
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Member, Steering Committee, AOSERP
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INTERIM REPORT ON ECOLOGICAL STUDIES ON THE LOWER TROPHIC LEVELS OF MUSKEG RIVERS WITHIN THE AOSERP STUDY AREA

DESCRIPTIVE SUMMARY

BACKGROUND

This project (AF 2.0.2) was conducted in 1977-78 as a planned complement of another project (AF 2.0.1) on invertebrate resources. The scope of this project was to investigate productivity in the oil sands region and it involved both baseline data collection and manipulation experiments. Artificial substrates were employed, water chemistry parameters were measured, taxonomic identifications were carried out, and productivity measurements were conducted in order to elucidate bacterial and algal productivity. Objectives set forth for the project were:

1. To determine the baseline numbers, biomass, taxonomic composition, and productivity of primary and secondary producers in the sediments, periphyton and suspended matter of muskeg rivers throughout the year;
2. To determine the input routes of organic and inorganic nutrients via groundwater, etc;
3. To determine the effects of siltation and stream substrate alterations upon primary productivity and total benthic respiration; and
4. To predict the effects of future oil sands development activities on the lower trophic levels of stream communities.

The activity in the project was planned in the context of a program that would involve additional work in 1978-79.

ASSESSMENT

In addition to AOSERP management review, the report for the project was reviewed by scientists at the University of Manitoba and the University of Alberta and the authors took opportunity respond to input from the referees. However, the content of the report does not necessarily reflect views of Alberta Environment or Environment Canada and the mention of trade names in the report does not constitute an endorsement. The Alberta Oil Sands Environmental Research Program accepts the report "Interim Report on Ecological Studies on the Lower Trophic Levels of Muskeg Rivers within the AOSERP Study Area" as a valid document to receive wide distribution and thanks the authors for their contribution.

S.B. Smith, Ph.D
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INTERIM REPORT ON ECOLOGICAL STUDIES ON THE LOWER TROPHIC LEVELS OF MUSKEG RIVERS WITHIN THE ALBERTA OIL SANDS ENVIRONMENTAL RESEARCH PROGRAM STUDY AREA

by

M.A. LOCK and R.R. WALLACE
Fisheries and Environment Canada

for

ALBERTA OIL SANDS ENVIRONMENTAL RESEARCH PROGRAM

May 1979

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ABSTRACT

A study of the baseline states of the bacteria and algae in three muskeg rivers located in the AOSERP study area in northeastern Alberta was carried out using natural and standardized granite substrates. The rivers were the Muskeg River, Steepbank River, and Hartley Creek.

The major proportion of algae and bacteria were found attached to rock surfaces as opposed to suspended in the river, and it appeared that at least in the case of the algae the suspended forms were primarily benthic in origin. The relationship between algae was examined. Principle bacterial and algal biomass peaks occured in early summer and early winter with minima occurring in August. The correlation of these two groups was positive and highly significant; however, the ATP living biomass indicator did not appear to be correlated with them and this phenomenon is discussed. Population fluctuations of bacteria and algae are discussed with relation to the physicochemical environment and evidence of the microbial control of some chemical species was presented. Additional possible machanisms for the decline of the bacterial/ algal films during August and mid-winter were offered.

An examination was made of the distribution of bacteria and algae on the upper and lower surface of rocks and also the structure of the films in which they are incorporated using transmission and scanning electron microscopy. Generally fewer organisms were found on the lower surfaces but in each case they were supported in a matrix of "polysaccharide-like" material. The role of this material as an adsorption site for inorganic and organic material and as a potential food source for benthic

invertebrates was discussed. Lastly, a study of the chemical composition of groundwater in the vicinity of the Muskeg River indicates that the nitrogen and phosphorus components of groundwater may be radically altered on their passage to the free water of the river channel.

ACKNOWLEDGEMENTS

We would like to thank J. Buchanan for her outstanding technical support both in the field and in the laboratory.

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We are also extremely grateful to J. Bell of the Environmental Protection Service for the sue of the Ortholux II microscope and to S. Malhotra of the Northern Forest Research Centre for use of the Zeiss spectrophotometer and Isocap 300 scintillation counter.

Special thanks are also due to our pilots who transported us efficiently and safely in the field, especially F. Wiskar and D. White, also their engineers J. LaRoux and E. Taylor for numerous small services.

We finally wish to express our sincere thanks to D. Hadler and C. Boyle for their valuable help with logistics and communications in the field and particularly M. Hirst for office services and p. Debnam for photographic services in Edmonton.

Thsi research project AF 2.0.2 was funded by the Alberta Oil Sands Environmental Research Program, a joint Alberta-Canada research program established to fund, direct, and co-ordinate environmental research in the Athabasca Oil Sands Area of northeastern Alberta.

1. GENERAL INTRODUCTION

Until recently, the microbial component (bacteria, algae, fungi) of river ecosystems has been virtually ignored; however, it is already apparent that the activity of these organisms is the principle agent which determines the capacity of a river to remove organic compounds from the water (Lock and Hynes 1976; McDowell and Fisher 1976). Indeed, the microorganisms of a river (both suspended and attached) can control the levels of nitrogen and phosphorus (Eichenberger 1975), and possibly many other chemical species which are present in the free water. Some of the mechanisms for this have been identified, but determinations of the rates of transfer of chemical species between biotic and abiotic components of ecosystems are only just beginning (Schindler et al. 1975). However, through measurement and understanding of these processes we will ultimately be able to predict the changes which occur in the chemical composition of the water which are observed with water quality monitoring programs, in particular, the biologically important elements, carbon nitrogen and phosphorus. By the same token through understanding the complex interrelationships between the living microorganisms and the inorganic matter flowing around them, we will be much more likely able to predict the effect of physical and chemical disturbances. Such effects could include the elimination of a group by a toxic substance or the stimulation of bacteria and algal growth through domestic effluents as might be in the case in the Muskeg River drainage basin (Figure 1) following development of the proposed Fort Hills townsite. Such disturbances of the natural communities will result in new baseline states in the water quality, which, in turn, could determine the domestic and recreational potential for such waters.

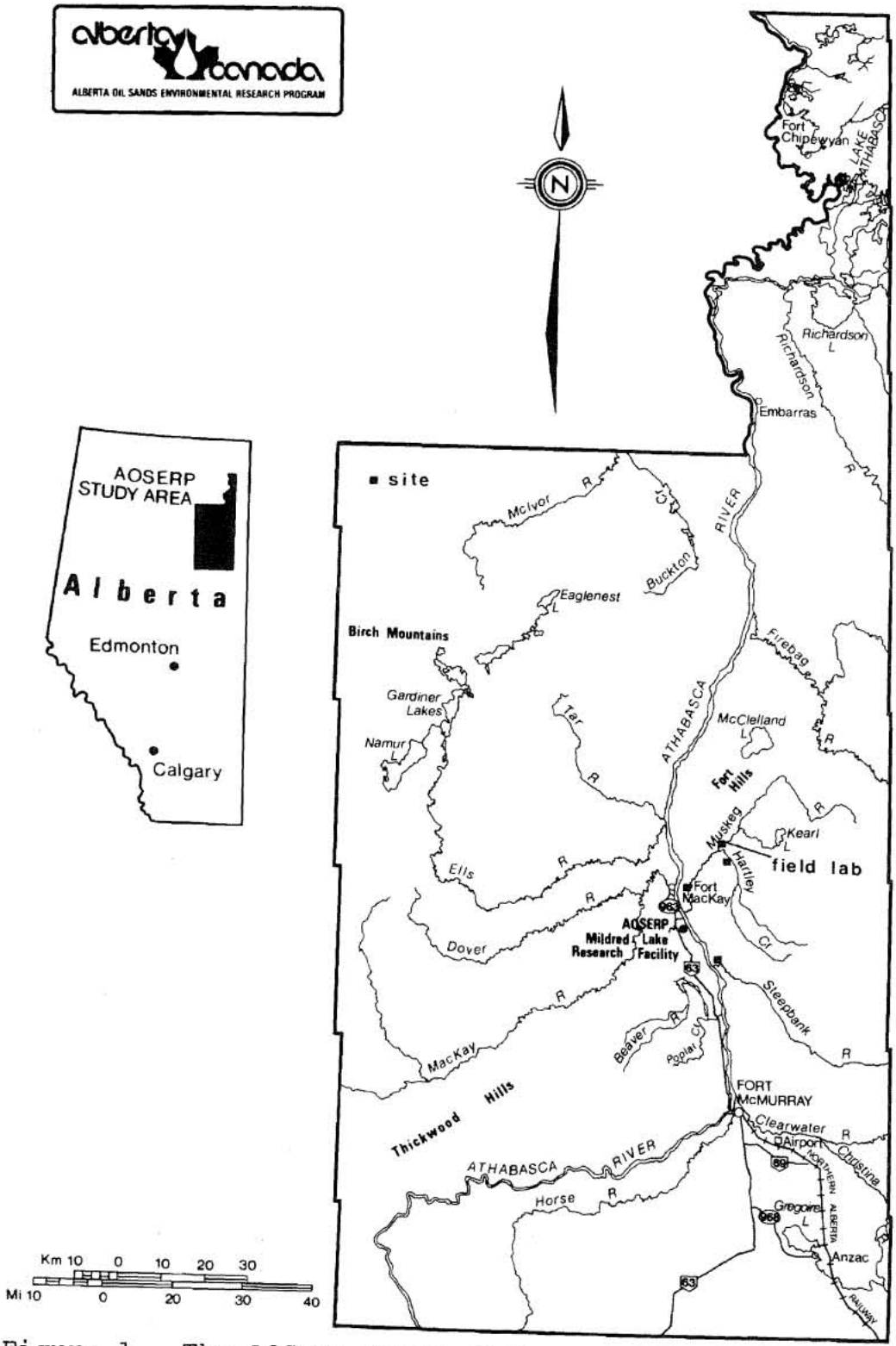

Figure 1. The AOSERP study area.

As well as their function as agents controlling some aspects of water quality, aquatic microorganisms also have the primary function as a food source for the next trophic level, the benthic invertebrates. Bacteria and fungi through their versatile biochemical ability are able to assimilate organic matter such as dissolved organic nitrogen and carbon compounds which are unavailable to invertebrates and convert it into microbial protoplasm which is a good food source for them. They are also able to convert cellulose from plant material, such as dead leaves and twigs, from a food source which only few stream organisms can digest to readily-assimilable microbial protoplasm. Much of the dissolved and particulate organic matter which bacteria assimilate washes into rivers from the surrounding land and provides an additional source of energy to the river in addition to that fixed by the algae through photosynthesis (Fisher and Likens 1973). As yet, the extent to which algal protoplasm is directly available to stream invertebrates as a food source is unclear, however, one of the principle pathways would include the action of bacteria as intermediaries, as outlined above.

The objectives of the study were as follows:

1. To determine the baseline numbers, biomass, taxonomic composition and productivity of primary and secondary producers in the sediments, periphyton and suspended matter of muskeg rivers throughout the year.
2. To determine the input routes for organic and inorganic nutrients via groundwater.
3. To determine the effects of siltation and stream substrate alterations upon primary productivity and total benthic respiration.
4. To predict the effects of future oil sands development activites on the lower trophic

levels of stream communities.

In the first year of this study, we set out to determine the baseline states of the bacteria and algae of muskeg rivers. This included determinations of where the major populations occur in these rivers and studies of their seasonal population fluctuations and, where possible, relating these fluctuations to changes in the water chemistry of the river. The findings from this study will produce the required base for the subsequent determination of the productivity and potential capacity of the river to remove organic and inorganic substances from the water that may enter as a direct or indirect consequence of oil sands development.

Additionally, a concurrent study was carried out in conjunction with the present study to examine the effects of hydrocarbons upon benthic river communities. The objective was to obtain and analyse the data from the bacterial and algal component and the findings are fully reported in a separate report (Barton and Wallace In prep.).

2. POPULATIONS BIOLOGY, SPECIES COMPOSITION, AND PRODUCTIVITY OF BACTERIA AND ALGAE IN MUSKEG RIVERS

Principal Researchers: M.A. Lock, S. Charlton and R.R. Wallace

2.1 INTRODUCTION

Ecological studies on rivers have been primarily concerned with benthic invertebrates or fish (Hynes 1970; Whitton 1975). The lower trophic levels, which support the macrobenthic communities and, ultimately, the fishes, have received considerably less attention. Most work on the lower trophic levels has been done on algae, with few studies being directed toward the fungi

and bacteria. Additionally, many of these studies have been concerned with the suspended microbial component of the river or where benthic communities were investigated, this was done using glass slides, a standard technique for investigating attached bacteria and algae (Sladeckova 1962). This last approach is subject to criticism on two accounts: first, slides are often cultured in the main flow of water away from the stream bed, and second, but perhaps more importantly, many of the racks used to suspend the slides radically alter the flow regime from that experienced by the upper surfaces of gravel and rubble of the stream bed. This would likely have a concomitant effect upon the types of organisms colonizing the surface of the slide and also in the subsequent development of the community (Lock and John in prep.), and would not be a true reflection of the natural communities.

Only recently has the partitioning of the bacterial and algal communities been investigated (Geesey et al. in prep.). In their studies on an alpine stream system, they clearly demonstrated the importance, in numerical terms, of the attached communities. On making the comparison between the numbers of bacteria $m\ell^{1}$ of stream water and those on 1 cm^{2}, of stream substrate, the latter were found to be greater by a factor of 10 - 100. The present study was designed to investigate the population biology and suspended/attached partitioning of the bacterial and algal components of three muskeg rivers, the Muskeg and Steepbank rivers and Hartley Creek, over a 12 month period. The study included the collection of information during the winter period as the rivers of the AOSERP study area are at 0°C, with extensive ice over, for up to half the year. The general objective was to describe the baseline states of the bacteria and algae in brown-water rivers and to

discuss these against a background of physicochemical factors.

Such studies do much to enlarge our base of knowledge about brown-water, muskeg rivers about which relatively little is known. Such an understanding is fundamental to predicting the response of such rivers to natural and man-made disturbances resulting from oil sands development.

In this study, we determined the numbers of bacteria and algae by direct counting, the biomass of algae as indicated by chlorophyll *a* levels and an indicator of total living biomass, adenosine tri-phosphate (ATP). The latter biomass indicator responds to all organisms that are living and thus contain a pool of ATP, i.e. bacteria, algae, fungi, protozoa, micro-invertebrates and macroinvertebrates.

2.2 MATERIALS AND METHODS

Sites were located on the Muskeg River, 10 km from the mouth, the Steepbank River, 1 km from the mouth, and Hartley Creek, 5.8 km from the confluence with the Muskeg River (Figure 1).

Two water samples were taken, one aseptically for bacterial enumeration (250 mℓ) and one for chlorophyll *a* determinations (1 ℓ). The sample for chlorophyll *a* was filtered through a Gelman E fibreglass filter and magnesium carbonate sprinkled over the retained material. The filter was folded, wrapped in aluminum foil, and upon return to the field laboratory (Figure 1), was frozen at -18°C. Determinations of chlorophyll *a* were carried out on the frozen material at the main laboratory in Edmonton, usually within two to three weeks of collection. The chlorophyll was extracted using 15 mℓ of 90% acetone, homogenized for 30 s with a Brinkmann Polytron PCU-2 homogenizer at maximum set-

ting and allowed to stand in the dark at -18°C for 24 hours. Chlorophyll *a* was determined using the methods of Moss (1967a, 1967b) which incorporate a correction for phaeophytin.

Immediately after collection of the samples for bacterial enumeration, five standard 3 mℓ subsamples (Jones and Simon 1975) were filtered to dryness through a sterile, 25 mm, 0.2 µm Nuclepore filter using a sterile Millipore filtration apparatus. Each filter was then lightly attached to a Millipore media pad in a sterile Petri dish by two small spots of rubber cement applied to the periphery. Two to three drops of 0.5% glutaraldehyde on a small ball of cotton wool were added to fix and preserve each sample. On return to the laboratory, the filters were stained with 0.01% Acridine Orange in potassium phosphate buffer for 2 min and then destained with iso-propanol in a Millipore filtration apparatus. The stained membranes were air dried, mounted on a glass slide containing a drop of immersion oil (Cargille Laboratories) and covered with a coverslip (Geesey and Costerton 1978). The membranes were viewed with a Leitz Ortholux II microscope fitted with a Fluorescence vertical illuminator with a flat-field objective at a magnification of 1250X. Illumination was provided by an Osram ultra high pressure mercury lamp (200W/4) with an excitation filter setting of 5 and a suppression filter setting of 3. Bacteria fluorescing orange or green were counted in 10 different 0.0047 mm^2 fields. In general, 20 - 100 cells were counted in each field (Jones and Simon 1975).

To enable standardized comparisons of the attached communities to be made between the three rivers, we elected to produce slabs of natural rock and allow these to become colonized with attached organisms. Initial attempts to produce slabs from the limestone of

the study area failed because of its highly fractured nature thus we turned to granite which was found in small quantities in all three rivers. For the substrates, discs were made by coring a large granite boulder then slicing the core into 1 cm thick discs, 15 cm in diameter. These discs were standardized natural substrates which were allowed to colonize naturally on the bed of the stream, thus ensuring continuity with the existing benthic communities and exposing them to the flow regime associated with the river bed. Twenty-four discs were placed into the Muskeg and Steepbank rivers and nine into Hartley Creek on 15 May 1978. The discs were placed in a block formation in the mid part of the channel. Colonization was allowed to continue for two months prior to sampling the discs in the Muskeg River and Hartley Creek, and three months in the Steepbank River (Eichenberger and Wuhrmann 1975). Sampling of the epilithon prior to this was by using slabs of a fine-grained naturally-occurring red sandstone at each of the three sampling sites.

Enumeration of bacteria attached to the upper surfaces of the rock was based upon the method of Geesey and Costerton (1978) and by removing 5 replicate 4 cm^2 areas of epilithon within the bounds of template with a sterile scalpel. Five separate discs were randomly chosen on each sampling date. Detached material was transferred to sterile vials containing 10 mℓ of freshly prepared sterile 0.5% Glutaraldehyde (EM grade, Ladd Industries) in 0.067 m Cacodylate buffer (Sigma Chemical Company) equilibrated to river temperature. On return to the laboratory, bacteria were dispersed by blending the sample for 30 s at setting 5 on a Brinkmann Polytron PCU-2 homogenizer using a PT-10 generator. Initial tests had demonstrated that this was the shortest time needed to obtain a maximum dispersion of the bacteria. Clean-

ing of the probe between samples was achieved by running the generator at maximum speed in 10 mℓ of cacodylate buffer for 90 s. Following appropriate dilution, a standard volume (Jones and Simon 1975) of 3 mℓ of the suspension was filtered onto sterile 0.2 µm Nuclepore filters and stained and viewed as above. All the solutions had been filter-sterilized and, in the case of the buffers, autoclaved. Periodic checks for contamination were done at all stages of the analyses and these yielded negative results.

Determination of ATP biomass involved the on-site aseptic removal of 4 cm^2 area of epilithon from five separate rocks and immediate extraction of ATP in 4 mℓ of boiling Tris [Tris - (hydroxymethyl) aminomethane] buffer (Holm-Hansen and Booth 1966). The samples were frozen on return to the field laboratory, approximately one hour later. ATP was determined using the Luciferin - Luciferase assay (Holm-Hansen and Booth 1966). Approximately 12 hours prior to assaying for ATP, a crude freeze-dried extract of Luciferin - Luciferase from firefly lanterns (Sigma FLE-50) was reconstituted with water and held at 5°C. Immediately prior to assaying, the samples were thawed, centrifuged at 15,000 RPM for 5 min and then 0.5 mℓ of the supernatent was added to 2.0 mℓ of arsenate buffer (50 mM sodium arsenate, 5 mM potassium phosphate, 5 mM magnesium chloride - pH 7.8). The reaction was initiated by the addition of 0.1 mℓ of the firefly lantern extract, vortexed for 5 s and the photon emission counted exactly 60 s later over a 0.1 minute period in an Isocap 300 scintillation counter set for tritium with the photo multiplier tube "out of coincidence" (Stanley and Williams 1969). All solutions were equilibrated to 0°C in an ice-bath. A standard curve was produced each time using equine muscle ATP (Sigma FF-ATP). All glassware

was acid-washed before use and rinsed three times with distilled water.

Chlorophyll *a* was determined by scraping 4 cm^2 areas of epilithon from five separate rocks from each depth and transferring these to vials containing filtered river water. The contents were filtered through a Gelman E fibreglass filter and magnesium carbonate was sprinkled onto the retained material. The filters were then folded and wrapped in aluminum foil and frozen on return to the field laboratory. Pigments were extracted and chlorophyll *a* concentration were determined as above.

Epilithon for direct counts of algae was obtained by scraping five replicate 4 cm^2 areas using a scalpel and template. The material was transferred to a vial containing 10 mℓ of distilled water plus seven drops of Lugols iodine as a preservative. Algae was identified and counted using the Utermöhl technique (Utermöhl 1958).

Additional information on discharge, water quality and temperature of the three rivers was provided by R. Seidner, Water Quality Control Branch, Edmonton (Pers. comm. and In prep.).

2.3 RESULTS

The three rivers were sampled on a monthly basis with more frequent sampling taking place during the open water season. On Hartley Creek and the Steepbank River, it was possible to sample from May through to December 1977, after which time the rivers had frozen to the bottom at the sampling sites. In January, free water was found at the Steepbank River and it was possible to get water samples for analysis of the suspended component of the microbial community. In contrast, it was feasible to obtain a complete monthly set of epilithon and suspended samples at the Muskeg

River through to March.

The seasonal dynamics of the epilithon, the attached communities, will be considered first, and in this regard, the Muskeg River and Hartley Creek are most similar with the Steepbank River being generally similar to them both. The bacteria of the Muskeg River and Hartley Creek increased after ice break-up to a maximum of around 1 - 2 x 10^8 cm^{-2} in July, but then fell dramatically to around 10^7 bacteria cm^{-2} by the middle of August. Populations then stabilized for 2-3 weeks, but began to rise again to reach a maximum in late October of 1.3 x 10^8 cm^{-2} at the Muskeg River and 1 x 10^8 cm^{-2} at Hartley Creek, the last value prior to the freezing of the substratum in December (Figure 2, Tables 1 and 2). The Steepbank River epilithon bacteria rose to a very abrupt peak in mid-July of 1.6 x 10^8 cm^{-2}, then fell rapidly to 8.6 x 10^6 at the end of August, after which it increased to only 1.4 x 10^7 by the end of October (Figure 4, Table 3). After the early winter peak of bacteria in October, the populations appeared to decline, thus the bacterial population curves seem to be essentially bi-modal, with peaks occurring in early summer and probably early winter reaching a minimum in mid-summer (Figures 2 - 4, Tables 1 - 3).

The seasonal dynamics of algal biomass as determined by chlorophyll *a* followed a similar pattern to that of the bacteria. In the Muskeg River and Hartley Creek, chlorophyll *a* reached a peak in July of 8.4 and 3.3 ng cm^{-2}, respectively (Figures 2 and 3, Tables 1 and 2). This was followed by a marked decline from mid-July to mid-August to around 0.2 ng cm^{-2}. In September, the algal biomass began to rise again to reach a peak in December of 22.6 ng cm^{-2} on the Muskeg River and 3.0 ng cm^{-2} on Hartley Creek, the last sample which could be obtained prior to the freezing of the

Table 1. Epilithic and suspended microbial biomass determinations on the Muskeg River.

Date	Epilithic ℓc			Suspended ℓc	
	Bacteria [a] (Number cm^{-2})	Chlorophyll *a* [b] ($\mu g\ cm^{-2}$)	ATP [b] ($ng\ cm^{-2}$)	Bacteria [a] (Number ml^{-1})	Chlorophyll *a* [b] ($\mu g\ \ell^{-1}$)
2 May , 1977	$1.1 \pm 0.2 \times 10^8$	2.1 ± 0.3	-	$2.6 \pm 0.3 \times 10^5$	1.0
5 June, 1977	$1.1 \pm 0.1 \times 10^8$	1.5 ± 0.2	82.6 ± 14.8	$1.2 \pm 0.2 \times 10^6$	2.9
21 June, 1977	$1.3 \pm 0.1 \times 10^8$	1.6 ± 0.3	4.7 ± 1.5	$1.2 \pm 0.1 \times 10^6$	1.7
8 July, 1977	$2.3 \pm 0.2 \times 10^8$	8.4 ± 0.9	1.6 ± 0.1	$1.2 \pm 0.2 \times 10^6$	1.0
15 August, 1977	$1.1 \pm 0.1 \times 10^7$	0.2 ± 0	8.7 ± 2.5	$1.0 \pm 0.1 \times 10^6$	1.1
31 August, 1977	$8.5 \pm 1.5 \times 10^6$	-	9.4 ± 2.1	$6.5 \pm 0.7 \times 10^5$	-
20 September, 1977	$3.9 \pm 0.6 \times 10^7$	0.4 ± 0.1	5.0 ± 1.6	$1.1 \pm 0.1 \times 10^6$	1.7
29 October, 1977	$1.3 \pm 0.1 \times 10^8$	20.1 ± 1.0	25.4 ± 4.7	$2.0 \pm 0.2 \times 10^5$	0.1
8 December, 1977	$1.2 \pm 0.1 \times 10^8$	22.6 ± 2.4	5.4 ± 1.6	$8.6 \pm 0.5 \times 10^5$	<0.1
20 January , 1978	$4.5 \pm 0.6 \times 10^7$	0.8 ± 0.1	[c]	$7.8 \pm 0.5 \times 10^6$	<0.1
10 February, 1978	$4.1 \pm 0.6 \times 10^7$	0.4 ± 0.1	[c]	$1.6 \pm 0.1 \times 10^6$	[c]
19 March, 1978	[c]	[c]	[c]	[c]	[c]

[a] Geometric mean with 95% confidence limits (Elliot 1971)

[b] Mean ± 1 S.E.

[c] not analysed

Table 2. Epilithic and suspended microbial biomass determinations on Hartley Creek.

	Epilithic ℓc			Suspended ℓc	
	Bacteria[a] (Number cm^{-2})	Chlorophyll a[b] ($\mu g\ cm^{-2}$)	ATP[b] ($ng\ cm^{-2}$)	Bacteria[a] (Number ml^{-1})	Chlorophyll a[b] ($\mu g\ \ell^{-1}$)
13 May, 1977	$2.70 \pm 0.5 \times 10^{7}$	0.8 ± 0.6	100	$9.17 \pm 1.3 \times 10^{5}$	1.2
6 June, 1977	$9.76 \pm 1.3 \times 10^{7}$	1.1 ± 0.2	44.41 ± 14.97	$7.21 \pm 0.7 \times 10^{5}$	0.4
23 June, 1977	$1.40 \pm 0.1 \times 10^{8}$	1.9 ± 0.5	2.39 ± 0.49	$5.66 \pm 1.3 \times 10^{5}$	0.8
13 July, 1977	$1.36 \pm 0.1 \times 10^{8}$	3.3 ± 0.5	1.93 ± 0.11	$9.80 \pm 0.2 \times 10^{5}$	0.9
16 August, 1977	$6.84 \pm 1.3 \times 10^{6}$	0.2 ± 0.1	16.44 ± 4.49	$9.94 \pm 0.5 \times 10^{5}$	2.0
30 August, 1977	$7.69 \pm 1.3 \times 10^{6}$	<0.1	4.37 ± 0.69	$1.08 \pm 0.1 \times 10^{6}$	<0.1
22 September, 1977	$1.43 \pm 0.2 \times 10^{7}$	0.1 ± 0	6.30 ± 0.32	$6.28 \pm 0.7 \times 10^{5}$	<0.1
27 October, 1977	$2.18 \pm 0.2 \times 10^{7}$	0.8 ± 0.1	28.85 ± 1.01	$1.96 \pm 0.1 \times 10^{5}$	0.8
8 December, 1977	$1.01 \pm 0.5 \times 10^{8}$	3.0 ± 0.1	~ 10 - 13	$5.08 \pm 0.5 \times 10^{5}$	c
January, 1978	Frozen - in	Frozen - in	Frozen - in	Frozen - in	Frozen - in
February, 1978	Frozen - in	Frozen - in	Frozen - in	Frozen - in	Frozen - in
March, 1978	Frozen - in	Frozen - in	Frozen - in	Frozen - in	Frozen - in

a Geometric mean with 95% confidence limits (Elliot 1971)

b Mean ±1 S.E.

c Not analysed.

Table 3. Epilithic and suspended microbial biomass determinations on the Steepbank River.

Date	Epilithic ℓc			Suspended ℓc	
	Bacteria [a] (Number cm^{-2})	Chlorophyll *a* [b] ($\mu g\ cm^{-2}$)	ATP [b] ($ng\ cm^{-2}$)	Bacteria [a] (Number ml^{-1})	Chlorophyll *a* [b] ($\mu g\ \ell^{-1}$)
12 May, 1977	-	-	-	$1.0 \pm 0.1 \times 10^5$	1.2
1 June, 1977	$5.4 \pm 0.7 \times 10^6$	0.3 ± 0.1	11.3 ± 4.1	$2.1 \pm 0.5 \times 10^4$	1.0
22 June, 1977	$9.7 \pm 0.4 \times 10^6$	<0.1	2.9 ± 0.4	$4.3 \pm 0.4 \times 10^5$	1.9
13 July, 1977	$1.6 \pm 0.1 \times 10^8$	9.6 ± 0.8	3.0 ± 0.6	$8.2 \pm 1.2 \times 10^5$	4.8
17 August, 1977	$1.3 \pm 0.4 \times 10^7$	0.1 ± 0	5.1 ± 0.4	$1.3 \pm 0.1 \times 10^6$	0.7
31 August, 1977	$8.6 \pm 1.4 \times 10^6$	-	4.5 ± 0.4	$8.9 \pm 0.7 \times 10^5$	-
23 September, 1977	$1.3 \pm 0.2 \times 10^7$	0.1 ± 0	3.5 ± 0.3	$4.4 \pm 0.6 \times 10^5$	1.8
28 October, 1977	$1.4 \pm 0.1 \times 10^7$	0.3 ± 0.1	24.8 ± 4.7	$1.7 \pm 0.3 \times 10^5$	c
12 December, 1977	$7.0 \pm 1.2 \times 10^6$	0.1 ± 0	5.9 ± 0.9	$4.5 \pm 0.4 \times 10^5$	c
1 January, 1978	Frozen - in	Frozen - in	Frozen - in	$5.6 \pm 0.4 \times 10^5$	<0.1
10 February, 1978	Frozen - in	Frozen - in	Frozen - in	Frozen - in	Frozen - in
19 March, 1978	Frozen - in	Frozen - in	Frozen - in	c	c

a Geometric mean with 95% confidence limits (Elliot 1971)

b Mean ± 1 S.E.

c Not analysed.

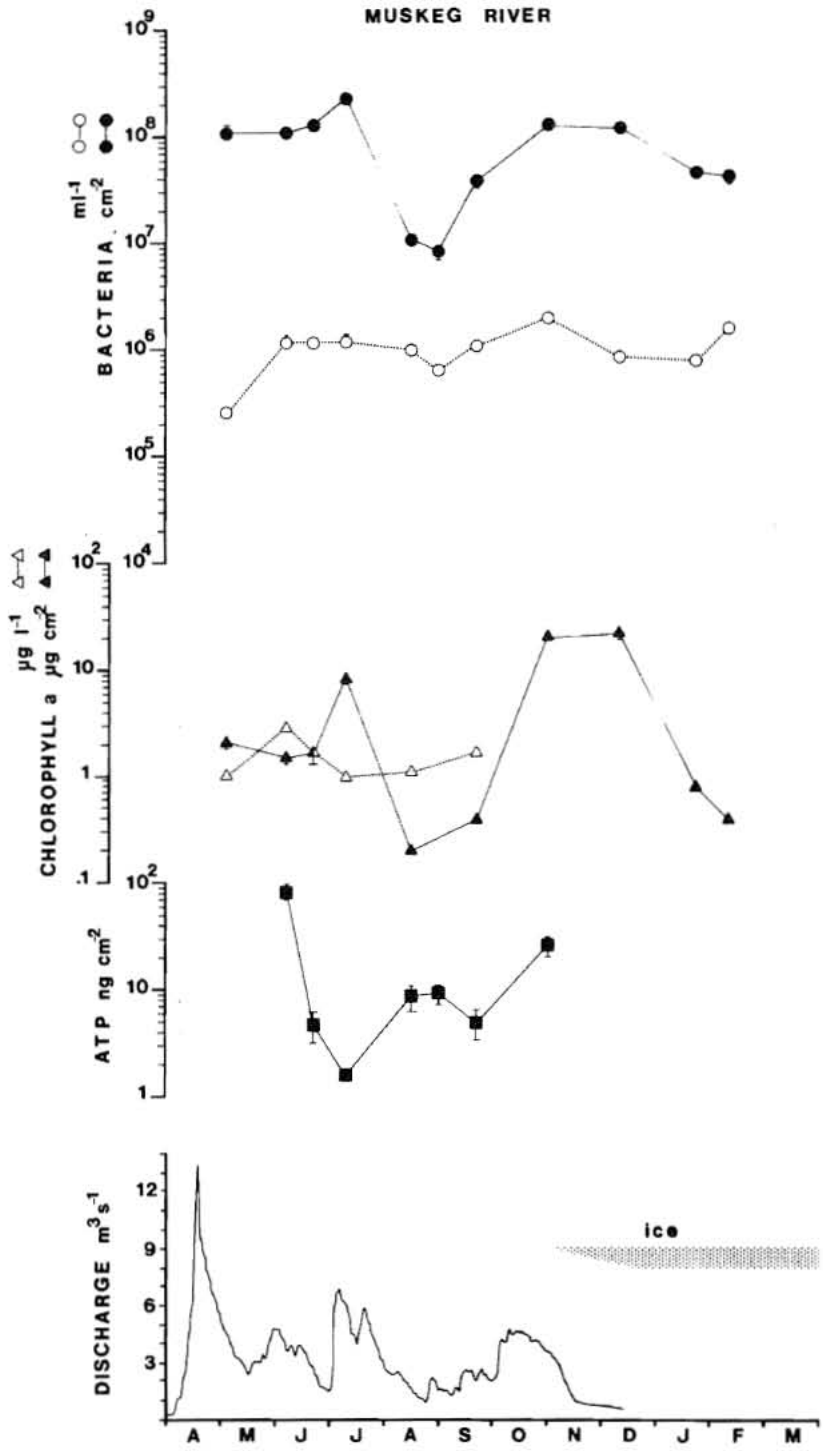

Figure 2. Epilithic and suspended microbial biomass determinations and discharge measurements for the Muskeg River.

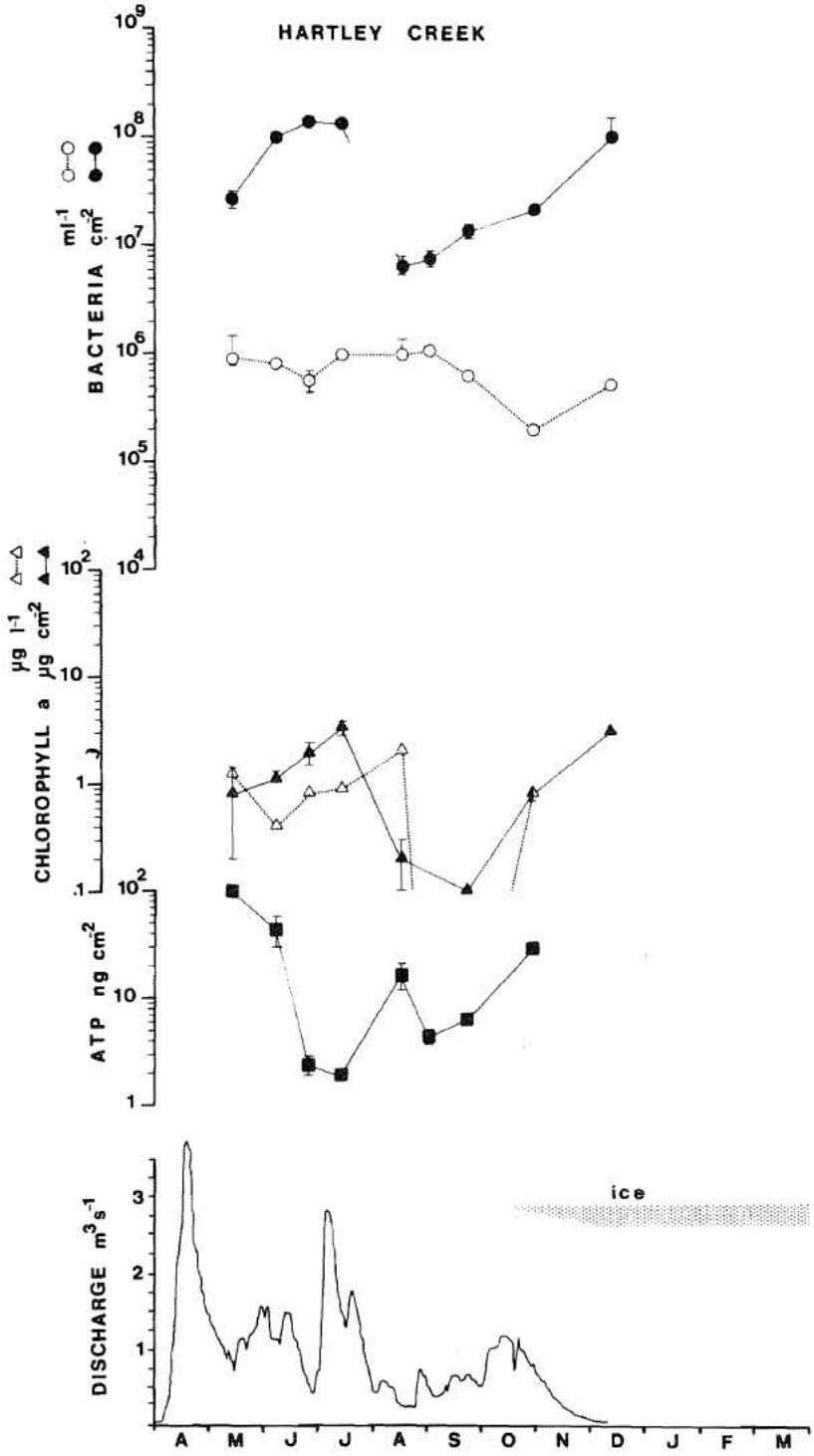

Figure 3. Epilithic and suspended microbial biomass determinations and discharge measurements for Hartley Creek.

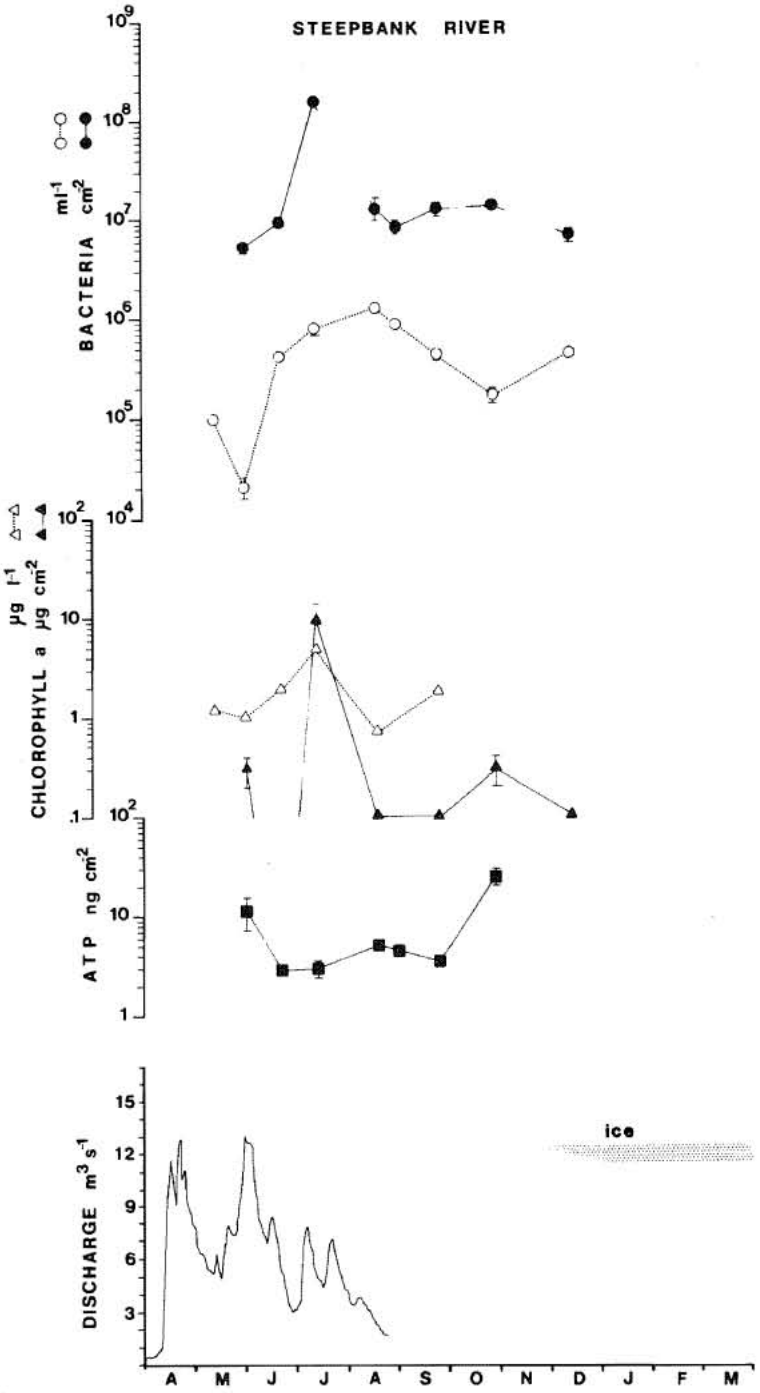

Figure 4. Epilithic and suspended microbial biomass determinations and discharge measurements for the Steepbank River.

substrate. Levels of chlorophyll *a* in the Steepbank River were generally low (0.2 μg cm^{-2}), however, a large peak in July was evident with a much smaller peak occurring in October (Figure 4, Table 3).

The seasonal curves of ATP, an indicator of living biomass, were similar in each of the three rivers (Figures 2 - 4, Tables 1 - 3), however, the pattern was very much different from those generated by the bacteria and algae. The ATP levels started out relatively high, just after ice-out, and then declined until the end of June followed by an increase which lasted until mid-August. After the peak in August, the ATP levels dropped slightly, reaching a low at the end of August of around 5 ng cm^{-2}. This was followed by a steep rise to around 25-30 ng cm^{-2} by the end of October.

In order to determine if there was a relationship between attached bacteria algae and ATP, we conducted a correlation analysis (Bailey 1959) of this biomass data. Each river exhibited a highly significant ($p < 0.01 - 0.001$) positive correlation between algal biomass and bacterial numbers cm^{-2} (Table 4). The same analysis for algal biomass/ATP and bacterial numbers/ATP revealed that there was no significant correlation between these parameters (Table 4).

Examination of the data on suspended bacteria and algae reveals no particularly consistent seasonal trends between the three rivers. The numbers of bacteria generally ranged from 10^5 - 10^6 $m\ell^{-1}$ with the only consistent feature of the curves being a drop in numbers during October, after which time they proceeded to rise. The levels of chlorophyll *a* in the three rivers were generally between 0.5 - 2.0 ng ℓ^{-1}, with no consistent trends. It is appropriate at this point to consider the possible origins of the suspended bacteria and algae. A reasonable assumption is that suspended algae and prob-

Table 4. Correlation coefficient values (r) between the 3 epilithon components, number of bacteria cm^{-2}, chlorophyll *a* cm^{-2} and ATP cm^{-2}, of the Muskeg River, Steepbank River and Hartley Creek.

Epilithon Component	Muskeg River		Steepbank River		Hartley Creek	
	r	df	r	df	r	df
Bacteria/Chlorophyll *a*	+0.914[a]	8	+0.994[b]	4	+0.845[a]	6
Bacteria/ATP	+0.038[c]	6	-0.207[c]	6	-0.231[c]	7
Chlorophyll *a*/ATP	-0.210[c]	5	-0.329[c]	4	-0.336[c]	6

[a] $p<0.01$

[b] $p<0.001$

[c] not significant

ably bacteria, are derived from the epilithon by detachment, and in Section 2.2, it is shown that the suspended algae are indeed largely attached forms. Given that detachment is the principle mode by which the epilithic forms of bacteria and algae get into the water column, the rate of detachment could be dependent upon the discharge of the river and/or the biomass of the epilithon. This hypothesis was tested by a correlation analysis (Bailey 1959) examining the association between suspended algal/bacterial biomass and river discharge on the day of sampling (Table 5), and the algal/bacterial biomass of the epilithon on that day. Visual inspection of the biomass data for the three rivers revealed no obvious link between discharge or epilithic biomass, and this is borne out by the correlation analysis (Table 6). There was no significant positive or negative correlation between the parameters tested except between the suspended algal biomass and the epilithic biomass in Hartley Creek. This produced an r value of +0.967 which, with 2 degrees of freedom, was only significant at the 95% level.

Assuming that the benthic bacteria and algae are supplying the suspended microorganisms to the river, this is apparently being done at a rate independent of discharge (i.e. high discharges do not bring more microorganisms into suspension), and generally independent of the epilithic biomass (i.e. at times of high attached biomass, greater numbers of microorganisms do not become suspended). It therefore appears that the numbers of suspended bacteria and algae are being regulated by factors as yet undetermined.

The water quality data available for the three rivers are presented in Tables 7 - 9, and Figures 5 - 7, and temperature data for the three rivers are presented in Figure 8.

Table 5. Discharge on day of sampling for the Muskeg River, Steepbank River and Hartley Creek, $m^3 s^{-1}$ (R. Seidner, Water Quality Control Branch, Edmonton, Pers. Comm.).

Month	Muskeg River	Steepbank River	Hartley Creek
May	5.04	3.20	0.97
June	4.56	4.79	1.24
June	2.92	2.81	0.86
July	6.51	5.04	1.56
August	1.76	1.50	0.28
August	1.60	-	0.52
September	2.63	-	0.52
October	3.68	-	0.78
December	0.59	-	0.02

Table 6. Corellation coefficient values(r) between suspended bacteria and algae versus discharge on the day of sampling and corresponding biomass in the epilithon.

	Muskeg River		Steepbank River		Hartley Creek	
	r	df	r	df	r	df
Discharge/Bacteria	-0.086[b]	4	+0.229[b]	3	-0.716[b]	4
Discharge/Chlorophyll *a*	+0.047[b]	7	-0.318[b]	3	+0.177[b]	7
Epilithon bacteria/ Suspended bacteria	-0.029[b]	9	-0.130[b]	7	+0.264[b]	6
Epilithon Chlorophyll *a*/ Suspended Chlorophyll *a*	-0.339[b]	4	-0.433[b]	4	+0.967[a]	2

[a] $p<0.05$

[b] Not Significant

Table 7. Water quality data for the Muskeg River over the study period April 1977 - November 1977, mg ℓ^{-1}

Parameter	Month							
	April 18	May 16	June 20	July 17	Aug 16	Sept 13	Oct	Nov 8
Total filterable residue	80	139	150	192	176	277	184	205
pH	7.5	7.9	8.1	7.8	8.0	8.0	8.1	7.7
Conductivity	126	220	250	320	294	420	295	310
Total organic phosphorus	0.07	0.03	0.03	0.03	0.02	0.02	0.04	0.02
PO_4 - P	0.02	<0.01	0.006	0.006	0.006	<0.003	0.006	0.004
Total organic nitrogen	0.86	1.07	1.26	0.98	1.55	0.66	1.40	0.87
$NO_3 + NO_2$ - N	<0.01	<0.01	0.028	0.014	0.003	0.004	0.100	0.050
NH_4 - N	0.05	<0.01	0.03	0.01	0.39	0.02	0.07	0.09
Dissolved organic carbon	0.08	34.0	22.5	24.5	20.0	19.5	29.0	18.5
Tannin + Lignins	1.2	1.50	1.45	1.60	1.75	1.50	1.70	1.00
C/N ratio	9.3	31.8	17.9	25.0	12.9	29.5	20.7	21.3

Table 8. Water quality data for Hartley Creek over the study period April 1977 - March 1978, mg ℓ^{-1}

Parameter	April	May	June	July	Aug	Sept	Oct	Nov
Total filterable residue	74	117	112	118	138	141	140	135
pH	7.3	8.3	7.9	7.7	8.0	7.8	7.9	7.8
Conductivity	115	175	190	196	230	214	214	205
Total organic phosphorus	0.05	0.03	0.01	0.02	0.02	0.01	0.02	0.002
PO_4 - P	0.01	<0.01	0.011	0.006	0.008	0.004	0.007	0.004
Total organic nitrogen	0.81	0.93	0.80	0.98	1.47	0.65	0.72	0.94
$NO_2 + NO_2$ - N	<0.01	<0.01	0.024	0.02	0.003	0.004	0.005	0.023
NH_4 - N	0.04	0.09	0.01	<0.01	0.13	0.03	0.04	0.16
Dissolved organic carbon	12.0	28.0	11.5	27.0	23.5	22.0	28.5	20.0
Tannins + Lignins	0.5	1.4	1.6	1.6	1.6	1.3	1.4	1.0
C/N ratio	14.8	30.1	14.4	27.6	16.0	33.8	39.6	21.2

Table 9. Water quality data for the Steepbank River over the study period April 1977 - March 1978, mg ℓ^{-1}

Parameter	Month							
	April 18	May 18	June 20	July 13	Aug 15	Sept 13	Oct 12	Nov 7
Total Filterable residue	69	107	86	116	138	165	146	169
pH	7.0	7.6	8.2	7.8	8.0	8.1	8.1	8.0
Conductivity	110	172	159	193	230	250	222	256
Total organic phosphorus	0.15	0.06	0.03	0.04	0.02	0.02	0.04	0.02
PO_4 - P	0.02	<0.01	0.007	0.007	0.011	0.006	0.009	0.006
Total organic nitrogen	0.77	0.76	0.93	1.29	1.86	1.65	1.41	0.66
$NO_3 + NO_2$ - N	<0.01	<0.01	0.003	0.009	0.074	0.014	0.004	0.04
NH_4 - N	0.04	0.01	0.03	0.01	0.17	0.02	0.03	0.02
Dissolved organic carbon	11.0	23.0	15.5	22.0	18.0	14.5	28.0	14.5
Tannins + Lignins	1.7	1.8	1.9	1.6	1.2	2.0	1.2	0.9
C/N ratio	14.3	30.3	16.7	17.1	9.7	8.8	19.9	22.0

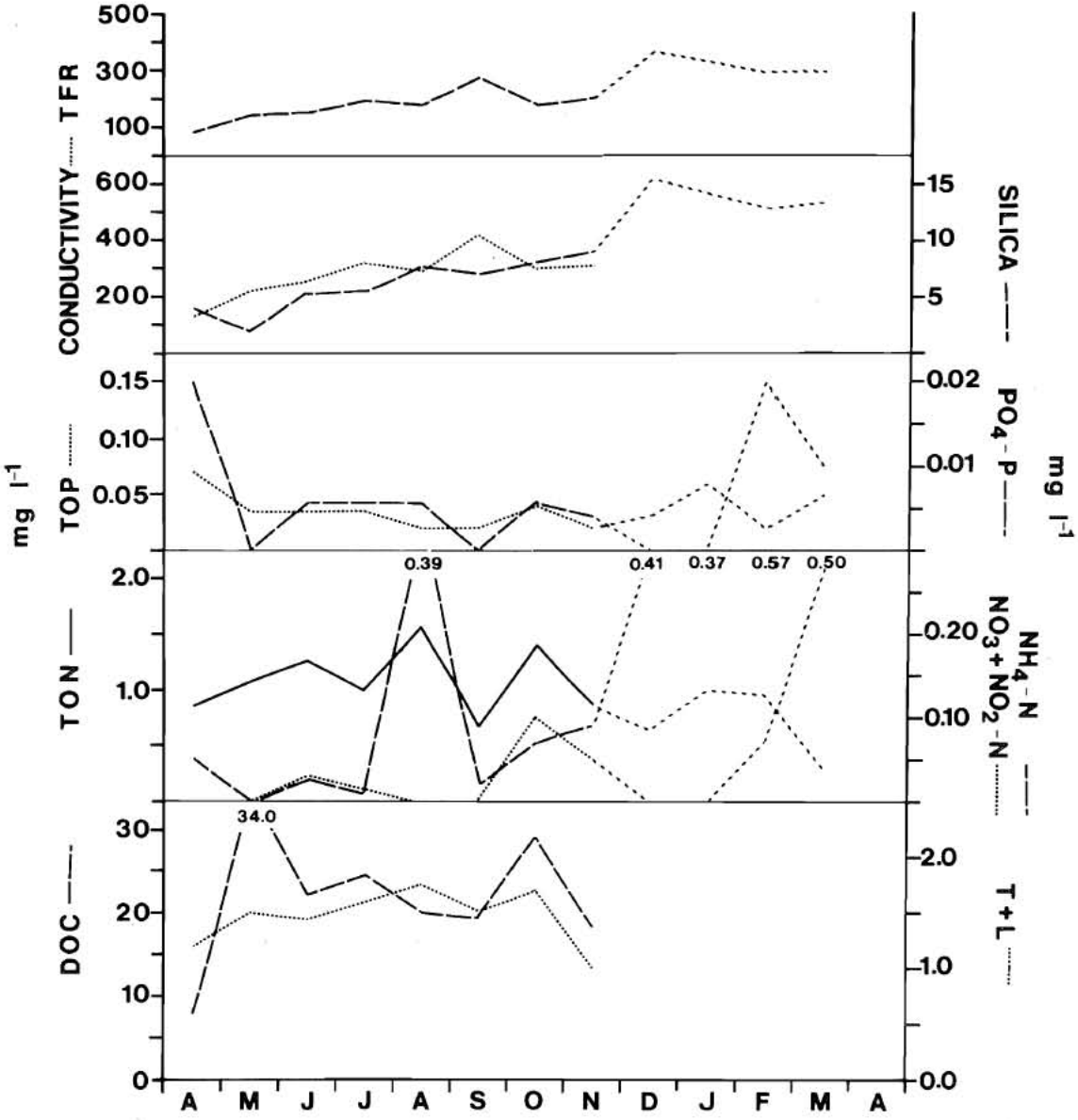

Figure 5. Water quality data for the Muskeg River over the study period.

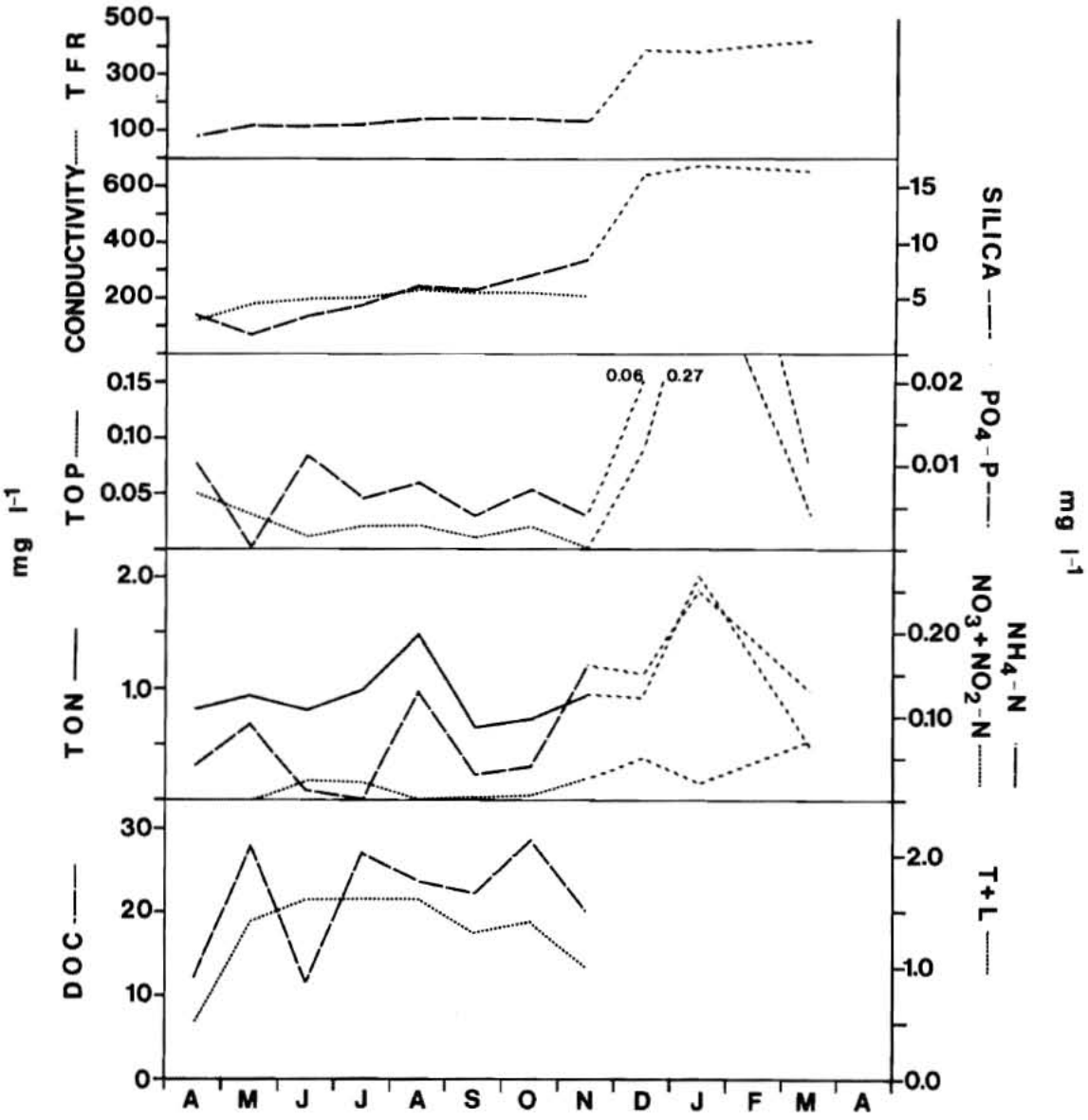

Figure 6. Water quality data for Hartley Creek over the study period.

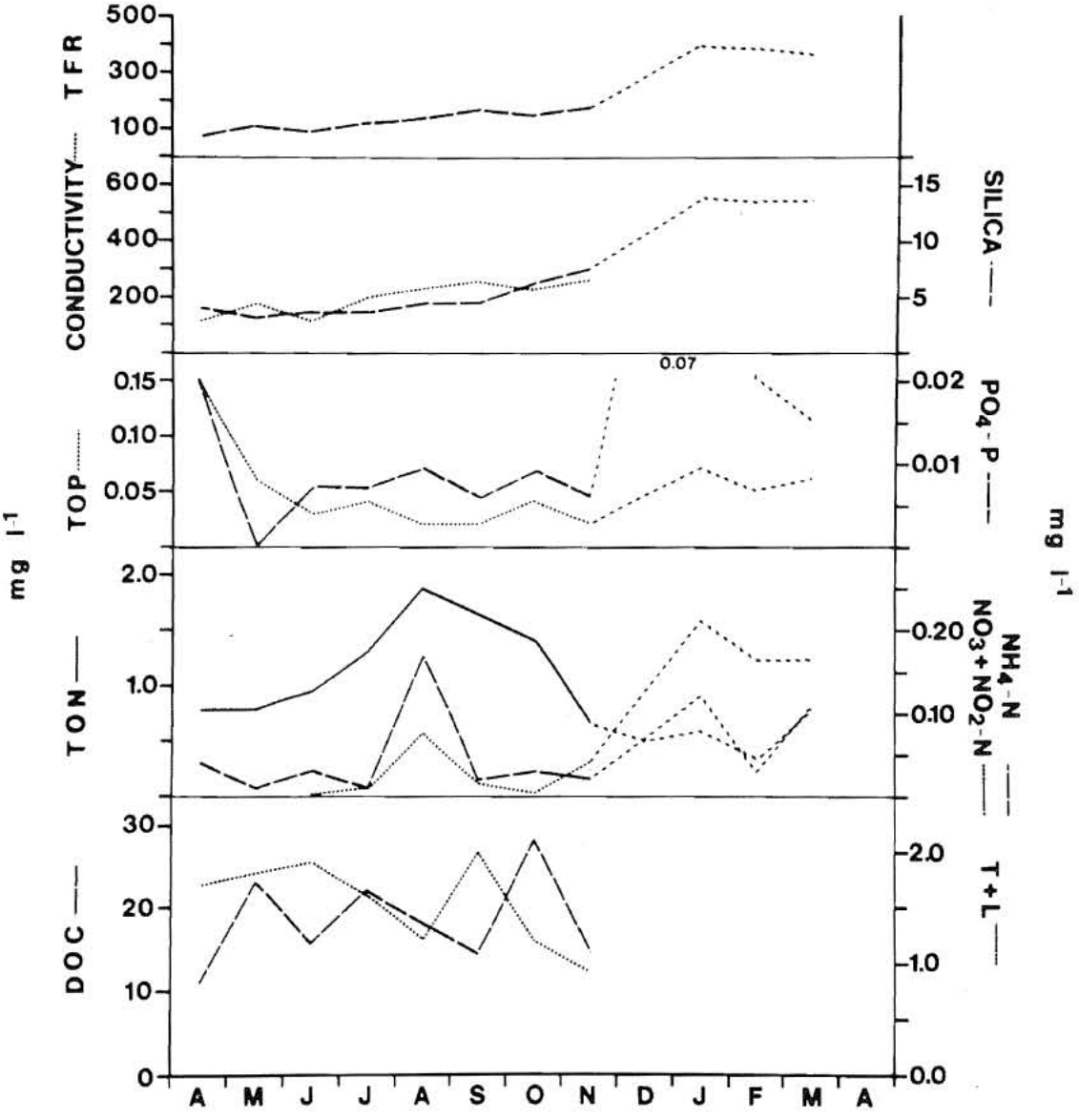

Figure 7. Water quality data for the Steepbank River over the study period.

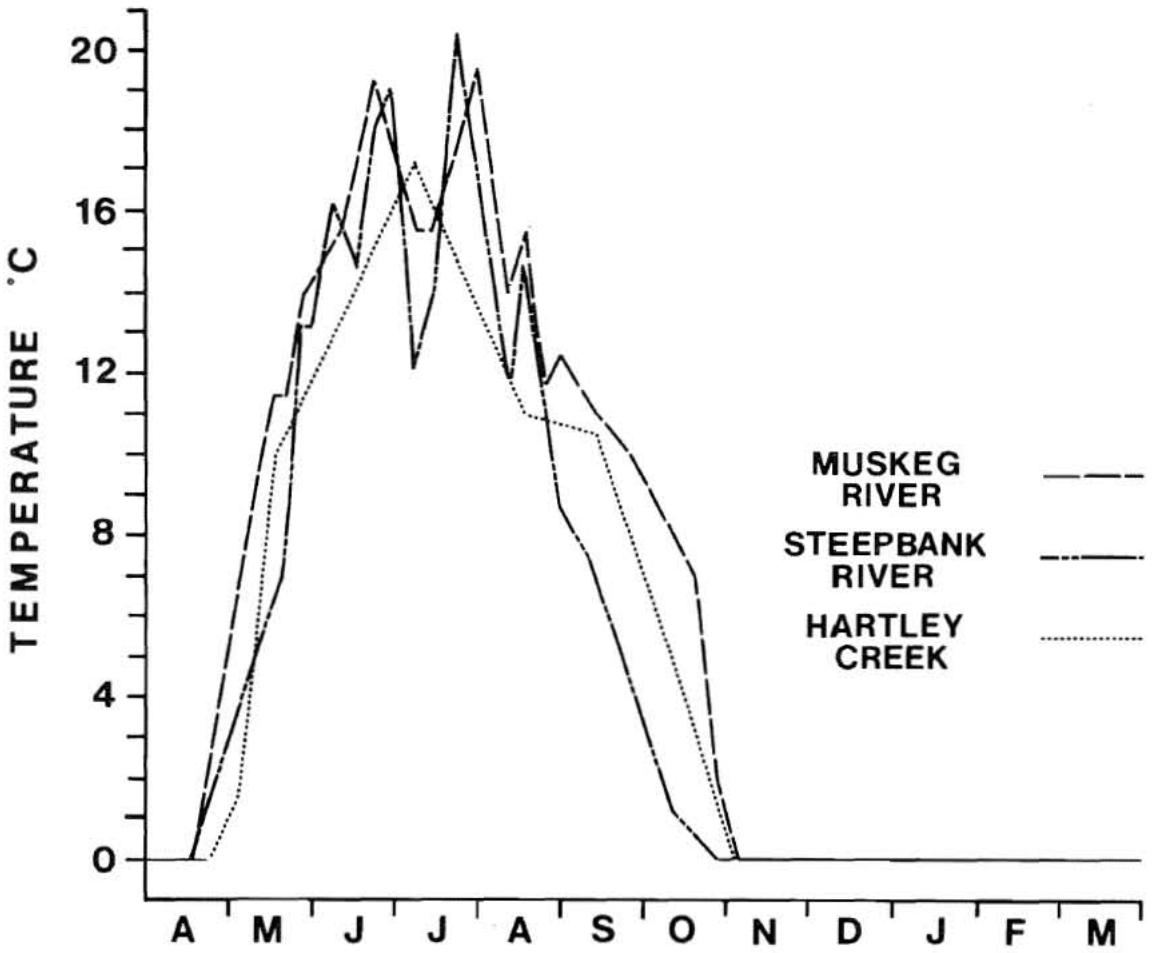

Figure 8. Temperature data for the Muskeg and Steepbank rivers and Hartley Creek over the study period.

2.4 DISCUSSION

The suspended and epilithic bacterial and algal population dynamics will now be discussed with relation to the chemical composition of the waters. Since the suspended bacteria are in intimate contact with the organic and inorganic components of the river water, these will be considered first.

Little correspondence between increasing levels of dissolved organic carbon (DOC) and increasing numbers of suspended bacteria was observed (Tables 7 - 9, Figures 5 - 7). However, there is an apparent association between an increasing concentration of total organic nitrogen (TON) and increasing bacterial numbers. This is not unexpected as the smaller the ratio of organic carbon/ organic nitrogen becomes (Tables 7 - 9), the more likely that the organic material which is present will be of a type readily utilized by bacteria (Wetzel 1975), thus permitting their increased growth. A consideration of the suspended algae reveals no obvious association between their population fluctuations and fluctuations of the major plant nutrients, phosphorus and nitrogen. However, the possibility exists that these algae are not metabolizing actively and would, therefore, not be expected to exert any signficant effect upon the water chemistry. However, this area warrants further study of both the algae and bacteria suspended in the river. A measure of the proportion of living or dead (or slowly metabolizing) cells would, in itself, give us an indication of the extent to which the suspended microbial component could exert a controlling influence on the water quality of these rivers.

Because of the very close association between population fluctuations of the epilithic bacteria and the algae, their population changes and water quality association will be considered together. However, first

it is necessary to examine the nature of this relationship. Although a significant correlation was demonstrated between population fluctuations in epilithic algae and the bacteria, that does not imply that the relationship is direct. However, it appears that there are two possible links between the algae and the bacteria, those being:

1. Epilithic algae could be responding to increased nutrient regeneration within the microbial film on the substrate as a consequence of activity of the large numbers of bacteria present in the film (for a detailed discussion of the structure of the film, see Section 3). This, of course, poses the question as to why the bacteria increase so dramatically after ice break-up. Turning again to the water quality data, we find no evidence of a massive increase in the organic or inorganic nutrients measured over the two periods, where bacterial numbers rapidly increased (early summer and early winter). Thus, if bacteria were increasing independently of the algae, then this was in response to a factor or factors as yet undetermined.
2. Epilithic bacteria respond to an increased growth of epilithic algae. This implies a utilization of extra-cellular products of algal photosynthesis including substances of high (polysaccharide matrix) and low (amino acids, sugars, etc.) molecular weight. In the case of the polysaccharide matrix, the response of bacteria could be direct, by utilization of the polysaccharide as a food source, or an indirect res-

ponse due to the ability of this matrix to concentrate organic and inorganic nutrients from the river water and make these available to the bacteria (Geesey and Costerton 1977).

In the case of this second hypothesis, an explanation for the original algal bloom and its subsequent decline is required. In this regard, an examination of the water quality data (Tables 7 - 9, Figures 5 - 7) does not reveal any massive increase in the major plant nutrients (PO_4 - P, NO_3 - NO_2 - N, and NH_4 - N) which might have stimulated algal growth. This assumes that the algae were initially nutrient limited. It is also interesting to note that the levels of silica in all studies increased steadily from ice break-up through the whole year (Tables 7 - 9, Figures 5 - 7). Thus, the possibility of silica controlling the bacillariophycean algae (Wetzel 1975) is also unlikely. Temperature (Figure 8) as a population regulator is also possible, the early summer peak of microorganisms being composed of a species assemblage adapted to warm water and the early winter peak being composed of species adapting to progressively colder conditions. The same could also be true for the effect of light intensity, the two factors, water temperature and light intensity, acting together. The subsequent mid-summer crash in the microbial populations might be due to the temperature and light optima of algae in this region being exceeded, but this hypothesis would not hold for the subsequent decline of the winter population. An additional hypothesis would be that, as the algal bacterial film increases, so also do the populations of protozoa and microinvertebrates (e.g. nematodes, oligochaetes, chironomids, etc.), and through a combination of grazing and possible mechanical damage to the integrity of the microbial film (see Section 3),

this causes it to be sloughed away by the current. This process could also be a contributing factor in the decline of the mid-summer population.

To summarize, the hypothesis favoured is that the bacterial populations are growing in response to an increasing algal population, and the subsequent collapse of the community being due in part to a breakdown of the mechanical integrity of the film, possibly brought about by burrowing and feeding activities of invertebrates inhabiting the film.

In order to present a more complete discussion of the interrelationship of biological phenomena and some aspects of the chemical fluctuations of the rivers, we have included the data from 1976-1977 as a substitute for the, as yet unavailable,data for the winter of 1977. The extrapolations of selected parameters are clearly indicated by the oblique slashed line.

In the three rivers, at the end of July, very high levels of NH_4 - N were recorded (Figures 5 - 7). This could be a result of bacterial breakdown of the debris resulting from the collapse of the bacterial-algal bloom of mid-July. The utilization of the substantial quantities of protein incorporated in the epilithon would result in large quantities of NH_3 being produced as a bacterial end-product of protein degradation (Rheinheimer 1974) which would become converted to NH_4 - N. This process probably took place in the interstices of the river bed where the sloughed off microbial film would have accumulated.

A similar phenomenon probably also took place during the winter months, except decomposition was taking place under partially anaerobic conditions (oxygen levels being below saturation values) resulting in the extremely elevated levels of both NH_4 - N and NO_3 + NO_2 - N. Depending upon the oxygen content of the water, the

NH_4 - N would be converted to nitrite and then nitrate through the bacteria *Nitrosomonas* sp. and *Nitrobacter* sp., respectively (Rheinheimer 1974). The increase in the levels of PO_4 - P is also probably due to microbially mediated processes (Rigler 1973). These relatively sudden increases in level are in contrast with the gradual increases which occur throughout the year (starting from ice break-up) in the silica content and the conductivity of the water. The latter presumably reflects the increasing proportion of the groundwater contribution to the river as base flow conditions are approached during the winter months.

In conclusion, it has been shown that the microorganisms, macroorganisms, and the chemical environment are interlinked. It is not possible to study any one in isolation from the rest. By determining how these components function as a system, we will best be able to determine the capacity of muskeg rivers to take up natural and unnatural chemical inputs.

3. POPULATION DYNAMICS AND SPECIES COMPOSITION OF ALGAE OF THE SEDIMENTS, RUBBLE, AND WATER OF TWO MUSKEG RIVERS.

Principal Researchers: S. Charlton, M.A. Lock, R.R. Wallace and M. Hickman

3.1 INTRODUCTION

Much has been written about factors that control phytoplankton dynamics, whereas attached communities have received far less attention (Round 1972). This is due to problems associated with sampling, and more particularly, with frequent large scale shifts in environmental conditions that arise in rivers (Round 1970; Jones 1974). However, in spite of these problems, studies have illustrated that benthic algae contribute

much to the standing crop and production in both lakes and rivers (Round 1964; Wetzel 1964; Moss 1967, 1968; Moss and Round 1967; Hargrave 1969; Hickman 1970, 1971a, 1971b, 1974a, 1974b, 1978a, 1978b; Hickman and Round 1970; Gruendling 1971; Kowalczewski et al. 1973; Klarer and Hickman 1975; Moore 1974a, 1974b; Marker 1976; Tai and Hodgkiss 1973, 1975). Marker (1976), Moore (1977), Cairns et al. (1970), Round (1973), and Westlake (1971) have illustrated that benthic communities are, by far, the most important primary producers in lotic systems. Direct comparisons among these various studies, however, remain nearly impossible due to the variation in sampling techniques. The most common and certainly the simpest technique employed for investigating benthic algae populations involves the use of artificial substrates, particularly glass slides. However, Seiver (1977), Tippett (1970), Wetzel (1975), Round (1970), Round and Hickman (1971) indicate that artificial substrates do not yield particularly useful results since they are selective to varying degrees and hence yield unnatural estimates of algal communities. Artificial substrates not only underestimate natural population size, but also yield unnatural shifts in species composition over both long and short-term studies. Glass slides, when placed in the lotic environment, also tend to accumulate phytoplankters and again rarely yield a truly attached community. The results obtained from such substrates do not have much ecological significance. Therefore, natural substrata were always used in this study for determinations of the natural algae populations, their fluctuations in population size, and species composition. Sampling was conducted on a weekly basis and in a quantitative manner.

The benthic algae are the most important photosynthetic primary producers in the Muskeg and Steepbank

rivers and will make for extremely valuable indicators of "water quality" (Patrick 1961, 1969). Therefore, accurate measurements of not only standing crop size and fluctuations, but also species composition and fluctuations are essential. Only through intensive studies and complete understanding of the natural algal communities can any useful predictive and/or management decisions be made.

Therefore, this study commenced in May 1977 to provide the necessary baseline information concerned with seasonal dynamics of the epilithon (that community of algae found attached to rocks), epipelon (that community of algae found on the submerged sediment), and phytoplankton of the Muskeg and Steepbank rivers.

3.2 MATERIALS AND METHODS

Samples were taken each week commencing 13 May 1977 from both the Muskeg and Steepbank rivers, at the sites used in Section 2.1, until the beginning of September when the sampling period was at approximately monthly intervals. Epilithon quadrats (400 cm^2) were placed in the river and all the material within them was removed. The quadrat was arbitrarily divided into two parts such that overlapping stones were included for sampling from one part and excluded from the other. The rocks within the quadrat were carefully removed and placed in a tray. Stones less than 1 cm in diameter were not included because such stones were observed to be easily dislodged and swept away.

The algae were removed from the rocks, almost immediately upon the latter being placed in the trays, using a stiff nylon bristle brush, as well as by scraping with a scalpel to remove any encrusting forms and placed in a known volume of water previously filtered through a Whatmann (GF/C) glass fibre filter paper to remove plank-

tonic algae. The samples were then returned to the laboratory for further processing.

A total of four stations were sampled in replicate on the Muskeg River. These included stones collected at depths of >50 cm, <10 cm, in riffle (≃8 cm) and midstream (≃26 cm). Two stations, 10 cm depth and midstream, were sampled on the Steepbank River.

In addition to the quadrat technique, precise estimates of algal standing crop were made for rock and naturally-occurring bitumen on the Steepbank River bed. Two 4 cm^2 scrapes were removed using a scalpel and some samples immediately preserved with Lugol's iodine while others were placed in containers for transportation to the laboratory.

In the laboratory, two 10 mℓ subsamples were removed from the samples obtained using the quadrat technique and preserved with Lugol's iodine for microscopic enumeration and identification. Further subsamples of known volume were also removed and filtered onto Whatmann GF/C glass filter paper for chlorophyll *a* analysis. Those had to be stored because of the primitive nature of the field laboratory (Figure 1), and therefore, each filter was buffered with anhydrous $MgCO_3$ and stored frozen.

For enumeration and identification, the inverted microscope technique of Lund, Kipling and LeCren (1958) and Utermöhl (1958) was used. Known volumes of each sample were pipetted into sedimentation chambers after thoroughly agitating the sample. The volume sedimented depended upon population size. Algae were always identified to species (for Keys used, see Appendix 1) where feasible and enumerations expressed as cells m^{-2}. Not less than 500 algae were counted per sample along transects. Indentification of diatoms was simplified by treatment with concentrated hydrogen peroxide and heat-

ing. Cleaned specimens were mounted in hyrax.

Further subsamples (10-20 mℓ) again, depending on population size, were filtered onto Whatmann GF/C filter paper for chlorophyll *a* analysis. The spectrophotometric method and equations of Moss (1967a, 1967b) were used to determine chlorophyll *a* content and results expressed as mg m^{-2} chlorophyll *a*.

Phytoplankton water was collected at one-half the actual stream depth for both chlorophyll *a* analysis, and enumeration and identification. Samples were always examined immediately before preservation in Lugol's iodine.

Epipelon quantitative samples were taken in shallow water at the edge of the Steepbank River site by pushing a perspex cylinder (internal diameter 9 cm),long enough to reach the water surface, into the sediment to delineate a known area (internal area 60 cm^2). A glass tube (Bore 0.4 - 0.5 cm) was connected to a polyethylene tube to a 500 mℓ polyethylene bottle. The glass tube was then moved over the sediment surface (top 5 mm) in order to suck up the mud surface. Algae were separated from the sediment by trapping them in lens tissue. This method removes up to 87.5% of the total population (Eaton and Moss 1966).

3.3 RESULTS

3.3.1 Epilithon

3.3.1.1 Steepbank River. Epilithic algal populations were small when the study commenced in early May, but quickly increased in size forming a late spring peak in late May/June (Figure 9). The populations then decreased in size reaching a summer minimum during July. Afterwards, the standing crop increased in size reaching a

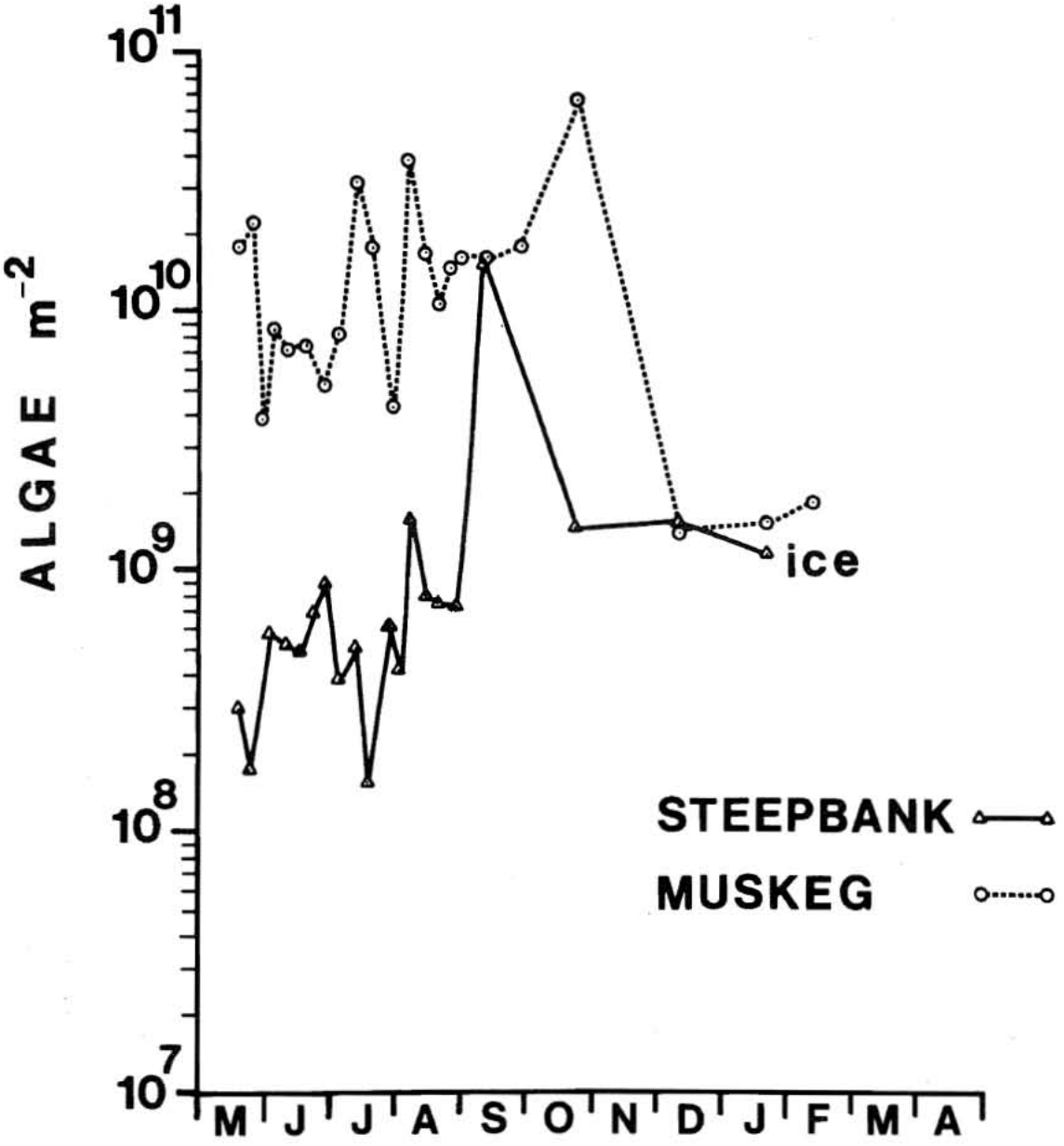

Figure 9. Total number of algae m^{-2} occurring in the Muskeg and Steepbank rivers over the study period.

maximum during October. Populations then decreased in size, but remained quite high compared with the populations encountered during the May/July period.

Cyanophycean algae were dominant throughout the entire study period followed by bacillariophycean (diatoms) and chlorophycean algae, respectively. The dominant cyanophycean species were *Phormidium*, *Lyngbya* and *Aphanocapsa* species. *Phormidium tenue* (Menegh.) Gomont, and *Lyngbya aergineo-caerulea* (Keutzing) Gomont were particularly important attached filamentous algae.

Maximum sized cyanophycean populations occurred in September just prior to the onset of rapidly declining water temperature (Figure 8, Section 2.1.3). The latter, together with the onset of winter, coincide with an increase in the numbers of diatoms present. *Synedra ulna* (Nitzsch.) Ehrenberg, *Nitzschia fonticola* (Grunow), *Achnanthes minutissima* (Keutzing), *Nivicula cryptocephala* (Keutzing), and *Gomphonema* species were the dominant diatom species occurring during the fall maximum.

Cladophora glomerata (L.) Keutzing and *Stigeoclonium pachydermum* (Prescott) were the dominant green algal species encountered in the Steepbank River during the midsummer peak of Chlorophyta. Standing crop fluctuations, determined by chlorophyll *a* content in the Steepbank River, showed a bimodal pattern (Figure 9). In addition to the major algal divisions indicated previously, three species of filamentous red algae were also encountered. The species which were most abundant were *Batrachospermum vagum* (Roth.) C.A. Agardh, and *Audouinella chalybea* (Lyngb.) Fries., and were present during spring, fall, and winter.

3.3.1.2 Muskeg River. Epilithic algal populations in the Muskeg River exceeded Steepbank benthic algal

populations throughout the study period. A decline in the total number of epilithic algae followed what was presumably a spring maximum. Epilithic populations remained high in number throughout the summer, but did exhibit a major decline during the period of maximum water temperatures (Figures 8 and 9, Section 2.1.3). A fall maximum occurred during late October, prior to ice formation. Maximum numbers of cyanophycean algae occurred during midsummer and fall with minimum numbers occurring during June-August. The dominant species were *Phormidium tenue* (Menegh.) Gomont, *Lyngbya aergineo-caerulea* (Keutzing) Gomont, *Aphanocapsa* sp., and *Chaemo-siphon incrustans* (Grunow).

Diatoms reached maximum numbers during periods of low water temperature in spring and fall. The dominant species were *Synedra ulna* (Nitzsch.) Ehrenberg, *Nitzschia fonticola* Grunow, *Achnanthes minutissima* Keutzing, and *Gomphonema olivaceum* (Lyngb.) Keutzing. Figure 9 clearly illustrates that maximum chlorophyll *a* content occurred in the Muskeg River during the fall period. Chlorophycean algal numbers fluctuated throughout the study period. Maximum numbers occurred during the months of June and July (Figure 9). The dominant species were *Draparnaldia plumosa* (Vauch.) C.A. Agardh, *D. acuta* (C.A. Agardy) Keutzing, *Cladophora glomerata* (L.) Keutzing, and *Ulothrix* species. Red algae, *Audouinella* sp. and *Batrachospermum* sp. were also observed to occur periodically in the Muskeg River, however, this algae was never dominant. *Batrachospermum vagum* (Roth) C.A. Agardh was observed during spring and fall. *Audouinella* sp. became particularly abundant during the winter period when ice covered the study area.

3.3.2 Phytoplankton

3.3.2.1 Steepbank River. The seasonal variation in the total chlorophyll *a* content of the phytoplankton is presented in Figure 11. Preliminary analysis of the samples indicated that a greater percentage of the species occurring in the water column are species which have become detached and been swept into the flowing water. The total chlorophyll *a* of the water column on all occasions was significantly less than that of the epilithic community, however, the pattern of total chlorophyll *a* (Figure 11) often peaked simultaneously with peaks of the midstream epilithic chlorophyll *a* (Figure 10).

3.3.2.2 Muskeg River. Seasonal variation in the total chlorophyll *a* content of the phytoplankton is presented in Figure 11. As indicated for the Steepbank River, a greater percentage of the species occurring in the phytoplankton were benthic forms which had been swept into the water column. The total chlorophyll *a* of the water column reached a maximum during May and following the spring flood. Minimal chlorophyll *a* occurred during the midsummer, followed by a September peak, and then declined toward the final sampling date in March. The peaks of chlorophyll *a* in the water column correspond to periods of minimal chlorophyll *a* in the epilithic community (Figure 10)

3.4 DISCUSSION

Moore (1977) attributed large summer variations in algal numbers to flooding, a deterioration of algal attachment characteristics, and high metabolic rates. However, it would appear that flooding is not necessarily the single most important factor in the Steepbank and

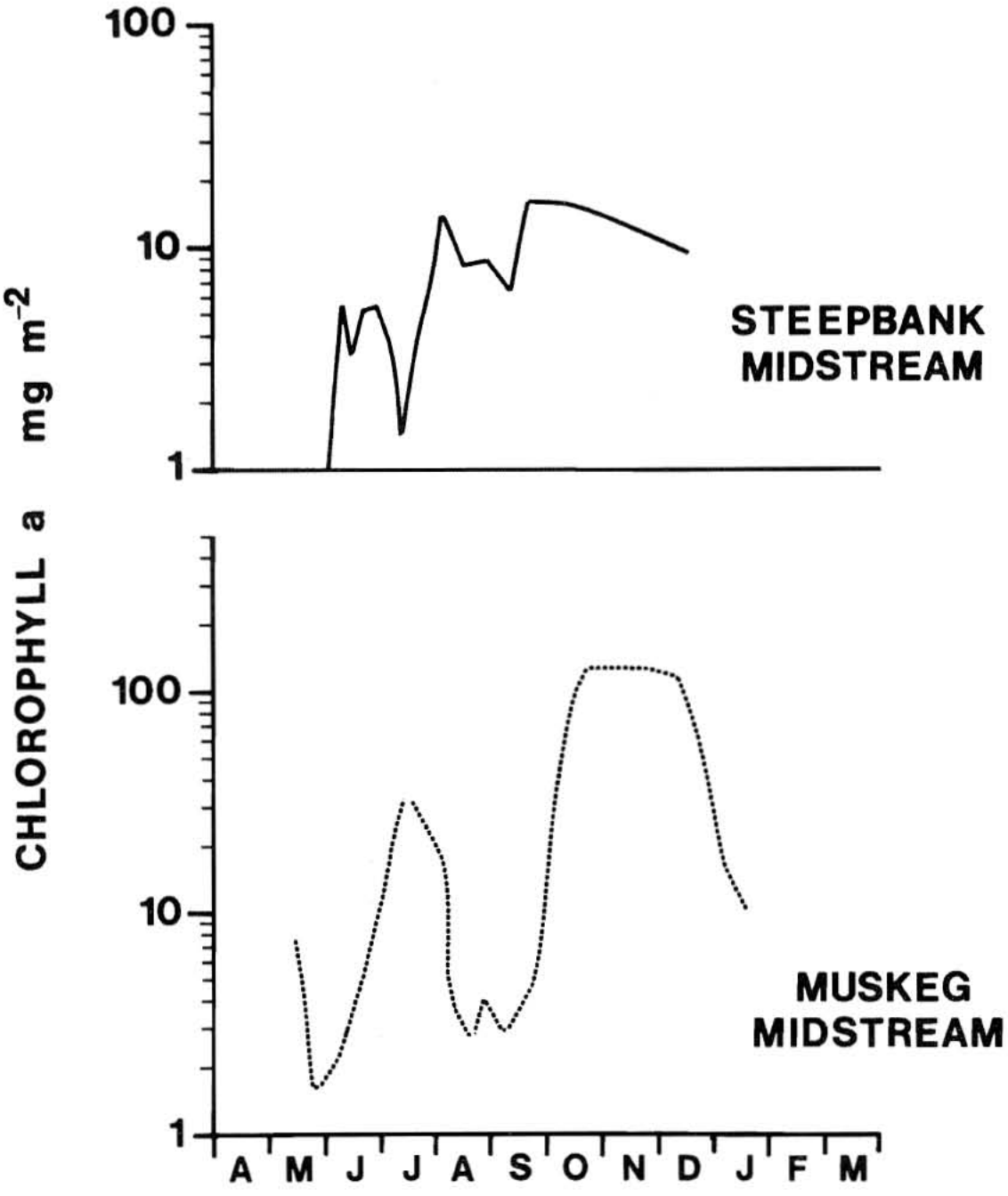

Figure 10. Chlorophyll in mg m^{-2} in the epilithon of the Muskeg and Steepbank rivers over the study period.

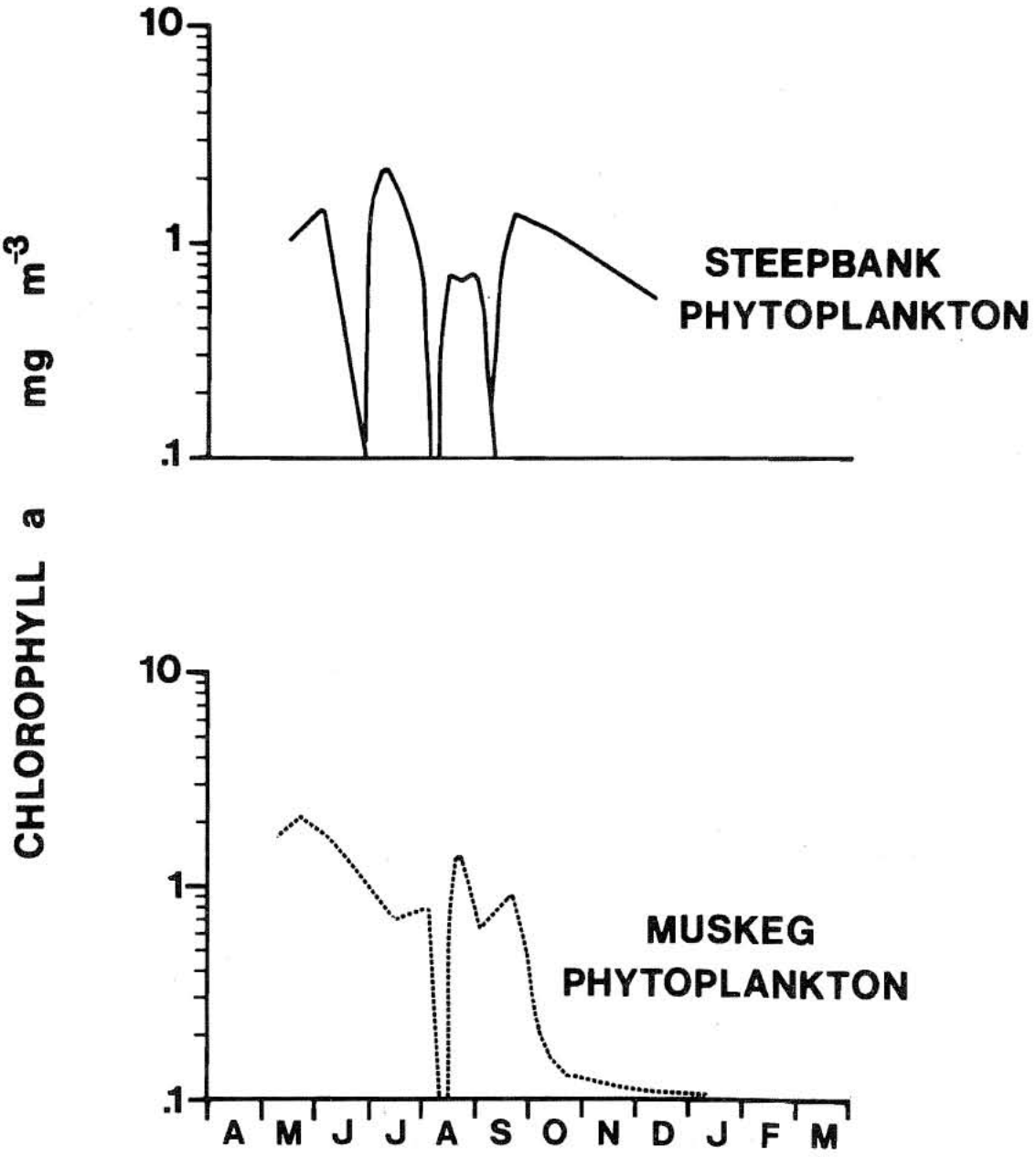

Figure 11. Chlorophyll *a* in mg m^{-3} from the phytoplankton of the Muskeg and Steepbank rivers over the study period.

Muskeg rivers, since periods of maximum discharge (Figures 2 and 4, Section 2.1.3) do not correspond to periods of minimal algal numbers (Figure 9). Therefore, periods of high discharge (flooding) do not necessarily result in massive detachment of benthic algae.

Algae were observed to form layers upon the river substrate. Films did form over the calcareous rock increasing the thickness; then, due to some mechanism, a deterioration of algal attachment occurred and much of the film was swept into the water column. During the formation of thick films, one could expect the area available for colonization to decrease. Thus, many benthic species attach to both the natural substrate and each other. This mode of accumulation of algal numbers, therefore, accounts for the large number of benthic algae per square meter of substrate. Secondly, the thick algal films may be subject to greater current effects than thin films. Thus, in the presence of thick films, where algae are attached to the substrate and each other, river flow may exaggerate fluctuations of benthic populations as they are swept into the water column. In addition to current effects, self-shading, growth inhibiting substances, nutrient availability, and photosynthetic efficiency around and within the film must be considered as important factors affecting the growth of benthic algae. Eichenberger and Wuhrmann (1975) indicate that, when the epilithic biomass is larger, the light intensity required for saturation of photosynthesis is less. This suggests again that photosynthesis by benthic organisms is limited by the exchange between organisms and their surrounding medium.

Benthic algae were observed to reach a maximum during the spring and fall sampling dates, particularly during the fall. These periods were characterized by reduced water temperature, and generally higher rates of

discharge for the Muskeg and Steepbank rivers during spring. The amount of light reaching the substrate during these periods was also less than that of midsummer when water depths were at a minimum and water temperatures reached a maximum. Parker, Samsel and Prescott (1973) indicated the specificity of a particular red algae *Batrachospermum vagum*, this species occurring during early and late summer as well as in winter under the ice. Parker, Samsel and Prescott (1973) demonstrated that this species inhabits highly aerated, clear cold streams which are shaded or only intermittently illuminated. Indeed, the environmental requirements of *Batrachospermum vagum* are fulfilled by the Steepbank sample site. However, this species was also observed to occur in the Muskeg River where shading from terrestrial plants does not occur. Thus, it would appear that the brown water which flows in the Steepbank and Muskeg rivers alters the light regime of the river substrata such that a shading effect occurs for benthic algae. M. Hickman (unpublished data, July 1977) indicated a 50% reduction of incident light at 1-2 meters for some Alberta lakes (e.g. Cold, Sylvan, Gull, etc.). However, a similar reduction of light occurs at 0.25 to 0.5 meters in the Muskeg and Steepbank rivers as well as other brown-water rivers (M. Hickman, unpublished data, June 1975). Therefore, brown-water streams are receiving a reduced amount of light at the substrate depth, compared with clear-water streams, and suggesting the need for an investigation of not only the quantity but also the quality of light available to benthic algae of brown-water streams.

Few conclusions can be reached concerning factors directly affecting benthic algal populations at this time, due to the limited amount of data concerning the physicochemical characteristics of the rivers. However, it would appear that benthic populations are at no

time subject to limited nutrients.

The benthic populations of the Steepbank and Muskeg rivers constitute a greater proportion of the algae than do the phytoplankton. This is confirmed by chlorophyll *a* analyses and final (midstream) or preliminary (phytoplankton) direct counts. The epilithic algae of the Muskeg and Steepbank rivers exhibit a bimodal peak occurring in spring and fall (Figures 12 - 14). These peaks of standing algal crop also occurred in the phytoplankton.

4. COMPOSITION AND STRUCTURE OF MICROBIAL FILMS COATING THE SURFACES OF ROCKS IN THE MUSKEG AND STEEPBANK RIVERS.

Principle Researchers: M.A. Lock, J.W. Costerton, R.R. Wallace and S. Charlton

4.1 INTRODUCTION

In Sections 2.1 and 2.2 we investigated the seasonal population biology of the bacteria and algae occupying the upper surfaces of rocks and granite discs, but no consideration was given to the communities growing on the lower surfaces of these substrates. Since these represent an extensive surface area available for grazing by invertebrates, the densities of bacteria and algae occurring there were determined and compared with density estimates taken from the upper surfaces at the same time. Further, information was obtained on the spatial relationships of the bacteria and algae and of the non-living structural components (detritus, biopolymers, etc.) of the film using transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Such knowledge was anticipated to be of assistance when formulating hypotheses on the relationships between bacteria, algae, other living organisms, and the

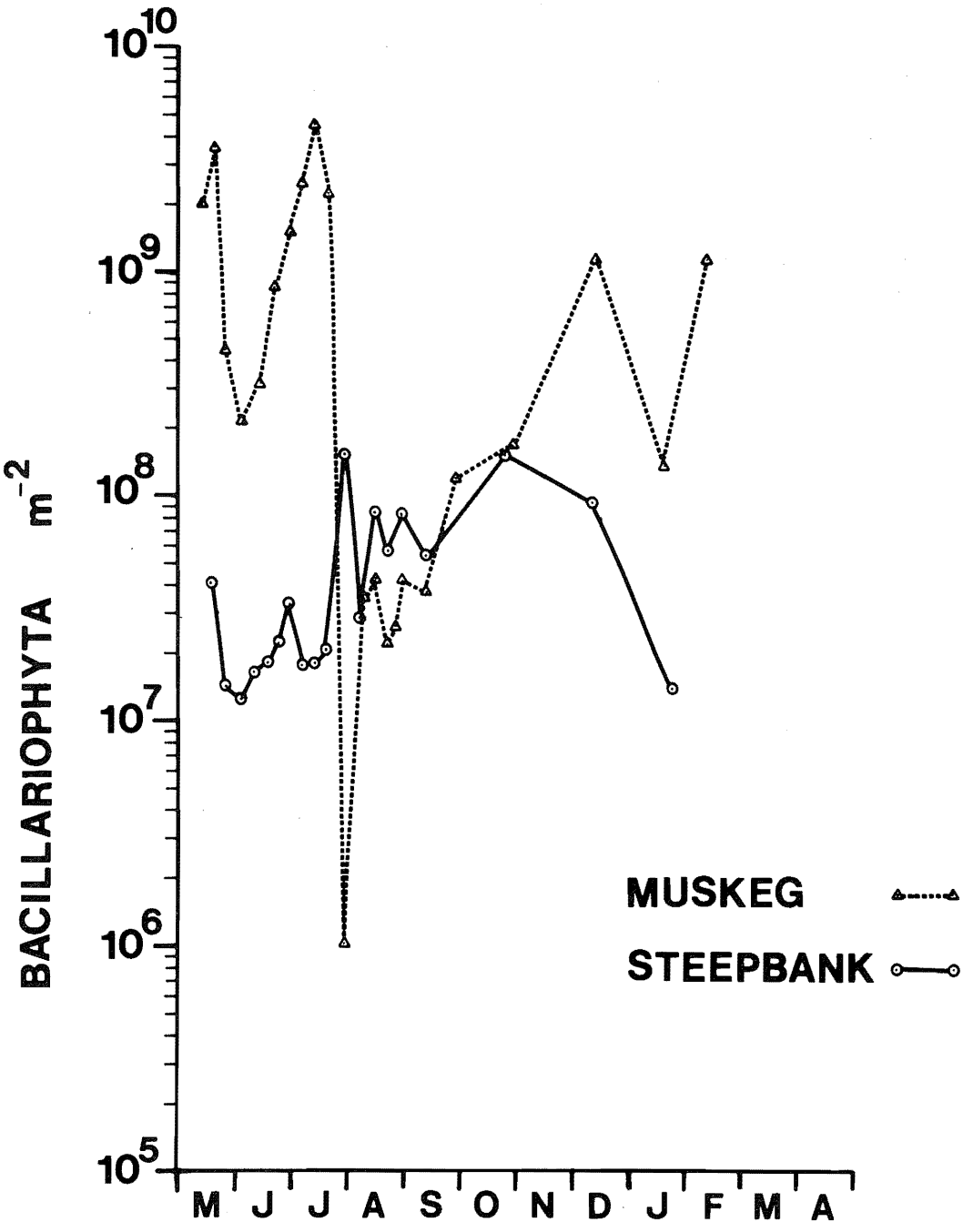

Figure 12. Numbers of Bacillariophyta m^{-2} in the Muskeg and Steepbank rivers over the study period.

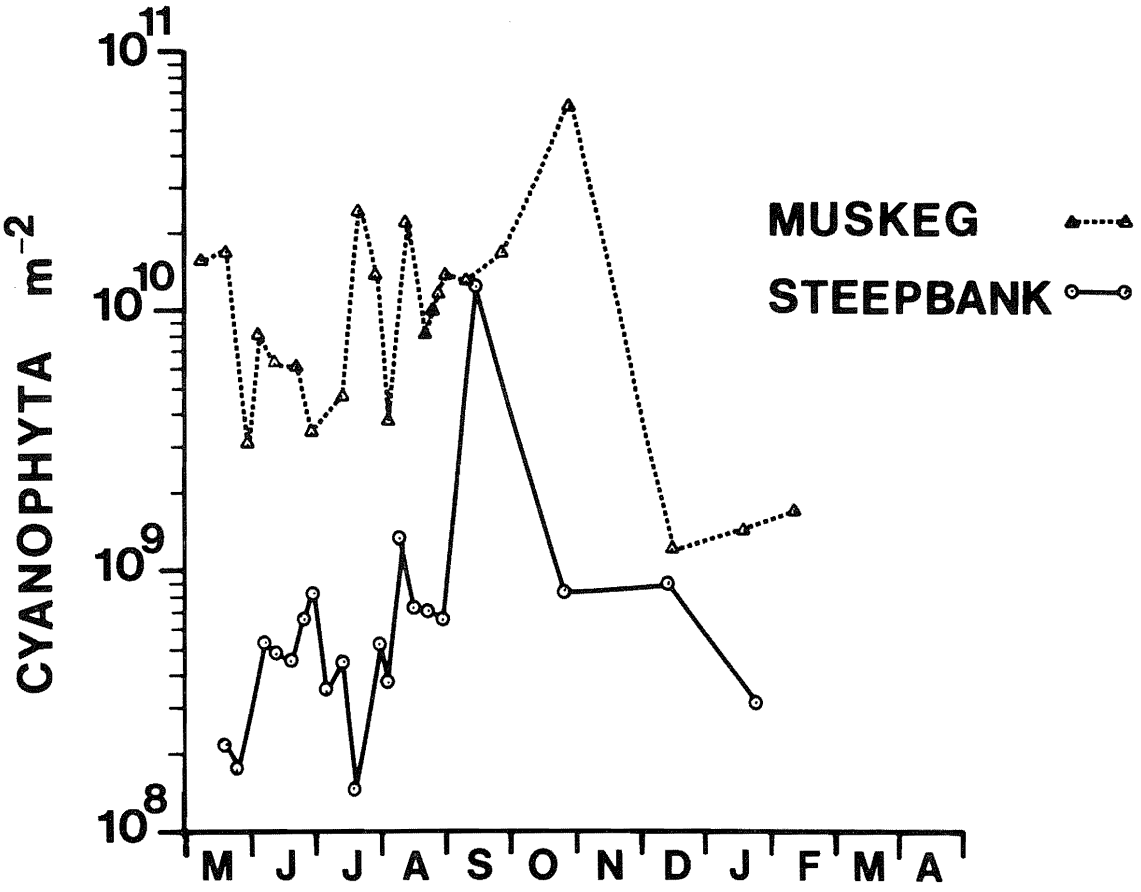

Figure 13. Numbers of Cyanophyta m^{-2} in the Muskeg and Steepbank rivers over the study period.

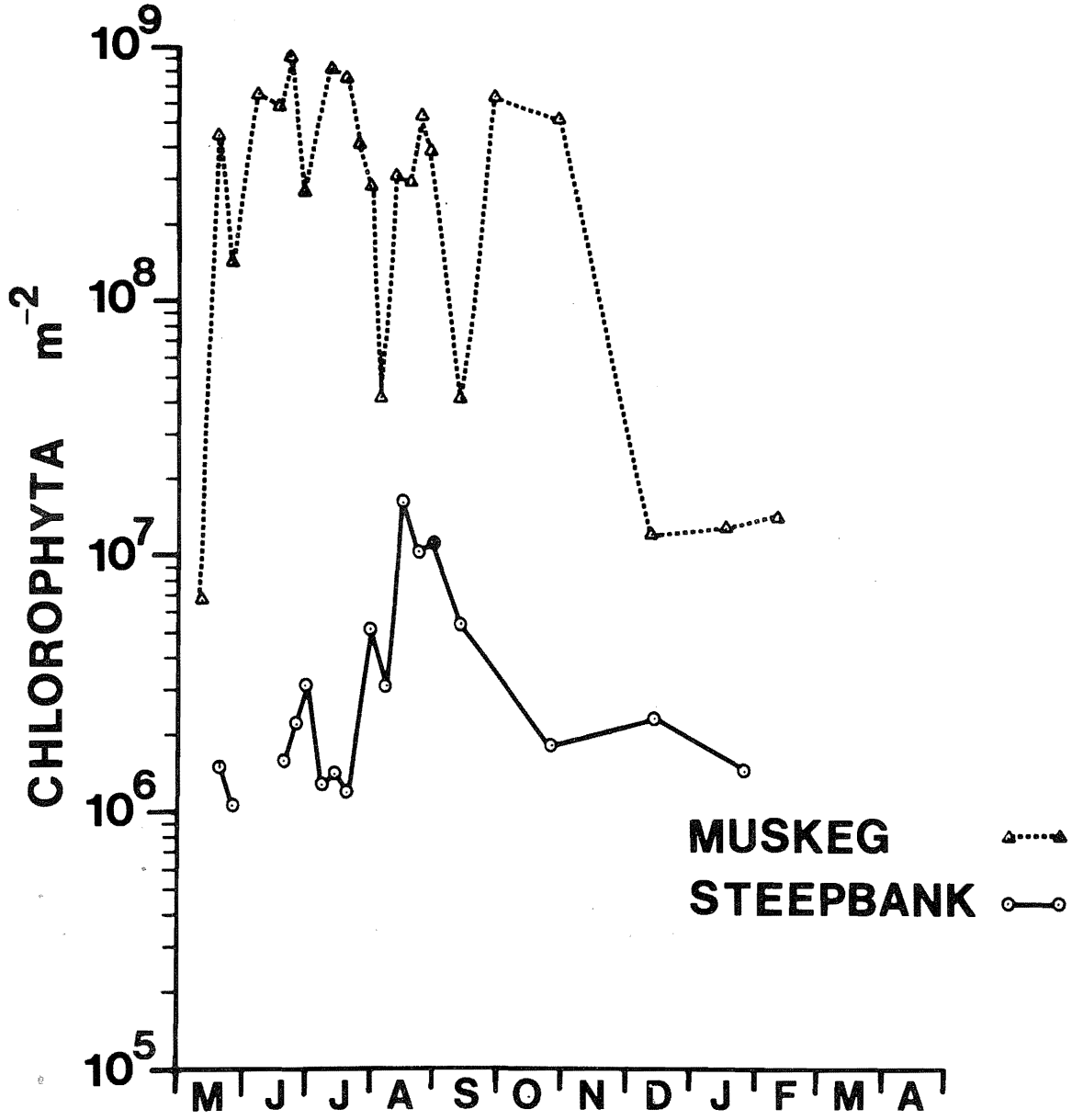

Figure 14. Numbers of Chlorophyta m^{-2} in the Muskeg and Steepbank rivers over the study period.

physicochemical aspects of muskeg rivers, an integral aspect of determining the capacity of the rivers to accommodate man-made disturbances.

4.2 MATERIALS AND METHODS

The studies were carried out at the sites noted previously for the Muskeg and Steepbank rivers (Section 2.1). Bacterial and algal enumerations and chlorophyll *a* and ATP determinations were carried out on the tops and bottoms of five granite discs using the methods of Section 2.1.2. Additionally, small slices of granite (as used for the discs) approximately 7 mm x 7 mm x 4 mm thick were placed in the river for colonization and subsequent viewing using SEM. Similar-sized slabs of epoxy resin (Vestopal) approximately 7 mm x 7 mm x 2 mm thick were also used for colonization and subsequent embedding and sectioning and viewing using TEM. The material for SEM was fixed in 0.5% Glutaraldehyde buffered with 0.067 m Cacodylate buffer. The same fixative was used for TEM material with the addition of 0.015% ruthenium red (for staining of the "polysaccharide-like" matrix). Preparation of the fixed material for viewing followed the method of Geesey et al. (1976) for TEM and Costerton and Geesey (1978) for SEM.

Both sets of substrates for electron microscopy were colonized in the river while attached to the upper or lower surface of a plexiglass plate using a small drop of rubber cement (for ease of removal after incubation). The dimensions of the plate used for incubation in the dark (i.e. the lower surface) were such that the granite and epoxy substrates were placed 9 cm in from the nearest edge, the plate being placed as close to the stream bed as possible. The mode of placement and the rendering of the plate opaque with aluminum foil meant that the microbial films developed

under the conditions normally encountered beneath the rocks of the stream bed. The plate for incubating the substrates for exposure to the light (upper surface) was also placed as close as possible to the bottom in order that they be exposed to the micro-flow regime associated with the rubble bottom.

The granite discs were put in place on 15 May 1977 in both rivers, and the substrates for SEM/TEM examination were put in place on 7 June 1977 for the Muskeg River and 17 June for the Steepbank River. A mid-channel location was used for all colonizations.

4.3 RESULTS

To date, only a preliminary examination of the algae by light microscopy has been made, however, the indications are that substantial populations of cyanophycean algae are present on the underside of the granite discs. The sampling of the Muskeg River in August was at a time when the microbial biomass was at its lowest (Section 2.1). Slightly fewer bacteria were found (Table 10) on the lower surface as opposed to the upper surface, a similar distribution of the levels of chlorophyll *a* (an algal biomass indicator), but nearly a 4-fold increase in the amount of ATP (a total living biomass indicator). During October, our sampling of the Muskeg and Steepbank rivers coincided with the early winter microbial biomass peak. In the Muskeg River, 10 times as many bacteria and 20 times as much chlorophyll *a* and nearly 4 times as much ATP were found on the upper surface as on the lower surface. However, the same comparison for the Steepbank River revealed a rather different picture on the underside of the discs, two types of film were apparent. One was the 'normal' dark brown film which was a "thin" (<0.5 mm) type, but the other was a light coloured "thick" (≃3 mm) type. In the

Table 10. The distribution of bacteria (mean with 95% confidence limits) chlorophyll *a* (mean ± S.E.) and ATP (mean ± S.E.) on the top and bottom surfaces of granite discs.

	AUGUST		OCTOBER			
	MUSKEG RIVER		MUSKEG RIVER		STEEPBANK RIVER	
Parameter	TOP n=5	BOTTOM n=5	TOP n=5	BOTTOM n=5	TOP n=5	BOTTOM n=5
Number of Bacteria cm^{-2}	$9.6 \pm 1.0 \times 10^{6}$	$7.3 \pm 1.3 \times 10^{6}$	$1.3 \pm 0.1 \times 10^{8}$	$1.8 \pm 0.2 \times 10^{7}$	$1.4 \pm 0.2 \times 10^{7}$	$7.2 \pm 1.1 \times 10^{7}$[a] $6.2 \pm 1.1 \times 10^{6}$[b]
Chlorophyll *a* $\mu g\ cm^{-2}$	0.16 ± 0.03	0.11 ± 0.03	20.10 ± 1.00	0.12 ± 0.03	0.32 ± 0.07	0.10 ± 0.03[b]
ATP $ng\ cm^{-2}$	12.6 ± 5.8	45.0 ± 23.2	25.4 ± 4.7	7.2 ± 1.9	24.8 ± 4.7	338.2 ± 10.0[a] 7.4 ± 1.3[b]

[a] "Thick" film

[b] "Thin" film

former, smaller amounts of chlorophyll *a* and ATP and numbers of bacteria were found in comparison with the upper surface, but the reverse was true for the "thick" film in which there was five times as many bacteria, and nearly 14 times as much ATP, the highest level recorded in any of the present studies (338.2 ng cm^{-2}).

For the electron microscopy study, 22 representative photographs were selected out of nearly 100 photographs taken which were themselves the result of over 20 hours of viewing of the samples with transmission and scanning electron microscopy. Beginning in August, on the Muskeg River, large numbers of cyanophycean algae are present on the substrates exposed to the light. Evidence for this comes from the ≃1 µm oval objects in the SEM photographs (Figures 15 and 16), some of which have the ends missing. The dimpled structure inside is possibly an intact thylakoid (Carr and Whitton 1973; Fogg et al. 1973) which are also seen in cross-section in the TEM (Figures 17 and 18). It is interesting that members of the Cyanophyta should be closest to the substrate, suggesting that this group might be the primary colonizer of new surfaces. The "polysaccharide-like" matrix is also well evidenced, demonstrating that approximately half the bulk of the film is non-living which may in itself represent a potential food source for benthic invertebrates. On the substrates which developed in the dark, we observed considerable numbers of filamentous cyanophycean (Figure 19) and bascillariophycean algae (Figure 19) along with a number of sessile ciliates (Figure 20). The high power picture in Figure 20 demonstrates the very delicate "fluffy" nature of the "polysaccharide" slime matrix. The corresponding TEM photographs (Figures 21 and 22) show several cells 1 µm across with a very distinct "stacked-plate" structure inside. These were abundant throughout all the sections

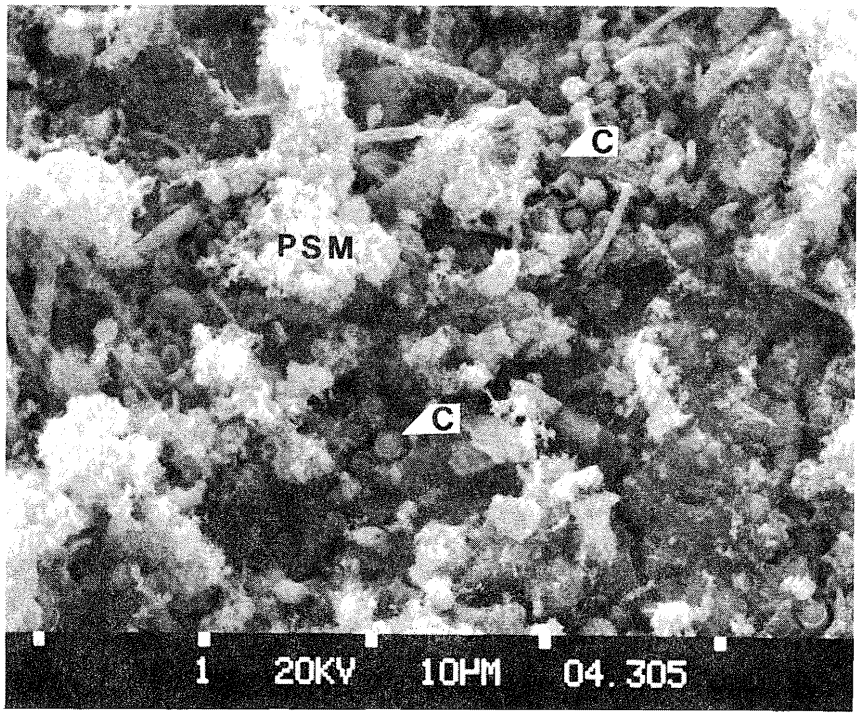

Figure 15. SEM of upper granite surface showing the presence of possible cyanophycean algae (C) and 'polysaccharide' matrix (PSM). Muskeg River, August 1977.

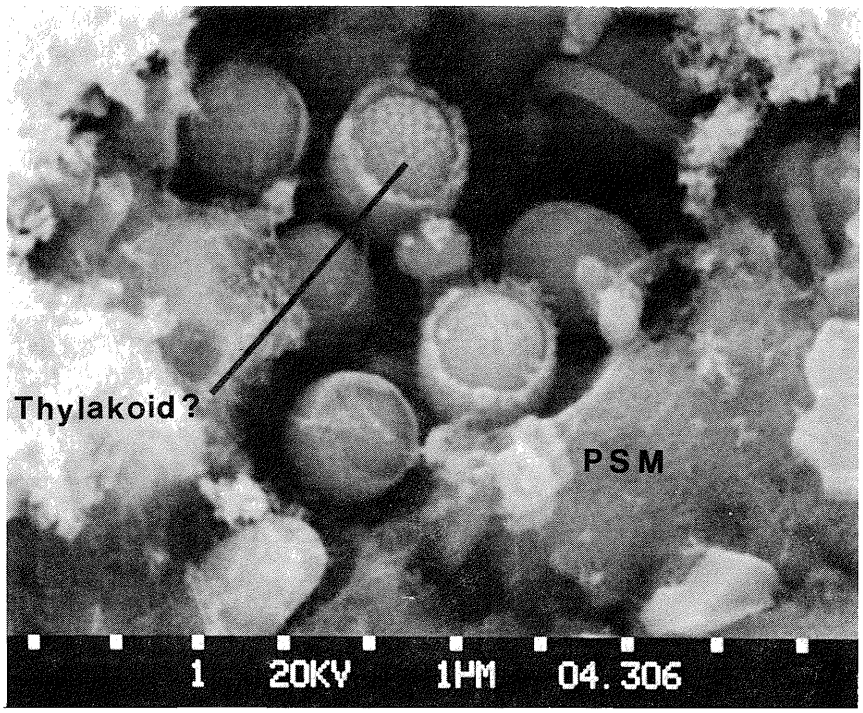

Figure 16. SEM of upper granite surface showing the presence of cyanophycean algae(?) close-up. The missing ends of the cells may be disrupted glycocalyx or cell wall material; if the latter, then the dimpled internal structure may be an intact thylakoid. PSM - 'Polysaccharide' matrix. Muskeg River, August 1977.

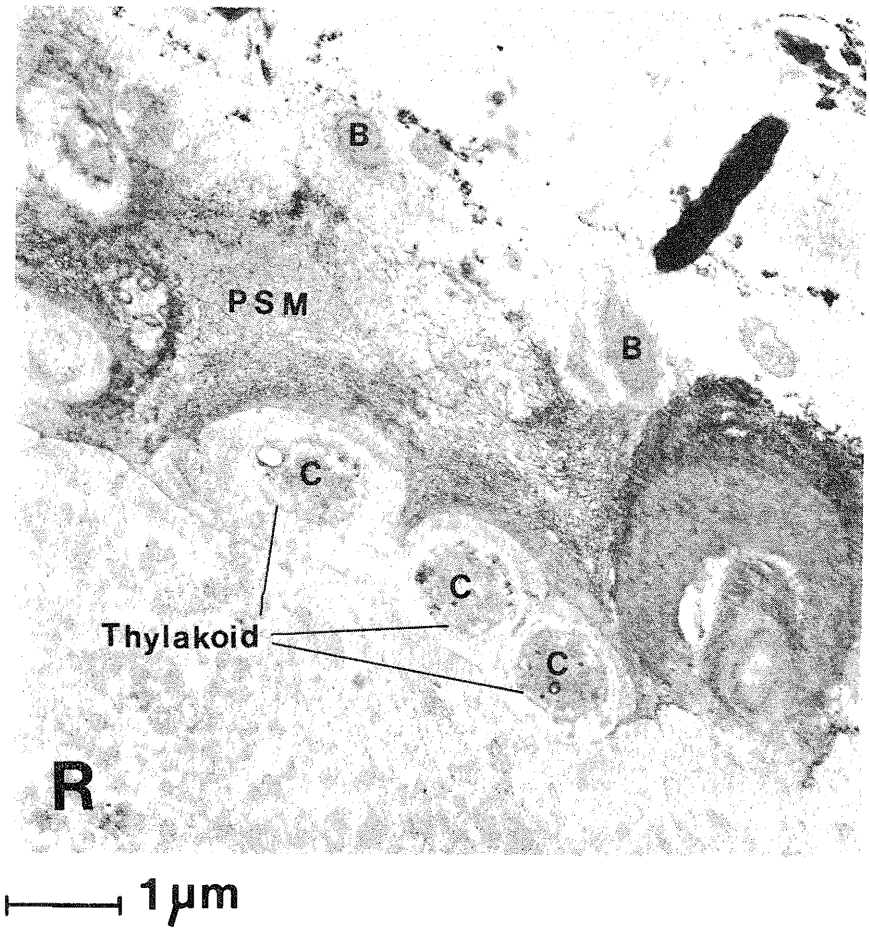

Figure 17. TEM of upper resin surface showing cyanophycean algae (C) attached directly to the resin substrate (R). A single thylakoid appears to be present in each cell. Bacteria (B) and algae are embedded in the 'polysaccharide' matrix (PSM). Muskeg River, August 1977.

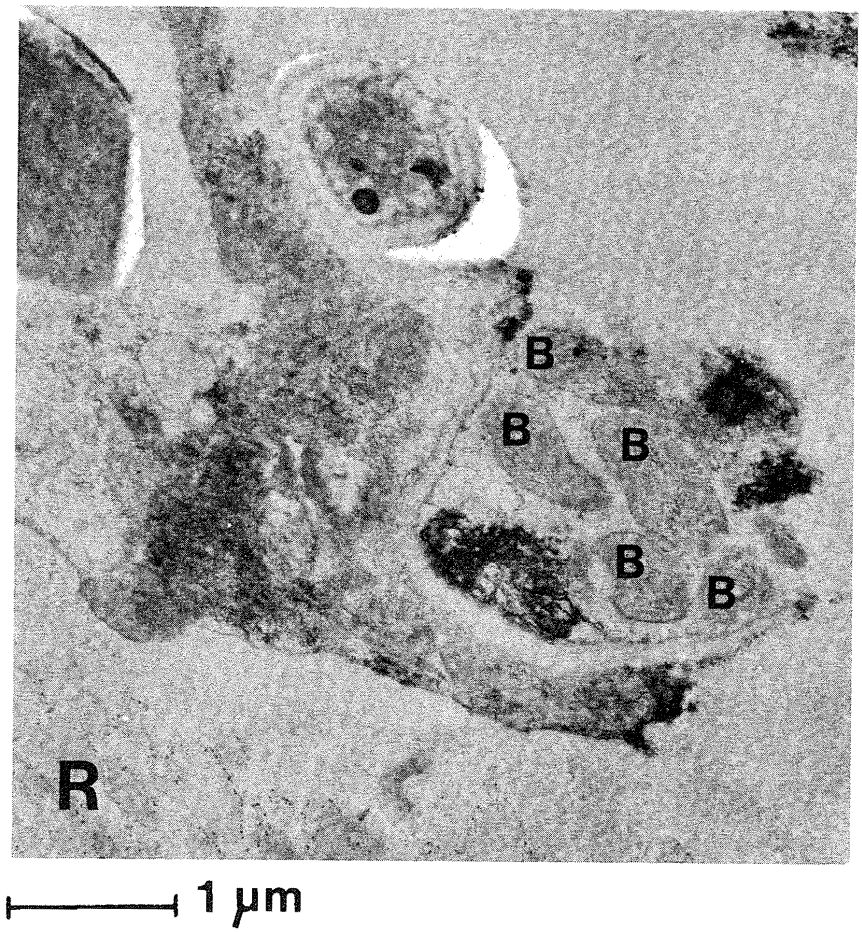

Figure 18. TEM of upper resin surface showing a micro-colony of bacteria (B) located close to the resin substrate (R). Muskeg River, August 1977.

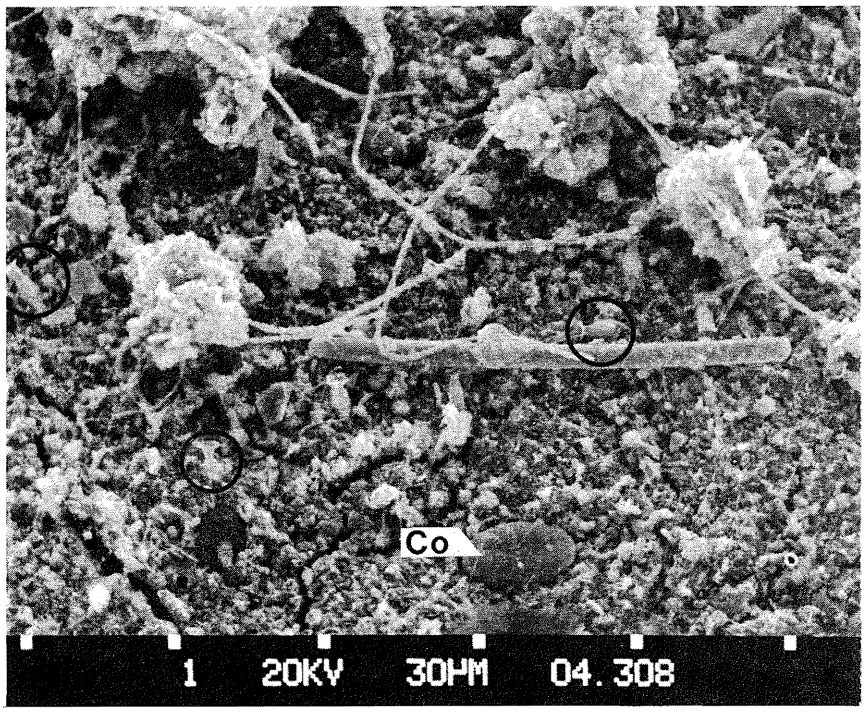

Figure 19. SEM of lower granite surface showing the presence of *Cocconeis* sp. (Co) and several peritrich ciliates (Ringed). Muskeg River, August 1977.

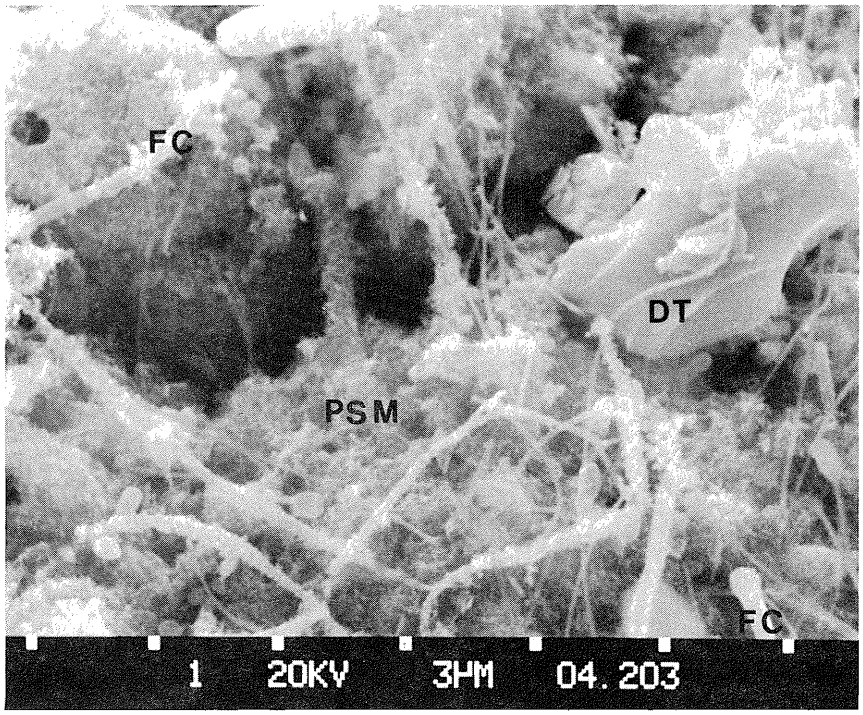

Figure 20. SEM of lower granite surface, showing extensive development of a polysaccharide matrix (PSM) with filamentous cyanophycean algae (FC) and detrital particles (DT). Muskeg River, August 1977.

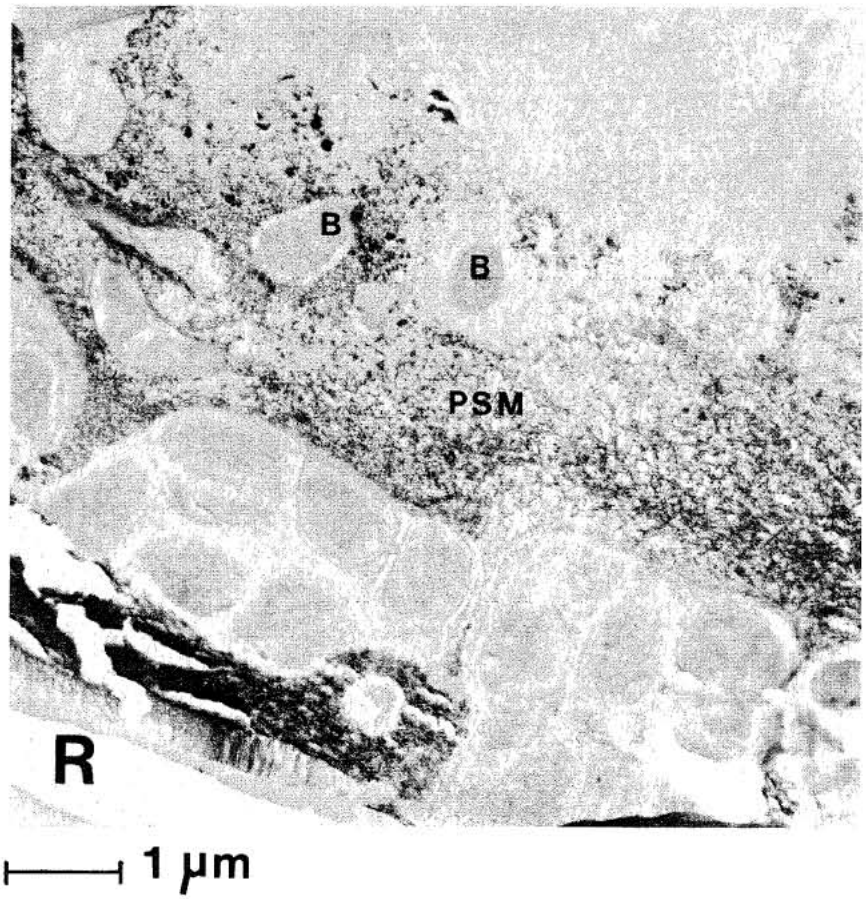

Figure 21. TEM of lower resin surface (R), showing the presence of bacteria (B) and large numbers of unidentified cells with a distinct 'stacked-plate' structure embedded in a 'polysaccharide' matrix (PSM). Muskeg River, August 1977.

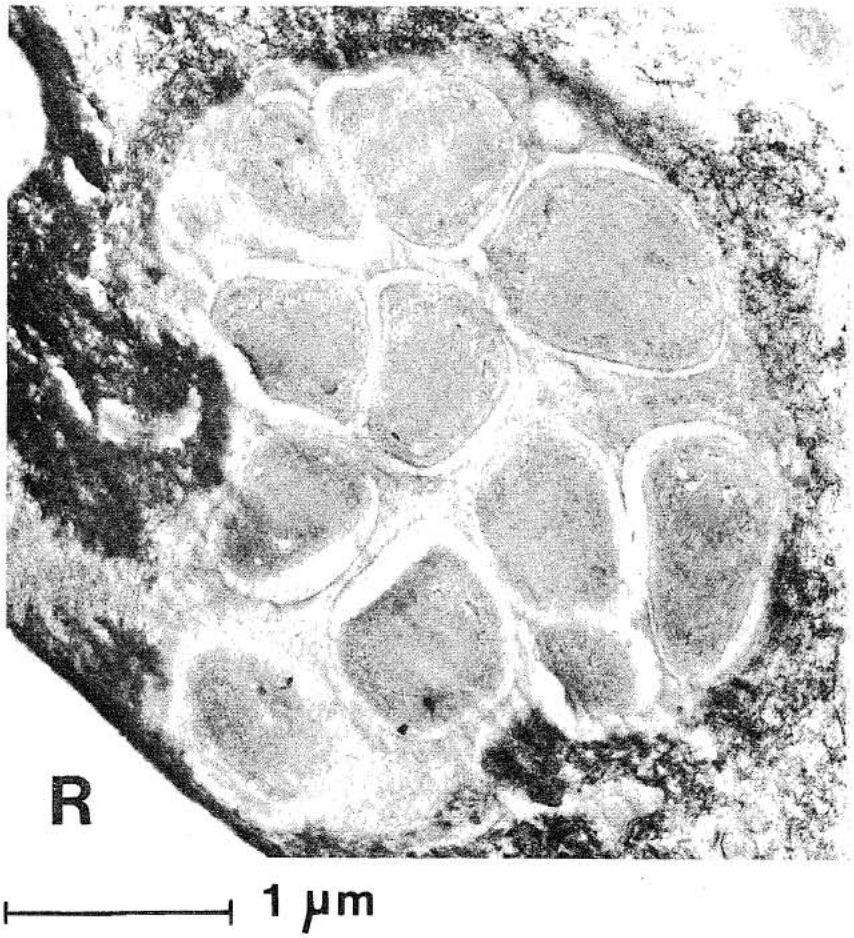

Figure 22. TEM of lower resin surface (R) showing a close-up of the unidentified cells with a 'stacked-plate' structure. Muskeg River, August 1977.

examined. As yet, these organisms are unidentified.

The samples taken in October in the Muskeg River were taken at a time when the development of the film was maximal (see Section 2.1.3). The slime was in the region of 2 - 3 mm thick and, on the lowest power of the scanning microscope, it was seen to be perforated with holes (Figure 23). These holes ranged from ≃0.1 mm to 0.5 mm in diameter. It is tempting to suggest that these are caused by the chironomid larvae and oligochaetes which inhabit the films. To take this suggestion further, this offers some circumstantial evidence for mechanical disruption of the film, and that could be a contributing cause for the population crash of algae and bacteria which occurred later in the year (Sections 2.1 and 2.2). Examination of the film under higher power shows it to be composed entirely of stalked bascillariophycean algae (Figure 24), very different in structure from the film produced in August. However, a TEM examination of the organisms occurring at the base of the film revealed large numbers of cyanophycean algae (Figure 25). It is problematical as to whether there would be sufficient light for photosynthesis to be in operation or whether they were in fact operating heterotrophically. Additionally, these algae seem to have etched their way into the epoxy resin substrate (Figures 25 and 26). If this is indeed so, it is possible that this could be an important facet of biodegradation of substrates, such as oil sand, which occurs in rivers. This possibility needs further investigation. The samples taken from the Muskeg River incubated under dark conditions had a much looser open film (Figures 27 and 28) than the one which had developed in August (Figures 17 and 18). Both bacillariophycean and cyanophycean algae were present.

The films which developed in the Steepbank

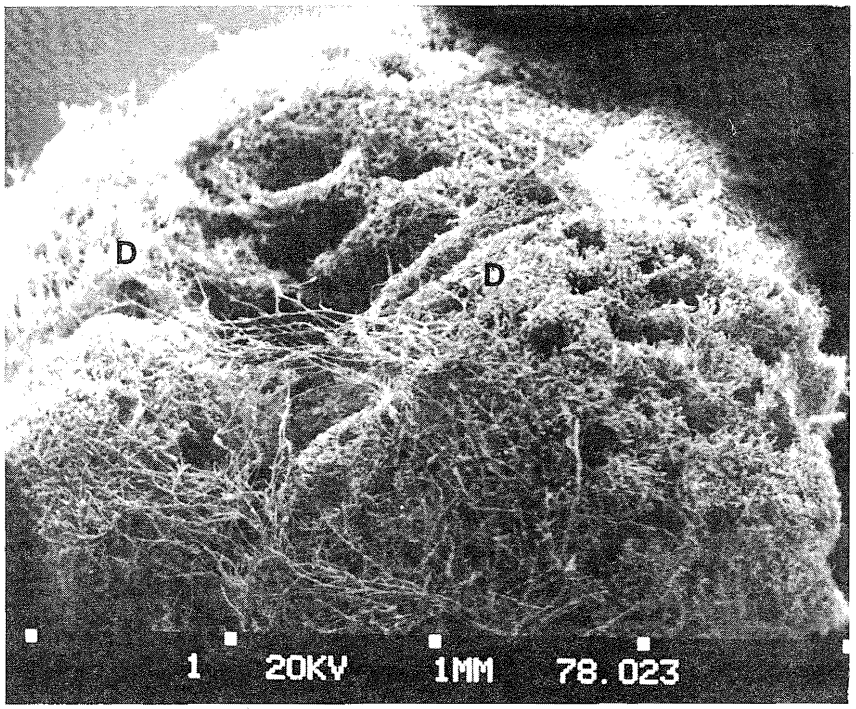

Figure 23. SEM of upper granite surface showing a thick mat (~ 3mm) of bacillariophycean algae (D) stalks perforated with holes possibly produced by chironamid larvae. Muskeg River , October 1977.

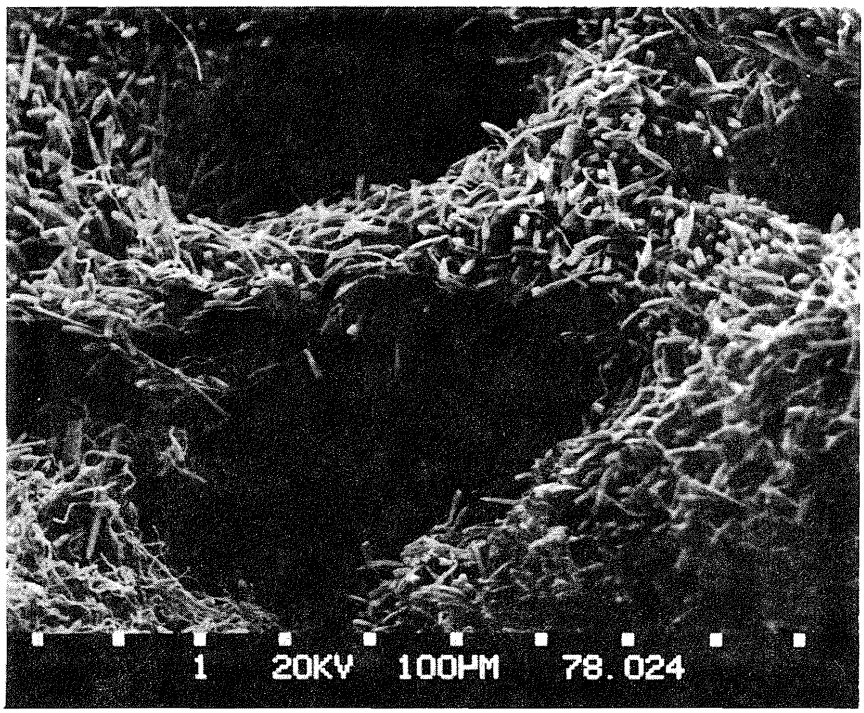

Figure 24. SEM of upper granite surface showing a close-up of the holes in Figure 23. The algae are primarily *Gomphonema* sp. and *Navicula* sp. Muskeg River, October 1977.

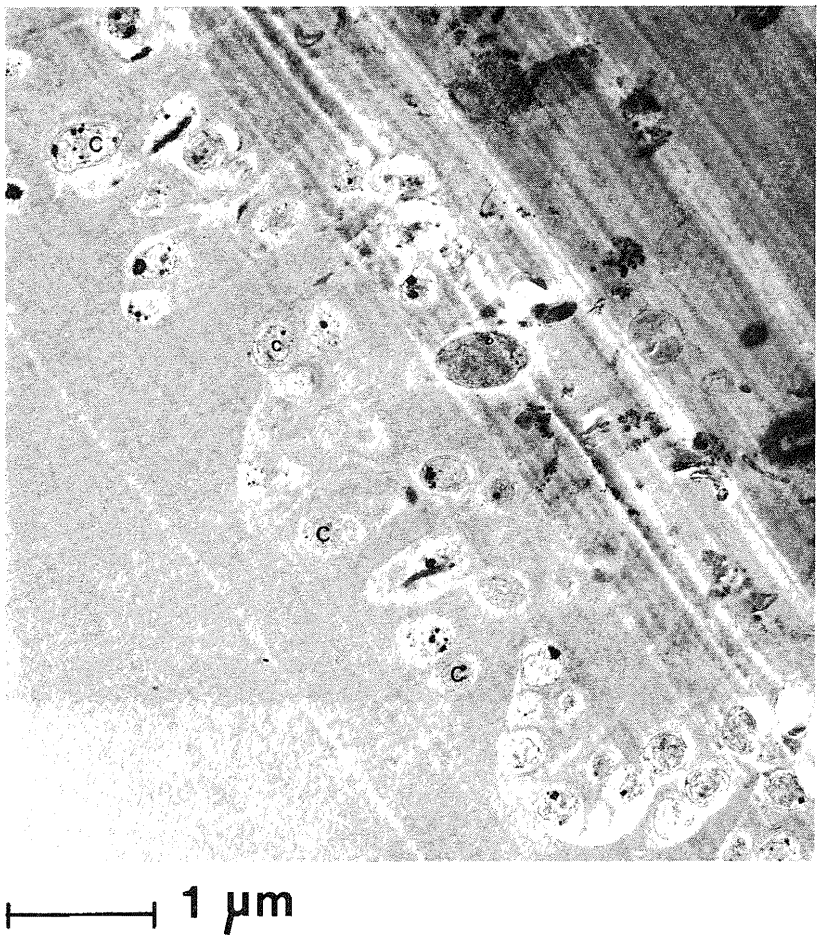

Figure 25. TEM of upper resin surface showing 'etching' by the cyanophycean (C) algae cells (?). Muskeg River, October 1977.

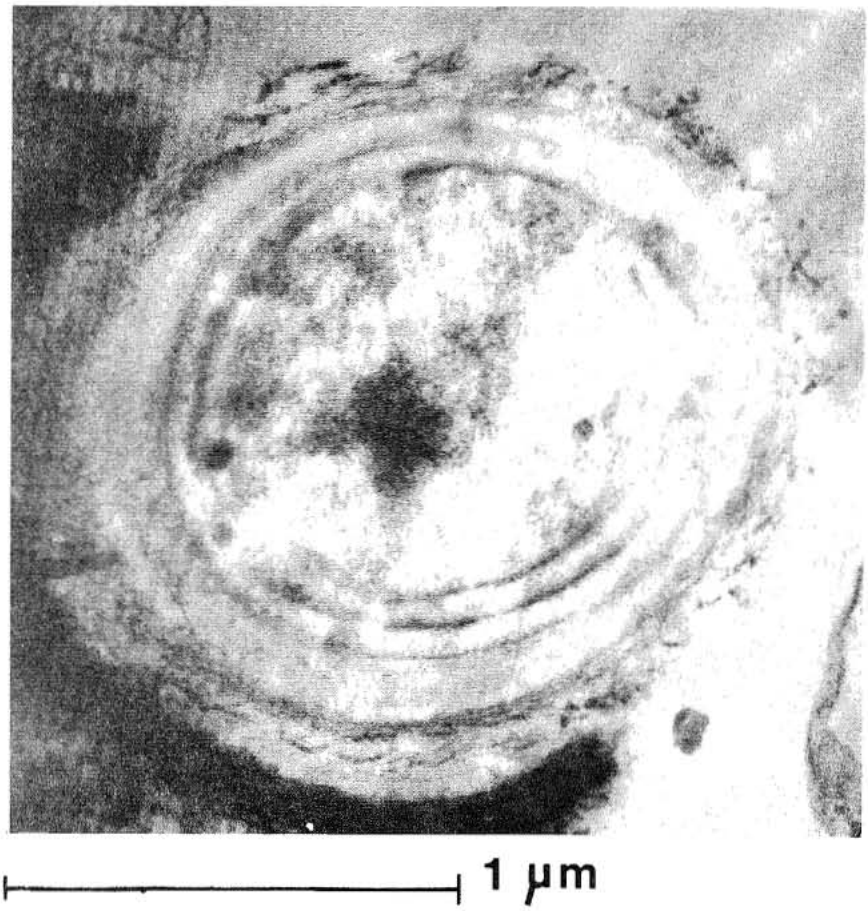

Figure 26. Close-up of a single cell in Figure 24. The structure suggests that they are cyanophycean algae. Muskeg River, October 1977.

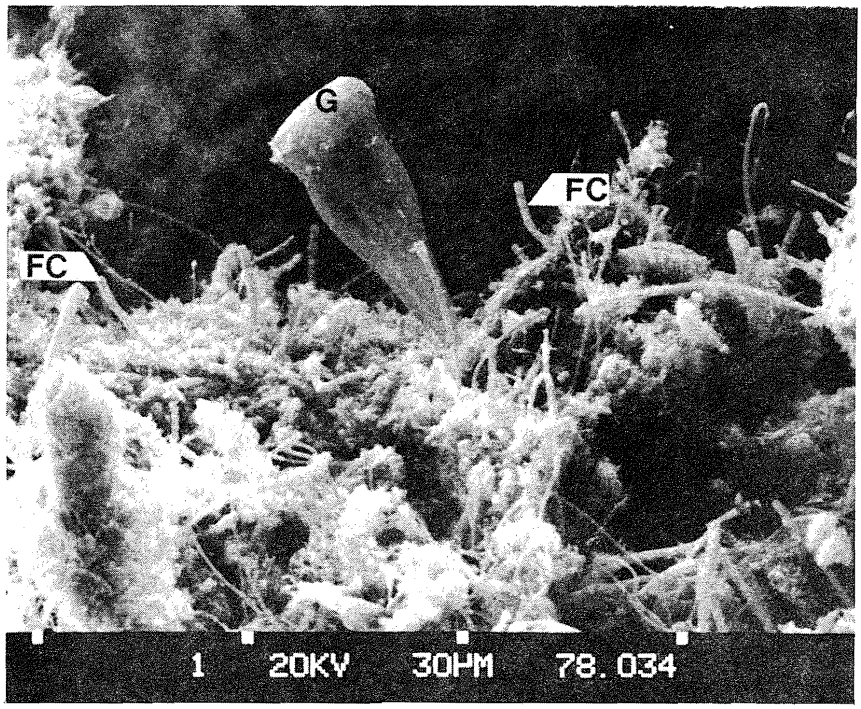

Figure 27. SEM of lower granite surface showing numerous filamentous cyanophycean algae (FC) and the bacillariophycean algae, *Gomphonema* sp. (G). Muskeg River, October 1977.

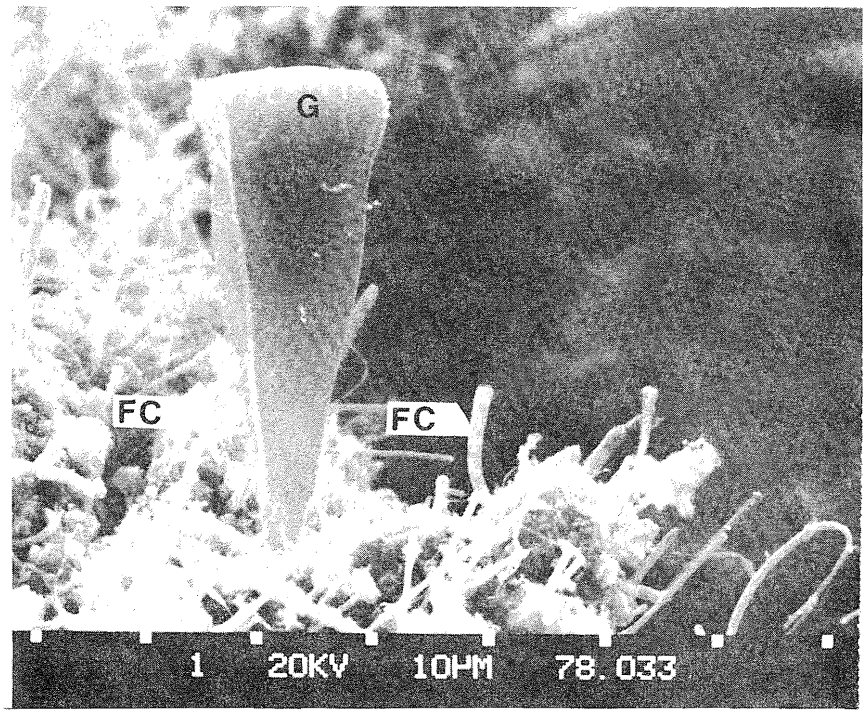

Figure 28. Close-up of Figure 26.

River in October were very much thinner than the corresponding ones in the Muskeg River. Figure 29 shows a micro-colony of filamentous cyanophycean algae only 2 mm across. A closer examination of the surface, once again, reveals large numbers of round cell about 1.0 - 1.5 nm in diameter Figure 30. These may be cyanophycean algae as in Figures 15 and 16, but diagnostic characters are not apparent in these specimens. In the dark, a large assemblage of these round structures is also found, but again, they cannot be identified conclusively Figure 32. The corresponding TEM photographs (Figures 33 and 34), however, indicate that presence of a large number of sausage-shaped structures, the tips of which may be the round structures in the SEM preparation. Their rather homogenous internal structure would suggest that these may be bacteria.

Lastly, a photograph of a "net" is included, possibly of an early instar trichopteran larvae. The net is attached at the junction of the granite slice and the base to which the piece of granite was attached. It measures only 1 mm in height and about 1.5 mm long (Figure 35). The last, Figure 36, is a high power photograph of a peritrich colony. On Figure 31, a number of such colonies were found over that substrate.

4.4 DISCUSSION

Since the granite substrates used for examination of the relative proportions of algae and bacteria had been in place for three months for the August sampling, and five months for the October sampling, it is likely that those communities would have stabilized (Eichenberger and Wuhrmann 1975), and would thus reflect the structure of the microbial community present in the river at that time. The same was noted for the substrates used for electron microscopy observation, as two

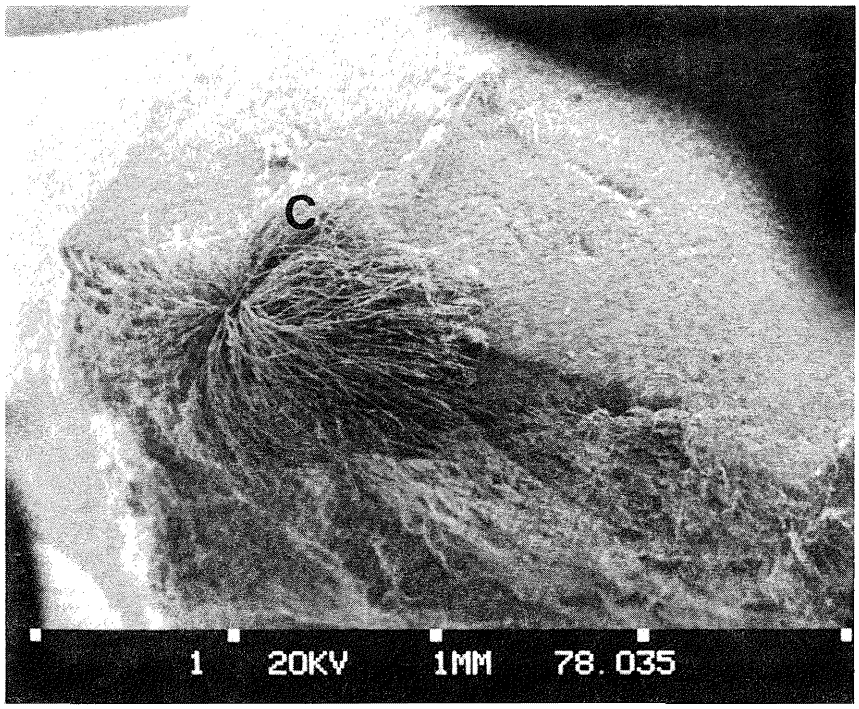

Figure 29. SEM of upper granite surface showing a large tuft of filamentous cyanophycean algae (C). Steepbank River, October 1977.

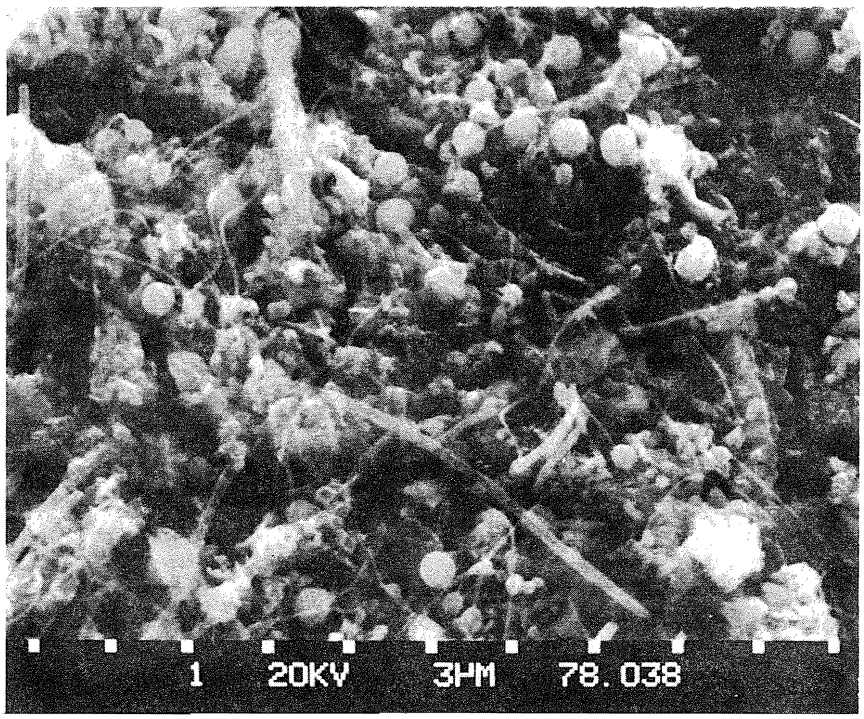

Figure 30. SEM of upper granite surface showing possible coccoid and filamentous cyanophycean algae. Steepbank River, October 1977.

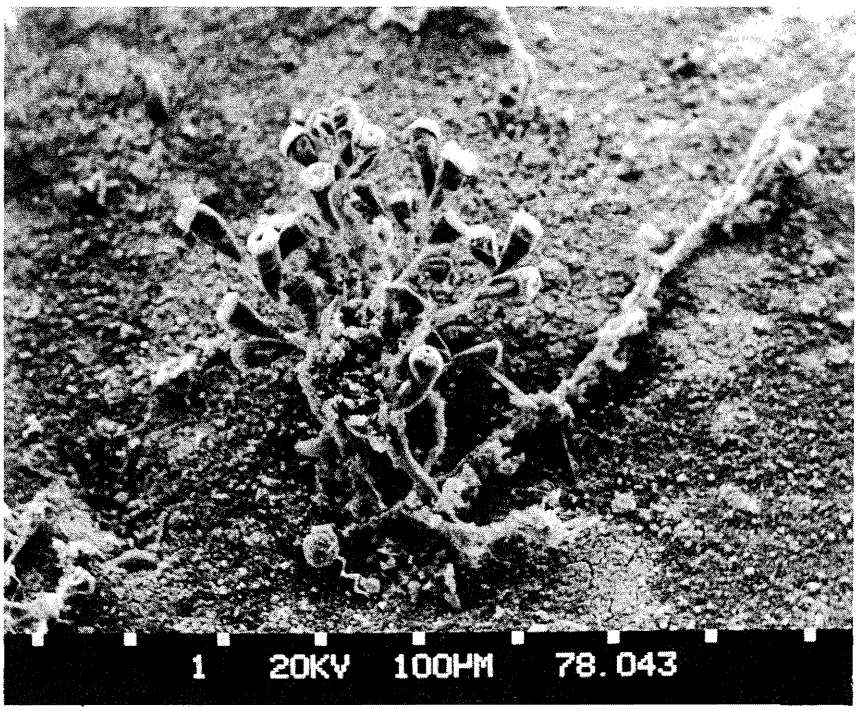

Figure 31. SEM of lower granite surface showing a colony of peritrich ciliates. Steepbank River, October 1977.

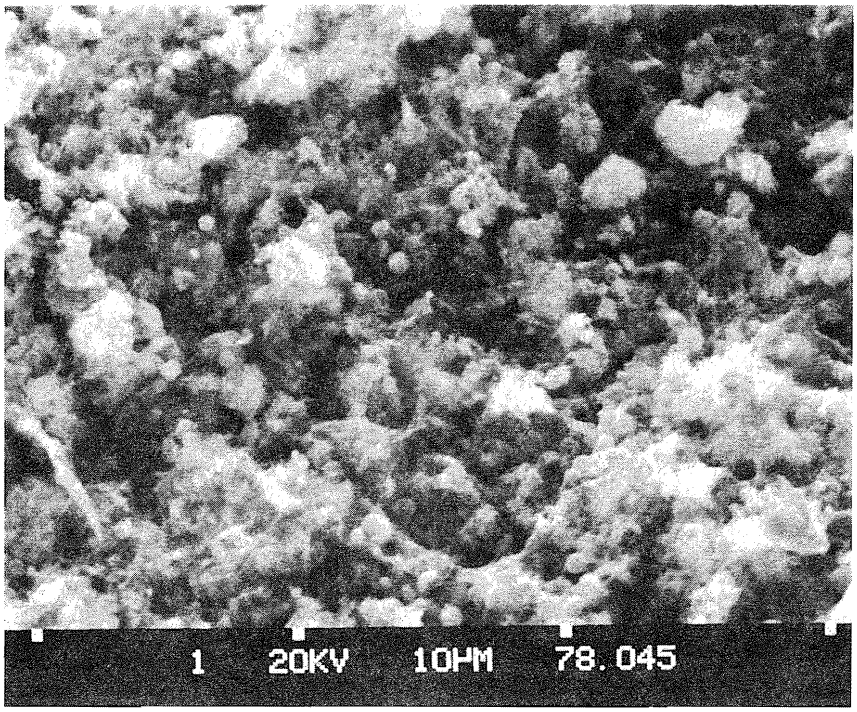

Figure 32. SEM of lower granite surface showing many round cells (∿1-2µm diam) which are possibly blue-green algae or bacteria. Steepbank River, October 1977.

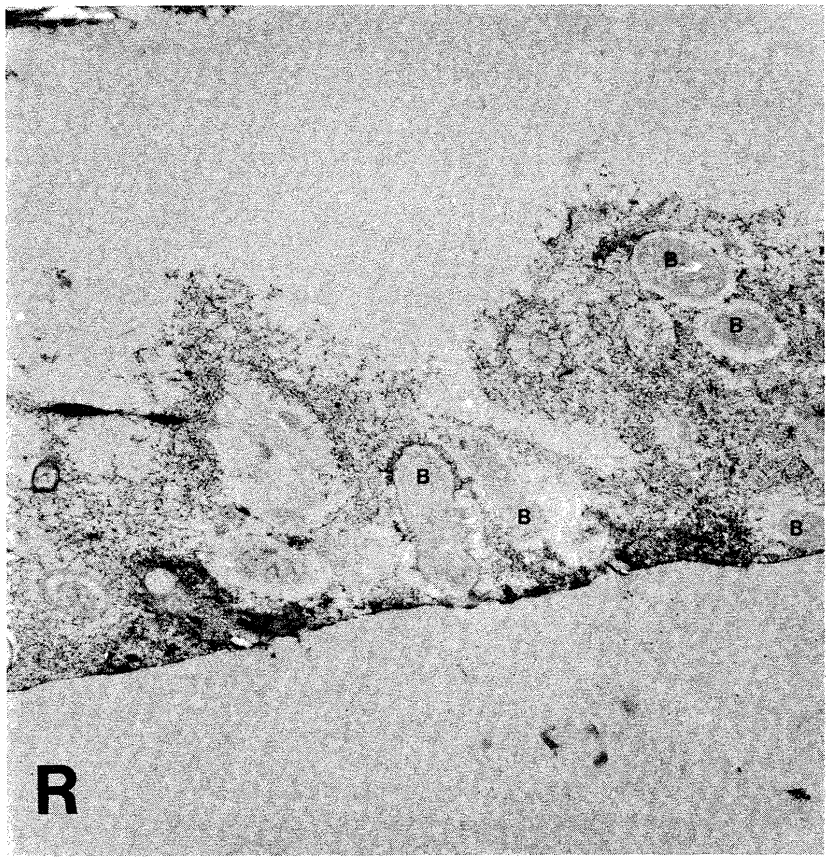

Figure 33. TEM of upper resin surface showing several cells which appear to be bacteria (B) including one which is attached to the resin surface (R). Steepbank River, October 1977.

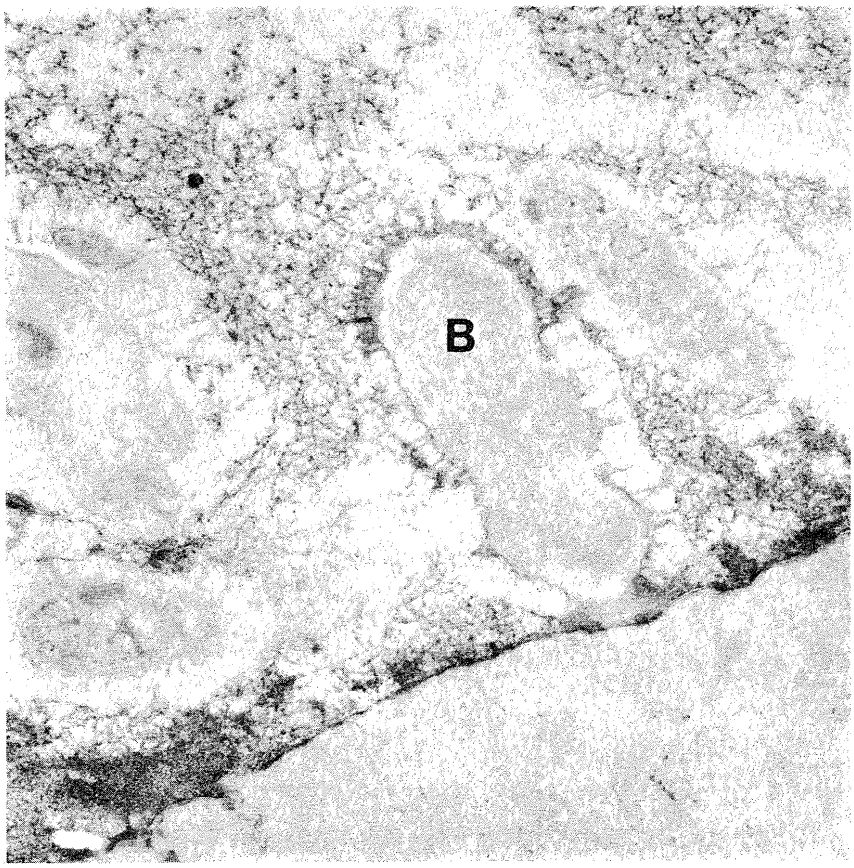

Figure 34. Close-up of attached cell in Figure 32. Steepbank River, October 1977.

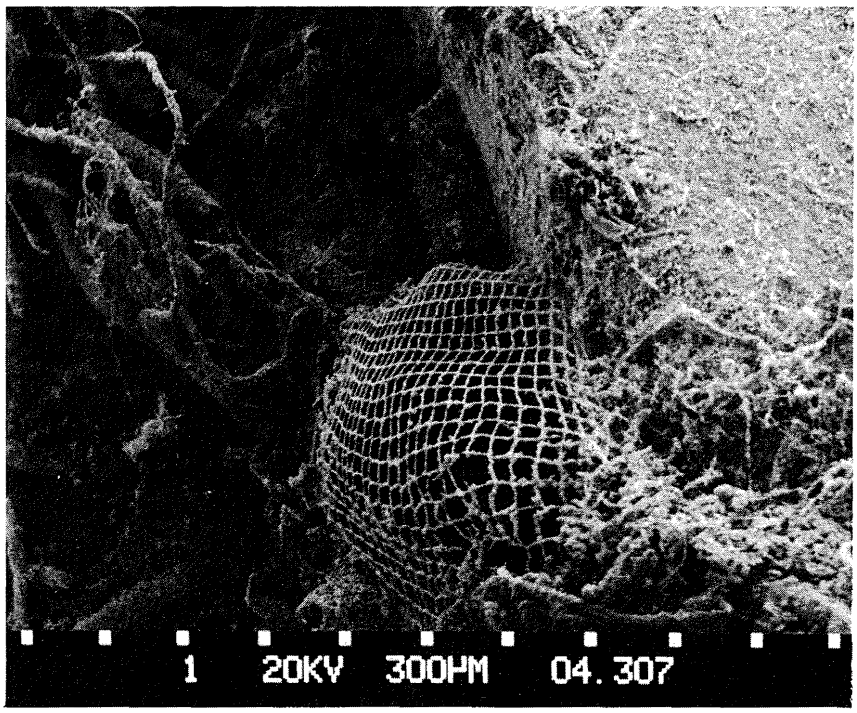

Figure 35. Net of a trichopteran larvae attached to granite chip. Steepbank River, October 1977.

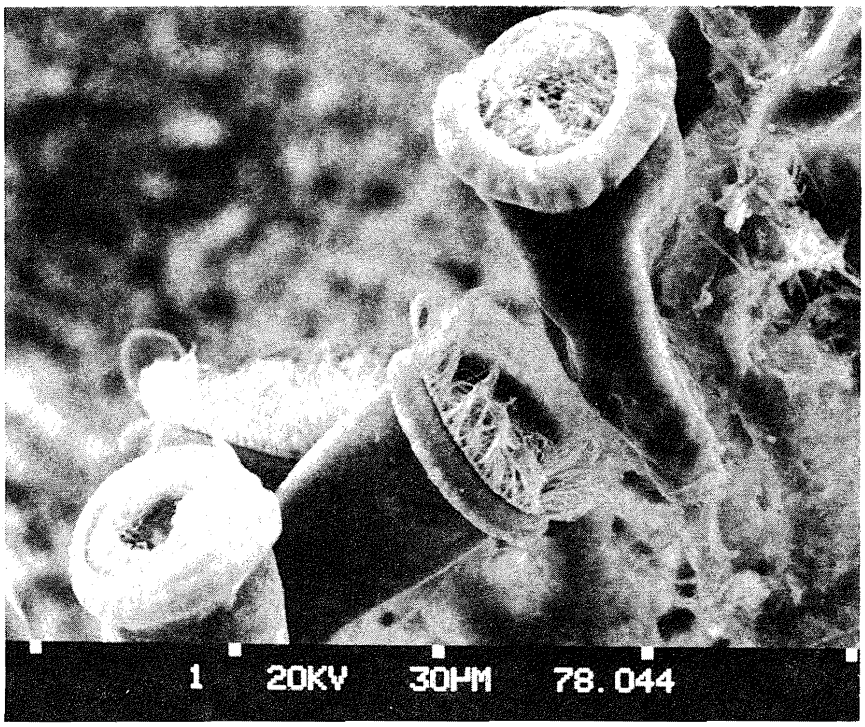

Figure 36. Close-up of peritrich ciliates in Figure 30.

months had elapsed by the time of the August sampling, and four months by the October sampling.

On the lower surfaces of the granite discs, substantial numbers of bacteria ($6 \times 10^6 - 2 \times 10^7$ cm^{-2}), low ($\simeq$0.1 ng cm^{-2}), but measurable levels of the algal biomass indicator chlorophyll *a* and variable amounts of ATP were found. In general, the microbial biomass was lower on the lower surfaces than on the upper surfaces of the discs. The exception to this was the exceedingly high level of ATP found in the "thick" film from the Steepbank River. An examination with a light microscope of this film revealed substantial colonies of a bryozoan. Thus, much of the ATP extracted from this particular film may have come from these animals. It may be that the levels of ATP recorded from the film more accurately reflect the biomass of the animal portion, especially as Section 2.1 demonstrated the lack of correspondence between ATP levels and those of bacteria and algae.

The results of the electron microscopy survey indicate the ubiquitous nature of the "polysaccharide" matrix which appears as a fine network in the TEM and as 'fluff' in the SEM. The role of this material is as yet undetermined, but because of its large surface area, it is likely to have ion exchange properties, the ability to adsorb organic molecules, or serve to maintain the concentration of bacterial exoenzymes within the film (Marshall 1976). All of these functions would serve to enhance the ability of the microorganisms to assimilate dissolved matter flowing down the river, the "self-purifying" function of flowing water systems.

Except for the very thick film which developed in the Muskeg River on the upper surfaces during October, the films from the upper and lower surfaces were rather similar in character. In particular, large numbers of 1 nm diameter cells were found throughout these films

which have developed in both the light (upper surface) and dark (lower surface). These may be cyanophycean algae, however, the presence of both cyanophycean and bacillariophycean algae was clearly demonstrated from the lower surface of the substrates from the Muskeg River in August (Figures 27 and 28). These do not give the appearance of being loosely attached casual members of the community, but rather they would seem to be growing there. It does, of course, remain to be determined whether the algae present are photosynthesizing at the low light levels recorded on the under-surfaces of rocks (≈0.1% of surface photosynthetically active radiation), or whether they are metabolizing heterotrophically, that is, actively taking up organic molecules from the river in the same manner as bacteria.

5. CHEMICAL COMPOSITION OF GROUNDWATER IN THE IMMEDIATE VICINITY OF THE MUSKEG RIVER.

Principle Researchers: M.A. Lock, R.R. Wallace

5.1 INTRODUCTION

At any point in a river, water may be entering or leaving the main flow, depending upon whether the aquifer is being recharged or is discharging at that point. Therefore, unless the system is balanced, the microbial communities within the stream bed will have a certain amount of water flowing over them from above or below. This water, with its associated organic and inorganic nutrients, could be of considerable importance as a food source to benthic microorganisms within the gravels, particularly in the situation where nutrient-rich groundwater flowed up through them on its way to the free water in the river channel. Such a situation was found in the interstitial waters at the interface of ground water and free water at a lake edge (John, Lock

and Gibbs 1977). This study set out to examine the spatial chemistry of the interstitial water in and close to the Muskeg River. If it is assumed the gravels and sediments of the river are completely inert, then it would be expected that the concentrations of chemical species would be identical at any point in the interstitial (gravel) waters. This study sets out to test this hypothesis. If our findings demonstrated that the interstitial waters are not chemically homogenous, then this would strongly suggest that the chemistry of the water was being modified by biological or geochemical processes.

5.2 MATERIALS AND METHODS

The area of river chosen for the study was the Muskeg River site used in Section 2.1. A water chemistry profile was obtained at three stations, midstream, stream edge, and 30 m from it. A set of sampling capsules was inserted into the gravels and soil at a series of depths using a percussion-driven spike. A 17 mm steel rod, sheathed with a 22 mm steel tube such that the solid nose cone of the steel rod just protruded, was hammered into the ground with the detachable driving head (Figure 37). On reaching the desired depth, the steel rod was withdrawn leaving the sheath in the ground. A plastic tube with a sampling capsule on the end was inserted down the sheath with a guide rod. The capsulses were made from a 10 cm^3 Auto Analyser cup with the sampling tubes set into these using a P.V.C. plug (Figure 37. When the capsule was in position, the sheath and guide rod were carefully withdrawn leaving the capsule at the desired depth. During this operation, the sheath was gently worked around so as to collapse the hole behind it. Because of the disturbance caused by the removal of the sheath, a minimum of 48 hours was allowed

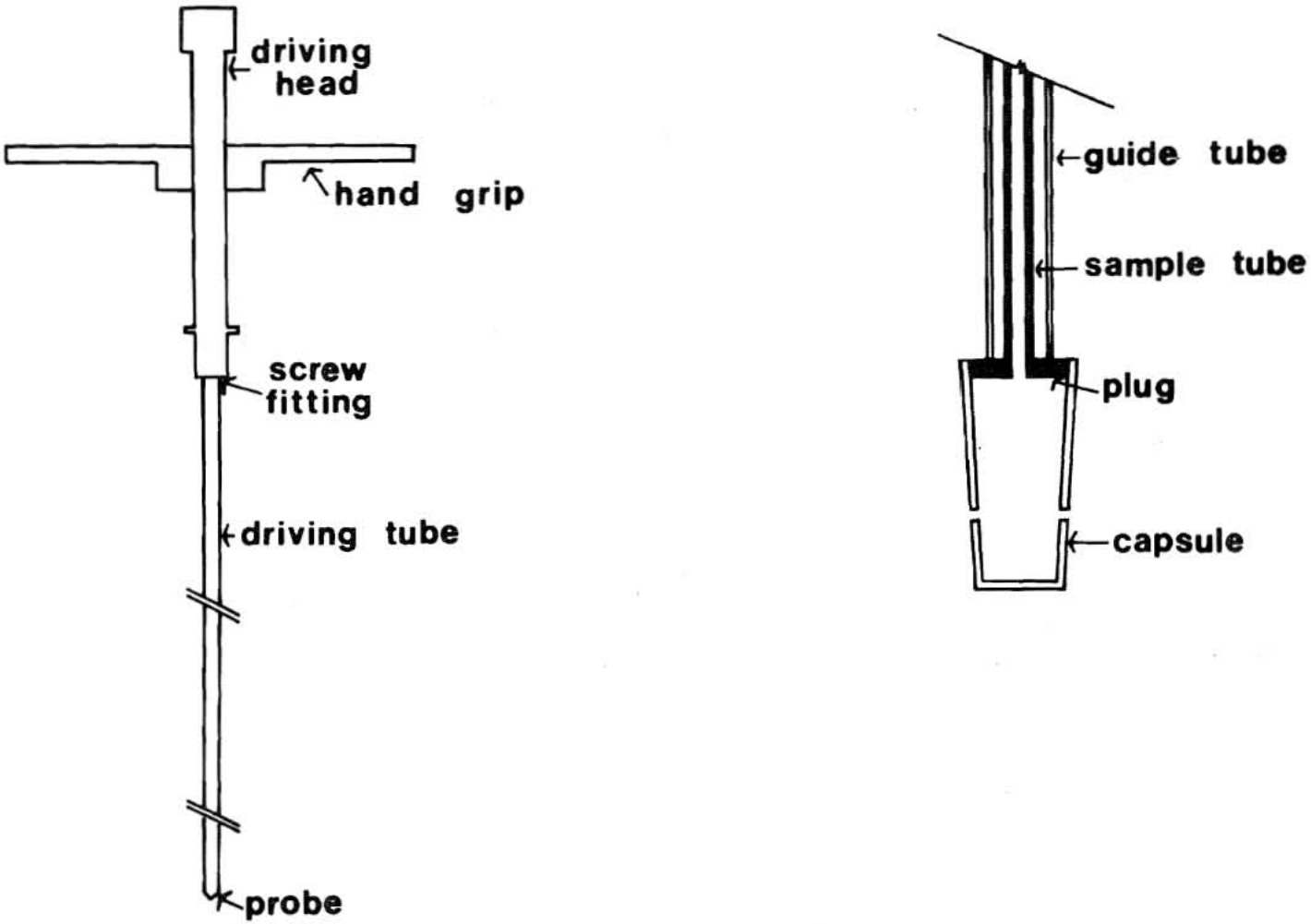

Figure 37. Percussion driven spike and sampling capsule for monitoring interstitial water quality.

prior to removing the sample. This was done by attaching the free end of the plastic tube to a 500 mℓ Erlenmyer flask with a side-arm and sucking the sample into this by the application of a hand vacuum pump. The first 100 mℓ were always discarded so that the tubing was flushed with the water about to be sampled.

Water samples were taken on 30 October 1977 for the following analyses, dissolved organic carbon, Kjeldahl nitrogen, total phosphorus, PO_4-P, NO_3 + NO_2-N, and NH_4-N. The subsample for dissolved organic carbon analysis was filtered through a precombusted fibreglass filter (0.45 µm), acidified, stored at 4° C, and analysed within three days of collection. The other samples were frozen at -18° C and then shipped to Chemex Limited for analysis. The samples for bacterial analysis were taken last to permit maximum flushing in order to reduce contamination. It was not possible to obtain aseptic samples, but they were collected and filtered directly onto 0.2 µm Nuclepore membranes using the method noted in Section 2.1.2. The total water volume removed was kept to a minimum to reduce the possibility of drawing in water from zones above and below the point of sampling.

5.3 RESULTS

On three out of nine depths sampled, we were unable to obtain information on the bacteria because of the exceedingly high clay/silt content of the water. In general, bacterial numbers $m\ell^{-1}$ were around 1×10^5 except at 0.5 m depth in the midstream where they were much higher at 7.5×10^5 $m\ell^{-1}$ (Figure 38, Table 11).

The levels of dissolved organic carbon (Figure 37, Table 11) were similar at all sampling stations, an average of 26.4 with a range of 23.7 - 33.5 mg ℓ^{-1}, the one high level of 33.5 mg ℓ was at 0.1 m in the midstream.

Table 11. Bacterial numbers and concentration of organic and inorganic nutrients in the interstitial water of the Muskeg River and its environs.

LOCATION and DEPTH	Bacteria no $m\ell^{-1}$	Dissolved Organic Carbon mg ℓ^{-1}	Dissolved Organic Nitrogen mg ℓ^{-1}	Dissolved Organic Phosphorus mg ℓ^{-1}	PO_4 - P mg ℓ^{-1}	NO_3 + NO_2 - N mg ℓ^{-1}	NH - N mg ℓ^{-1}
BANK							
(Below groundwater table)							
0.25	-	27.3	4.00	0.572	0.003	0.032	0.410
0.65	-	26.5	1.88	0.565	0.010	0.103	0.680
EDGE							
(Below groundwater table)							
0.1	9.0×10^4	27.8	2.39	0.408	0.012	0.235	0.170
0.9	2.3×10^4	24.9	0.75	0.165	0.003	0.024	0.330
1.9	4.6×10^4	24.1	0.68	0.255	0.011	0.029	0.290
MID-STREAM							
(Below sediment/water interface)							
0	1.5×10^6	24.9	0.4	0.024	0.004	0.043	0.060
0.1	8.5×10^4	33.5	1.62	0.213	0.007	0.051	0.090
0.25	1.6×10^5	24.9	1.64	0.199	0.007	0.048	0.090
0.50	7.5×10^5	24.9	1.26	0.208	0.015	0.052	0.120
0.70	-	23.7	2.01	0.548	0.012	0.090	0.220

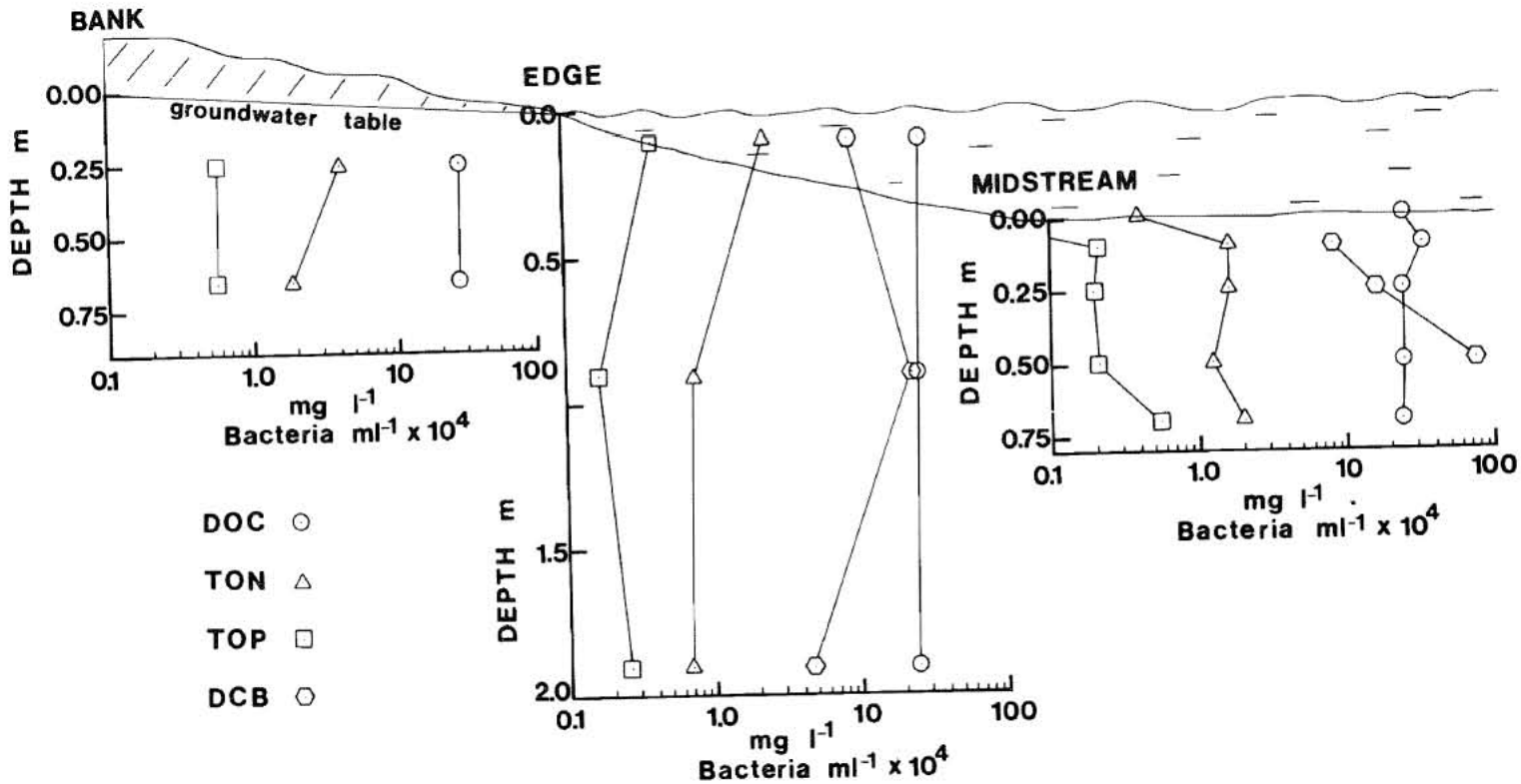

Figure 38. Numbers of bacteria ml^{-1} and concentration of organic nutrients in the interstitial water of the Muskeg River.
DOC - dissolved organic carbon
TON - total organic nitrogen
TOP - total organic phosphorus
DCB - direct counts of bacteria

The level in the river was 24.9 mg ℓ^{-1}. The levels of total organic nitrogen (Total Kjeldahl nitrogen - Inorganic N) were very much more variable, but all were very much higher than that of river water (0.5 mg ℓ^{-1}). At 0.25 m in the bank, very high organic nitrogen levels were found (4.00 mg ℓ^{-1}). At the edge of the river, at a depth of 0.1 m, a level of 2.39 mg ℓ^{-1} was recorded, the second highest level in the study, but at 0.9 and 1.9 m, the levels were very much lower, around 0.7 mg ℓ^{-1}. In the midstream, the levels were generally similar with the higher concentration being recorded at 0.70 m depth (Figure 38, Table 11). Total organic phosphorus levels (Figure 38) of the interstitial water ranged from around 0.570 mg ℓ^{-1} to 165 mg ℓ^{-1}. Highest levels were recorded in the bank 0.572 and 0.565 at 0.25 m and 0.65 m, respectively. The next highest level was in the midstream (0.548 mg ℓ^{-1}) at a depth of 0.7 m, followed by that at the edge (0.408 mg ℓ^{-1}) at a depth of 0.1 m. At the remaining depths, the total organic phosphorus was around 0.200 mg ℓ^{-1}. In comparison, the level of dissolved organic phosphorus of the river water was only 0.024 mg ℓ^{-1}. The concentration generally increased with depth for organic phosphorus, the exception being at 0.1 m at the river edge. There, the concentrations ranged from 0.003 mg to 0.015 mg ℓ^{-1}, while the concentration in the river was 0.004 mg PO_4-P ℓ^{-1}.

The levels of nitrate-nitrogen (Figure 39, Table 11) were generally around 0.020 - 0.050 mg ℓ^{-1}. There was, however, one very high value and this occurred again at the river edge at a depth of 0.1 m. The highest levels of ammonium nitrogen were recorded from the bank site, 0.410 and 0.680 mg ℓ^{-1}. Intermediate levels were found at depths of 0.9 and 1.9 m at the edge (around 0.300 mg ℓ^{-1}), however, the concentration was approximately half of this at 0.1 m (0.170 mg ℓ^{-1}). In

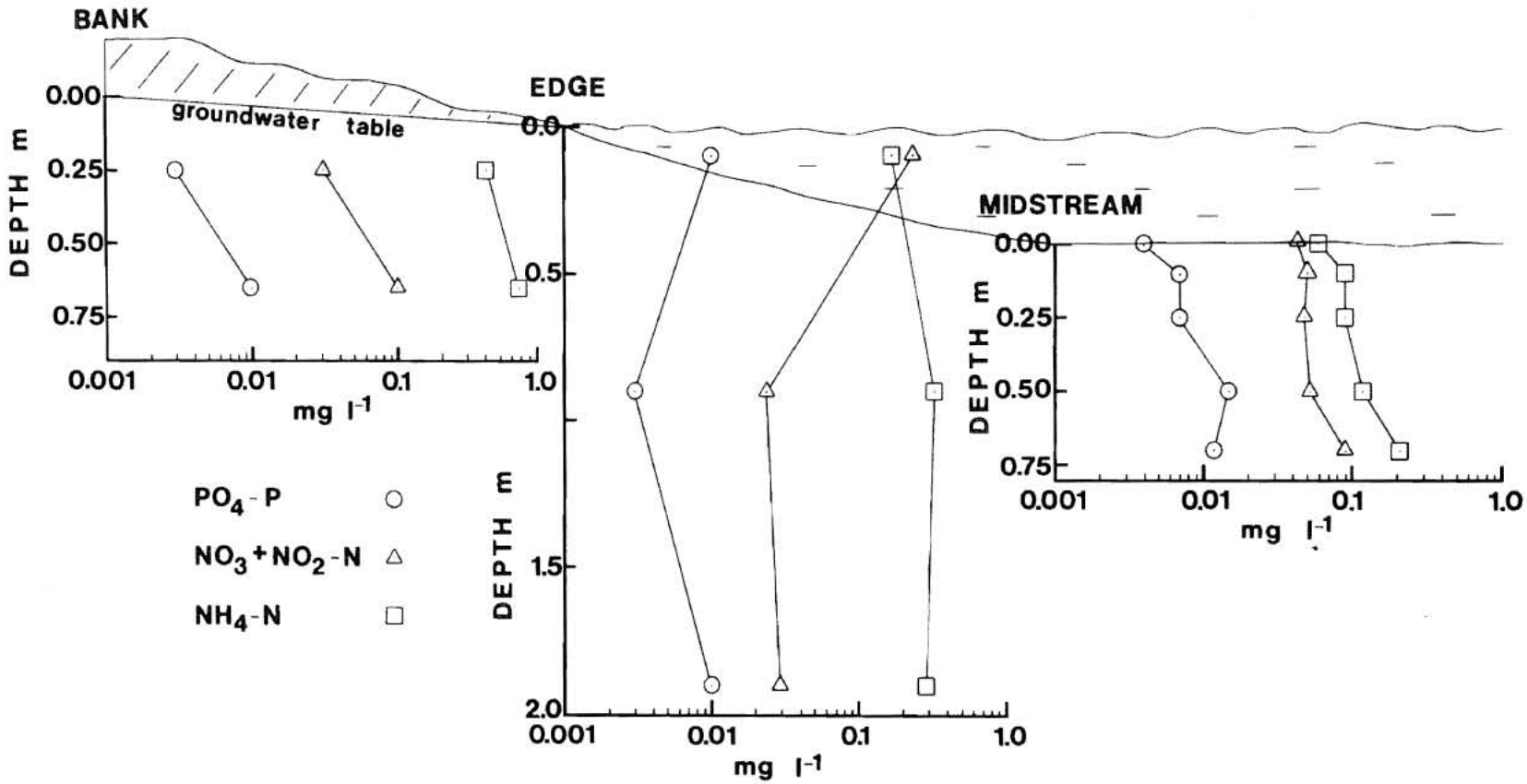

Figure 39. Concentration of inorganic nutrients in the interstitial water of the Muskeg River.

midstream, at 0.1 - 0.5 m depth, the concentration was around 0.100 mg ℓ^{-1}, but increased to 0.220 mg ℓ^{-1} at a depth of 0.70 m. The concentration of ammonium nitrogen in the river was only 0.060 mg ℓ^{-1}.

5.4 DISCUSSION

It is readily apparent that the interstitial water is not homogenous, except for dissolved organic carbon, a situation which has also been observed in a small alpine watershed in S. Alberta (P. Wallis pers. comm.). For dissolved organic phosphorus and ammonium-nitrogen, there is a trend of decreasing concentration toward the center of the river. This would suggest that if the net flow of water is toward the river, these two moieties are being converted into a particulate form by incorporation into microbial biomass or by chemical processes. Two other major anomalies occur at 0.1 m depth at the edge of the river and at the deepest sampling point in the midstream (0.7 m). At the former, there is an elevation in all parameters, except ammonium -nitrogen, which was lower than the concentration preceding it further down into the gravel. In contrast, the nitrate-nitrogen level was the highest recorded in the survey. The possibility, therefore, exists that, depending upon the flow of water, the microbial conversion of ammonium to nitrate, or vice versa, was occurring. It is also interesting to note that at this sampling point the total organic nitrogen was also high. This could be indicative of high microbial activity. However, such a suggestion is not borne out by the bacterial counts, but these, of course, only represent the bacteria found in the free water. We have no information on the attached populations which are likely to be proportionately higher (Section 2.1). At the deepest midstream station, high levels (approaching a 2-fold

increase over the other depths) were recorded for all chemical parameters measured, with the exception of dissolved organic carbon.

Therefore, from a biogeochemical standpoint, it appears that, if water is entering or leaving the substrata in this stretch of the river, it may not be undergoing purely physical or chemical processes. This study provides circumstantial evidence of immobilization and regeneration of organic and inorganic nutrients at different depths of the interstitial water. However, there is a much broader observation to be made regarding chemical composition of the river water (0 m in the mid-stream), as it is radically different from the interstitial water anywhere else, with most parameters being of a lower concentration.

If it is assumed that water is entering the river at this point, and that the situation here is representative of a considerable length of river, then a large proportion of the organic and inorganic nutrients are possibly being removed as the water flows up through the last 0.1 m of the stream bed. If this is indeed so, the most likely agent for the removal of such material is the microbial material coating the rocks and gravels. The cyclical activity of the bacteria and algae of the river could have a profound effect upon the chemistry of the river water.

There are, however, alternative non-biological hypotheses to explain the observed variability in the chemical composition of the water, in and below the stream:

1. Dispersive mixing of stream water and groundwater beneath the stream.
2. Variable flow path lengths and/or different origins for groundwater entering different areas of the stream.

It is, therefore, clear that further work is required to assess the role played by groundwater in the energy and nutrient dynamics of muskeg rivers.

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7. APPENDICES

7.1 KEYS USED IN THE IDENTIFICATION OF ALGAE

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7.2 SPECIES OF ALGAE IN THE MUSKEG AND STEEPBANK RIVERS.

BACILLARIOPHYTA

Achnanthes exigua Grunow
A. lanceolata (Brèb.) Grunow
A. lanceolata var. *rostrata* Hustedt
A. minutissima Kuetzing
Amphipleura pellucida Kuetzing
Amphora ovalis Kuetzing
Caloneis bacillum Grunow
Cocconeis pediculatus Ehrenberg
C. placentula Ehrenberg
C. placentula var. *lineata* (Ehrenberg) Cleve
Cyclotella sp.
Cymatopleura solea (Brèb.). W. Smith
Cymbella caespitosa (Kuetzing) Brun
C. gracilis (Rabenhorst)
C. lacustris (Agardh.) Cleve
C. prostrata Berkeley
C. ventricosa Kuetzing
Diatoma elongatum Agardh.
D. vulgare Bory
Epithemia argus Kuetzing
E. sorex Kuetzing
E. turgida (Ehrenberg) Kuetzing
E. turgida var. *westermanii* Keutzing
E. zebra (Ehrenberg) Kuetzing
Fragillaria capucina Desmazieres
F. construens (Ehrenberg) Grunow
F. virescens Ralfs
Gomphonema acuminatum Ehrenberg
G. constrictum Ehrenberg
G. gracile E. Cleve
G. intricatum (Kuetzing) Cleve

G. longiceps Ehrenberg
G. olivaceum (Lyngb.) Kuetzing
G. parvulum Kuetzing
Gyrosigma keutzingii Kuetzing
Melosira varians C. A. Agardh.
Meridion sp.
Navicula anglica Ralfs
N. bacillum Ehrenberg
N. cryptocephala Kuetzing
N. halophila Grunow
N. muralis Grunow
N. oblonga-subcapitata Kuetzing
N. pygmeae Kuetzing
N. rhyncocephala Kuetzing
N. viridula Kuetzing
Nitzschia sp.
N. acicularis W. Smith
N. amphibia Grunow
N. fonticola Grunow
N. perminuta Grunow
N. sublinearis Hustedt.
Pinnularia brevicostata Cleve
Rhapoldia gibba (Ehrenberg) H. Mull.
R. rhopala (Ehrenberg) Hustedt
Rhoicosphenia curvata (Kuetzing) Grunow
Stauroneis legumen Ehrenberg
Stephanodiscus sp.
Synedra acus Kuetzing
S. faciculata (Agardh.) Kuetzing
S. parasitica W. M. Smith
S. pulchella var. *minuta* (Ralfs) Kuetzing
S. rumpens Kuetzing
S. ulna (Nitzsch.) Ehrenberg
Tabellaria fenestrata (Lyngb.) Kuetzing

T. flocculosa (Roth.) Kuetzing

CYANOPHYTA

Anabaena sp.

A. wisconsinense Prescott

Aphanocapsa sp.

A. elachista West & West

A. pulchra West & West

Aphanozymenon flos-aquae Kuetzing

Calothrix breviarticulata West & West

C. epiphytica West & West

Chaemosiphon curvatas Nordstedt

C. incrustans Grunow

Chroocuccus turgidis (Kuetzing) Naegeli

Geotrichia longiarticulata G. S. West

Lyngbya sp.

L. aergineo-caerulea (Kuetzing) Gomont

L. epiphytica Hieronymus

L. nordgaardii Wille

L. taylorii Drouet & Strickland

Microcystis aeruginosa Kuetzing

Nostoc commune Vaucher

N. microscopicum Carmichael

N. paludosum Kuetzing

Oscillatoria agardhii Gomont

O. lacustris (Kleb.) Geitler

O. tenuis Kuetzing

O. tenuis var. *tergestina* Kuetzing

Rivularia haematites (D.C.) C. A. Agardh.

Schizothrix purpurescens (Kütz.) Gomont

S. tinctoria Gomont

Spirulina laxa G. M. Smith

Tolypothrix limbata Thuret

CHLOROPHYTA

Ankistrodesmus sp.
Bulbochaete sp.
Chaetophora incrassata (Huds.) Hazen
Chlamydomonas sp.
Cladophora glomerata (L.) Kuetzing
Closteriopsis longissima Lemmermann
Draparnaldia acuta (C. A. Agardh.) Kuetzing
D. plumosa (Vauch.) C. A. Agardh
Euglena gracilis Klebs
Merismopedia elegans (A. Braun) Kuetzing
Microspora pachyderma Wille
Pithophora varia Wille
Rhizoclonium hierglyphicum var. *hosfordii* (Agardh.) Keutzing
Scenedesmus obliquus (Turpin) Kuetzing
Spirogyra sp.
Stigeoclonium pachydermum Prescott
Ulothrix subconstricta G. S. West
U. tenerrima Kuetzing
U. zonata (Weber & Mohr) Kuetzing
Uronema elongatum Hodgetts

RHODOPHYTA

Audouinella chalybea (Lyngb.) Fries
A. pygmaea Kuetzing
Bactrachospermum vagum (Roth) C. A. Agardh.

CRYPTOPHYTA

Cryptomonas erosa Ehrenberg
C. minuta Ehrenberg
C. ovata Ehrenberg
Rhodomonas sp.

CHRYSOPHYTA

Chromulina sp.
Mallomonas caudata Iwanoff

8. AOSERP RESEARCH REPORTS

1.		AOSERP First Annual Report, 1975
2.	AF 4.1.1	Walleye and Goldeye Fisheries Investigations in the Peace-Athabasca Delta--1975
3.	HE 1.1.1	Structure of a Traditional Baseline Data System
4.	VE 2.2	A Preliminary Vegetation Survey of the Alberta Oil Sands Environmental Research Program Study Area
5.	HY 3.1	The Evaluation of Wastewaters from an Oil Sand Extraction Plant
6.		Housing for the North--The Stackwall System
7.	AF 3.1.1	A Synopsis of the Physical and Biological Limnology and Fisheries Programs within the Alberta Oil Sands Area
8.	AF 1.2.1	The Impact of Saline Waters upon Freshwater Biota (A Literature Review and Bibliography)
9.	ME 3.3	Preliminary Investigations into the Magnitude of Fog Occurrence and Associated Problems in the Oil Sands Area
10.	HE 2.1	Development of a Research Design Related to Archaeological Studies in the Athabasca Oil Sands Area
11.	AF 2.2.1	Life Cycles of Some Common Aquatic Insects of the Athabasca River, Alberta
12.	ME 1.7	Very High Resolution Meteorological Satellite Study of Oil Sands Weather: "a Feasibility Study"
13.	ME 2.3.1	Plume Dispersion Measurements from an Oil Sands Extraction Plant, March 1976
15.	ME 3.4	A Climatology of Low Level Air Trajectories in the Alberta Oil Sands Area
16.	ME 1.6	The Feasibility of a Weather Radar near Fort McMurray, Alberta
17.	AF 2.1.1	A Survey of Baseline Levels of Contaminants in Aquatic Biota of the AOSERP Study Area
18.	HY 1.1	Interim Compilation of Stream Gauging Data to December 1976 for the Alberta Oil Sands Environmental Research Program
19.	ME 4.1	Calculations of Annual Averaged Sulphur Dioxide Concentrations at Ground Level in the AOSERP Study Area
20.	HY 3.1.1	Characterization of Organic Constituents in Waters and Wastewaters of the Athabasca Oil Sands Mining Area

21.		AOSERP Second Annual Report, 1976-77
22.		
23.	AF 1.1.2	Acute Lethality of Mine Depressurization Water on Trout Perch and Rainbow Trout
24.	ME 4.2.1	Air System Winter Field Study in the AOSERP Study Area, February 1977.
25.	ME 3.5.1	Review of Pollutant Transformation Processes Relevant to the Alberta Oil Sands Area
26.	AF 4.5.1	Interim Report on an Intensive Study of the Fish Fauna of the Muskeg River Watershed of Northeastern Alberta
27.	ME 1.5.1	Meteorology and Air Quality Winter Field Study in the AOSERP Study Area, March 1976
28.	VE 2.1	Interim Report on a Soils Inventory in the Athabasca Oil Sands Area
29.	ME 2.2	An Inventory System for Atmospheric Emissions in the AOSERP Study Area
30.	ME 2.1	Ambient Air Quality in the AOSERP Study Area, 1977
31.	VE 2.3	Ecological Habitat Mapping of the AOSERP Study Area: Phase I
32.		AOSERP Third Annual Report, 1977-78
33.	TF 1.2	Relationships Between Habitats, Forages, and Carrying Capacity of Moose Range in northern Alberta. Part I: Moose Preferences for Habitat Strata and Forages.
34.	HY 2.4	Heavy Metals in Bottom Sediments of the Mainstem Athabasca River System in the AOSERP Study Area
35.	AF 4.9.1	The Effects of Sedimentation on the Aquatic Biota
36.	AF 4.8.1	Fall Fisheries Investigations in the Athabasca and Clearwater Rivers Upstream of Fort McMurray: Volume I
37.	HE 2.2.2	Community Studies: Fort McMurray, Anzac, Fort MacKay
38.	VE 7.1.1	Techniques for the Control of Small Mammals: A Review
39.	ME 1.0	The Climatology of the Alberta Oil Sands Environmental Research Program Study Area
40.	VE 7.1	Interim Report on Reclamation for Afforestation by Suitable Native and Introduced Tree and Shrub Species
41.	AF 3.5.1	Acute and Chronic Toxicity of Vanadium to Fish
42.	TF 1.1.4	Analysis of Fish Production Records for Registered Traplines in the AOSERP Study Area, 1970-75
43.	TF 6.1	A Socioeconomic Evaluation of the Recreational Fish and Wildlife Resources in Alberta, with Particular Reference to the AOSERP Study Area. Volume I: Summary and Conclusions
44.	VE 3.1	Interim Report on Symptomology and Threshold Levels of Air Pollutant Injury to Vegetation, 1975 to 1978
45.	VE 3.3	Interim Report on Physiology and Mechanisms of Air-Borne Pollutant Injury to Vegetation, 1975 to 1978

46.	VE 3.4	Interim Report on Ecological Benchmarking and Biomonitoring for Detection of Air-Borne Pollutant
47.	TF 1.1.1	A Visibility Bias Model for Aerial Surveys of Moose on the AOSERP Study Area
48.	HG 1.1	Interim Report on a Hydrogeological Investigation of the Muskeg River Basin, Alberta
49.	WS 1.3.3	The Ecology of Macrobenthic Invertebrate Communities in Hartley Creek, Northeastern Alberta
50.	ME 3.6	Literature Review on Pollution Deposition Processes
51.	HY 1.3	Interim Compilation of 1976 Suspended Sediment Data in the AOSERP Study Area
52.	ME 2.3.2	Plume Dispersion Measurements from an Oil Sands Extraction Plant, June 1977
53.	HY 3.1.2	Baseline States of Organic Constituents in the Athabasca River System Upstream of Fort McMurray
54.	WS 2.3	A Preliminary Study of Chemical and Microbial Characteristics of the Athabasca River in the Athabasca Oil Sands Area of Northeastern Alberta.
55.	HY 2.6	Microbial Populations in the Athabasca River
56.	AF 3.2.1	The Acute Toxicity of Saline Groundwater and of Vanadium to Fish and Aquatic Invertebrates
57.	LS 2.3.1	Ecological Habitat Mapping of the AOSERP Study Area (Supplement): Phase I

These reports are not available upon request. For further information about availability and location of depositories, please contact:

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