ALBERTA OIL SANDS ENVIRONMENTAL RESEARCH PROGRAM

A Review of Aquatic Biomontioring with Particular Reference to its Possible Use in the AOSERP Study Area

Project WS 3.5

June 1980

Sponsored jointly by

Alberta Environment

Environment Canada

Environnement Canada

15th Floor, Oxbridge Place
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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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A Review of Aquatic Biomonitoring
With Particular Reference to its Possible Use
in the AOSERP Study Area

Project WS 3.5

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

and

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

Sirs:

Enclosed is the report "A Review of Aquatic Biomonitoring with Particular Reference to its Possible Use in the AOSERP Study Area".

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

Respectfully,

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Chairman, Steering Committee, AOSERP
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A REVIEW OF AQUATIC BIOMONITORING WITH PARTICULAR REFERENCE TO ITS POSSIBLE USE IN THE AOSERP STUDY AREA

DESCRIPTIVE SUMMARY

Projected development of the Athabasca Oil Sands area probably will subject the Athabasca River drainage basin to increasing loads of pollutants. These can be expected to emanate from a variety of sources, including domestic sewage facilities, industrial effluents, saline groundwater from mining operations, fall-out of stack emissions, and possibly oil spills. The nature of such pollution would be variable and complex, and heavy metals, and organic substances, such as hydrocarbon derivatives.

Other studies are being conducted to assess the environmental effects of these substances through physical and chemical analyses of various effluents and natural regional water and also through bioassays involving fish and invertebrates. As of yet, no effort has been made to monitor the environmental effects of development through the use of field biomonitoring techniques.

The primary objective of this report was to review and assess the relevance of available aquatic biomonitoring techniques which could be applied in the AOSERP study area. This project was also intended to provide the initial step in defining the process of relating aquatic biomonitoring with water quality monitoring and assimilative capacity.

A draft of this report was reviewed by scientists in Alberta Environment, Environment Canada, University of British Columbia, and the oil sands industry and the authors were able to respond to the reviews in the final report.

The report, "A Review of Aquatic Biomonitoring with Particular Reference to its possible Use in the AOSERP Study Area", is recommended for distribution to selected Canadian libraries. The authors are thanked for their contribution.

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A REVIEW
OF AQUATIC BIOMONITORING
WITH PARTICULAR REFERENCE TO ITS POSSIBLE USE
IN THE AOSERP STUDY AREA

by

P.J. McCart
D.W. Mayhood

Aquatic Environments Limited

for

ALBERTA OIL SANDS ENVIRONMENTAL
RESEARCH PROGRAM

WS 3.5

June 1980

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ABSTRACT

The general principles, approaches, and methods of aquatic biomonitoring are outlined from a review of the literature, with emphasis on those aspects directly applicable to the Alberta Oil Sands Environmental Research Program (AOSERP) study area. It is argued that an aquatic biomonitoring program must be implemented in the study area to ensure that measures taken to protect the water systems are working, so that improvements may be made if need be. A series of suggestions for an aquatic biomonitoring program in the study area are made.

The report recommends that:

1. A biomonitoring program be initiated in the study area;
2. Routine biomonitoring be preceded by at least a year of preliminary studies to establish the methodology that should be applied routinely, over the long term;
3. Consideration be given to monitoring four major groups of aquatic organisms (benthic invertebrates, periphyton, sessile bacteria, and fish); and
4. The biomonitoring program should be the responsibility of a group specializing in such studies.

ACKNOWLEDGEMENTS

This report benefitted greatly from the concerns and suggestions presented by the participants in the biomonitoring workshop held 13 June 1979 in Edmonton. Subsequently, discussions were held with several interested parties to obtain additional suggestions for a possible aquatic biomonitoring program in the Athabasca Oil Sands area. These people were: A. Masuda, Pollution Control Division, Alberta Environment; P. Shewchuk, Standards and Approvals Division, Alberta Environment; Klaus Exner, Pollution Control Division, Alberta Environment; M. Aleksiuk and J. Retallack, Syncrude Canada Ltd.; and W. Cary and R. Martin, Suncor Limited. Many people reviewed a draft of the report and presented detailed comments on it. These included R. Seidner, AOSERP, Alberta Environment; M. Aleksiuk and J. Retallack, Syncrude Canada Ltd.; H. Boerger, Department of Biology, University of Calgary; J. Moore, Pollution Control Division, Alberta Environment; D. Robinson, Environmental Protection Service, Environment Canada; and one or more anonymous reviewers. All viewpoints expressed were taken into account for the final report, and we are grateful to those who contributed them. The final content of the report, however, represents the views of the authors alone.

We thank Cecilia Gossen for typing the draft and final report.

This research project WS 3.5 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 north-eastern Alberta.

1. INTRODUCTION

This report reviews information pertaining to the biomonitoring of aquatic communities, with special reference to the Athabasca Oil Sands area. The objectives of the review were, within the limits of the information currently available in the literature, to:

1. Review aquatic biomonitoring methodology with special attention to diversity indices;
2. Relate the kinds of water system perturbations likely to result from oil sands developments; and
3. Summarize the kinds of information likely to result from the application of various methodologies.

It has not been possible to exhaustively review the massive literature relating to aquatic biomonitoring. Instead, those papers in the biomonitoring literature which elucidated general principles, summarized widely used approaches and methods, or presented promising new approaches or methods were concentrated on. Emphasis was placed on those aspects directly relating to problems likely to be encountered in the Athabasca Oil Sands area and the literature describing aquatic communities within the Athabasca Oil Sands region. In addition to the review, a workshop was conducted which included participants from government and industry (Table 1) and discussions were held with individuals interested in the possibility of biomonitoring within the Alberta Oil Sands Environmental Research Program (AOSERP) study area. The report incorporates many of the concerns and suggestions expressed during the workshop and discussions with individuals.

Table 1. Participants in AOSERP Workshop on Aquatic Biomonitoring, 13 June 1979.

	Name	Affiliation
1.	Akena, A. Mark	Alberta Environment, Pollution Control Division
2.	Avramenko, Anne	Alberta Environment, AOSERP
3.	Beliveau, Dave	Alberta Environment, Pollution Control Division
4.	Bergstrom, Glen	Alberta Forestry Service, Forest Land Use Branch
5.	Borutski, David	Alberta Recreation, Parks and Wildlife, Fish and Wildlife Division, Fisheries Habitat
6.	Cary, Bill	Suncor
7.	Exner, Klaus	Alberta Environment, Pollution Control Division
8.	Findlay, Ron L.	Amoco Canada Petroleum Co.
9.	Hall, Ken J.	Westwater Research Centre
10.	Hammond, B.	Alberta Environment, Research Secretariat
11.	Hamilton, Hal	Alberta Environment, Pollution Control Division
12.	Johnston, Les	Alberta Environment, Standards and Approvals
13.	Khan, B.A.	Alberta Environment, AOSERP
14.	Lake, Bill	Alberta Environment, Pollution Control Division
15.	Lillie, L.	Alberta Environmental Centre
16.	Martin, Robert	Suncor
17.	Mayhood, Dave	Aquatic Environments Limited
18.	McCart, P.J.	Aquatic Environments Limited
19.	Munson, Barry	Alberta Environment, AOSERP
20.	Pledger, Dave	Alberta Environment, Pollution Control Division
21.	Retallack, J.	Syncrude Canada Ltd.
22.	Sargent, Randy	Alberta Environment, Pollution Control Division
23.	Seidner, Read	Alberta Environment, AOSERP
24.	Soluk, Dan	Alberta Environment, Pollution Control Division
25.	Smith, S.B.	Alberta Environment, AOSERP
26.	Thimm, Harald	Energy Resources Conservation Board
27.	Wuite, John	Alberta Environment, Planning Division
28.	Yonge, Eva	Alberta Environment, Pollution Control Division

2. SOME GENERAL CONSIDERATIONS

2.1 A DEFINITION

Biological monitoring of aquatic ecosystems assesses the responses of selected aquatic organisms to changes in their environment. In urban and industrial settings, this can be done:

1. *In-plant* (e.g., in the refinery), using either bioassay procedures or by more elaborate methods which measure such parameters as the respiratory response (e.g., opercular movements in fish), coughing reflex, etc., of test organisms; or
2. *In-stream*, by determining what changes have occurred in selected natural populations of aquatic organisms which can be ascribed to the discharge of liquid effluents or other materials (e.g., gases, sediments).

The latter, in-stream biomonitoring of natural populations of aquatic organisms, is the subject of this discussion.

2.2 PURPOSE

There are two basic reasons for undertaking a biomonitoring program:

1. To provide an early warning of incipient damage to biotic communities from an impacting agent; and
2. To determine if human activities, especially industrial development, are affecting biotic communities.

Aquatic biomonitoring in the AOSERP study area could be done for both reasons, but the latter reason is particularly convincing. Considerable effort has been put into measures to protect the environment in the development area, and it is important to determine that these measures are, in fact, working.

2.3 AN EXAMPLE

A good example of the kind of information that can be derived from biomonitoring is Patrick's (1977) studies of the Savannah River which forms the Georgia-South Carolina border. She

was able to correlate changes in the numbers of species in several groups of aquatic organisms (algae, arthropods, and fish) with various human activities in the river basin (Figure 1). The clearest responses were the changes in the numbers of algal species which:

1. Increased markedly as dam closure in 1955 led to reduced sediment loads in the river;
2. Declined as dredging in the late 1950's and early 1960's again raised sediment loads;
3. Increased again as dredging ceased;
4. Declined a second time as industrial pollution increased during the 1960's; and
5. Increased for a third time as more rigid controls on industrial pollution led to improved environmental conditions in the early 1970's.

Note that the data presented indicate only numbers of species within groups, a relatively insensitive parameter. Even so, Patrick's data demonstrate the kinds of information that can be generated by consistent application of biomonitoring techniques over a long period of time.

2.4 BASIC ASSUMPTIONS

Aquatic ecosystems are composed of a complex of interdependent organisms. Figure 2 indicates some of the interrelationships possible between major taxonomic and functional groups inhabiting streams. The relationships at the species level would be vastly more complex than this. The basic assumption of all in-stream biomonitoring is that changes in the environment, whether natural or man-made, will be reflected in changes in aquatic ecosystems and in the characteristics of individual components, either species or groups of species (communities), within them. Biomonitoring can be used to reflect both short-term and long-term changes in environmental conditions. Microbial populations, which may respond to changed environmental conditions within a matter of hours, certainly within a few days, can be useful in assessing

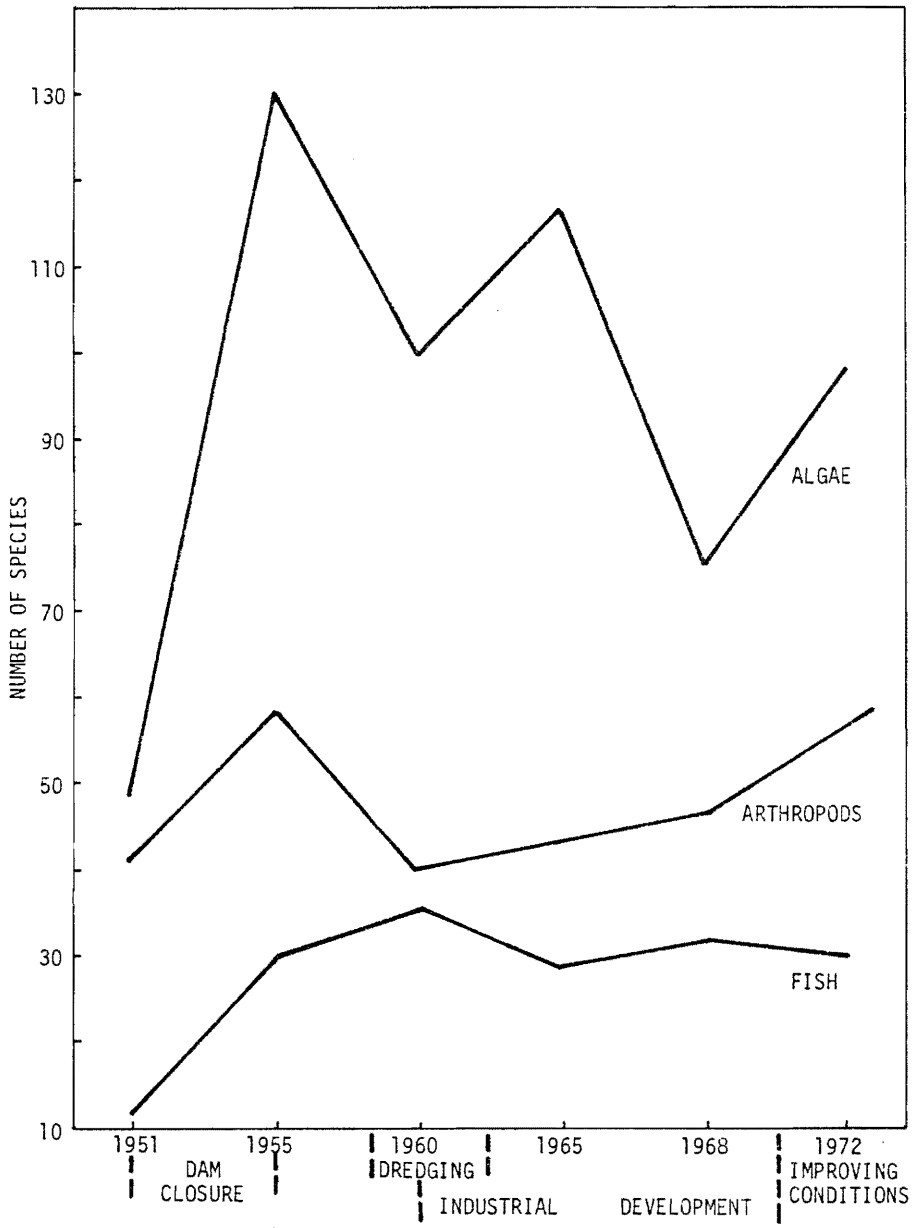

Figure 1. Changes in numbers of species of algae, arthropods, and fish in the Savannah River during the course of long-term biomonitoring studies. Data summarized from Patrick (1977).

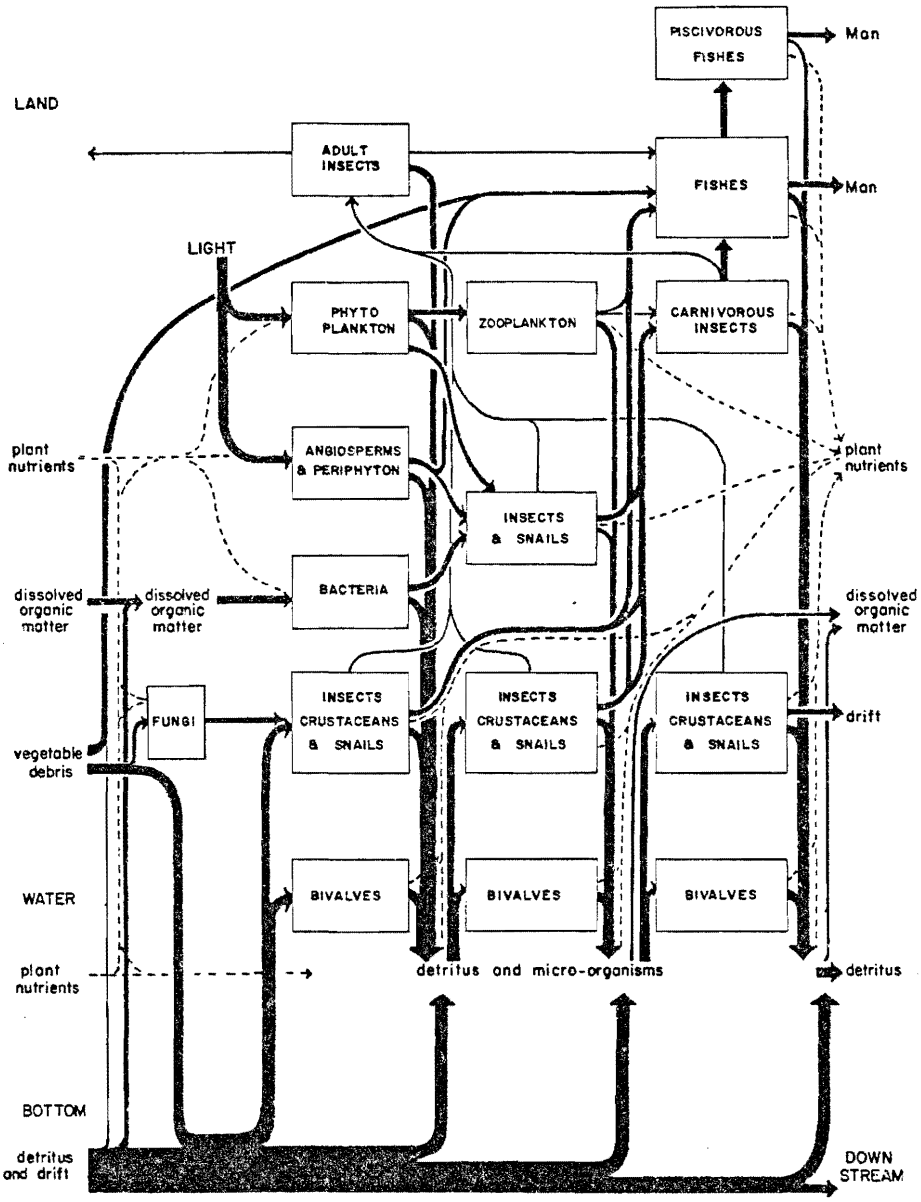

Figure 2. Major energy pathways of a stream ecosystem, illustrating the importance of benthic macro-invertebrates. (Adapted from Hynes 1970).

short-term change. Other groups, such as benthic invertebrates and, in some circumstances, fish, are better suited to assessing change over longer periods, from several months to years.

One of the basic tenets of biomonitoring is that it is not necessary to understand and monitor the aquatic community in general or even all the species within individual major groups (e.g., bacteria, protozoa, periphyton, benthic invertebrates) to establish that major environmental changes have taken place. Because of their interrelationships, if there are demonstrable changes in one segment of the aquatic community, it is likely that other segments have also been affected. Biomonitoring is applied, not pure, research and, for this reason, practicality and economics are an important consideration. Biomonitoring programs are established to provide an "index" of environmental change, not to measure all of the biological effects of such change, something that is, in any case, well beyond the present capabilities of the biological sciences. The aim should be to choose groups of organisms which are, first, thought to be sensitive to kinds of environmental change which are likely to occur; second, reasonably well known taxonomically and ecologically; and third, easily and efficiently sampled and analysed. The fact that organisms which meet these criteria may constitute only a small proportion of the total numbers of organisms or of the biomass of aquatic communities is not, in itself, a very persuasive criticism. As indicated, the value of biomonitoring lies in its application and the only really cogent question is, "Does it work?"

A good example of a biomonitoring program in which a small group of organisms has been used to assess or "index" the status of an entire community is the wide application of the coliform test. Coliform bacteria occur naturally but they are also a major constituent of the bacterial flora of domestic sewage. While pathogenic coliforms do occur, the major value of the coliform test is to determine the presence of significant quantities of untreated human waste including potentially serious pathogens. While it might be argued that it would be preferable to test for the pathogens

themselves (e.g., those causing typhoid, diphtheria, hepatitis, poliomyelitis), this is neither practical nor, in some cases, possible. While as an index the coliform test may be fallible, sometimes overestimating and sometimes underestimating the public danger, there is no question that the method works at the practical level and that society at large has benefited. It is one of the few biomonitoring techniques which is a basis for action (e.g., the closure of beaches and shellfishing areas and the improvement of sewage treatment facilities). While it is easier for the public, biological scientists among them, to perceive the danger from pathogenic organisms, the coliform index is closely analogous to other forms of biomonitoring which index the more subtle, longer term, and potentially more damaging effects of environmental degradation.

2.5 CHOICE OF ORGANISMS FOR BIOMONITORING

Hellawell (1977) has considered the problem of choice of organisms in biomonitoring studies. His literature survey (ibid.:73) indicated that "... most published work recommended the use of macroinvertebrates .... Algae were also highly recommended, but fish gained a surprisingly low score" (Figure 3). Hellawell further summarized the major advantages and disadvantages of the major groups of organisms represented in fresh waters (Table 2). As the result of his review, he recommended, at least initially, the adoption of macroinvertebrates for biological monitoring on the following grounds:

1. Popularity;
2. Availability of keys for most groups (except chironomid and trichopteran larvae);
3. A high "hysteresis" value because of their sedentary or relatively stationary habits (which allows meaningful spatial analyses of results) and relatively long life cycles (making temporal analyses possible); and
4. Heterogeneity--several phyla are represented and it is possible that at least some groups would respond to a given environmental change.

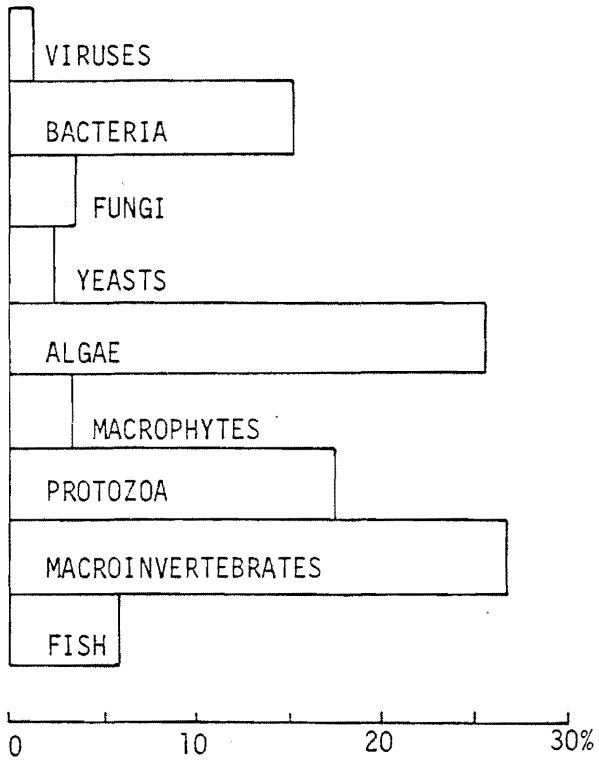

Figure 3. Percentage distribution of taxa recommended for use as indicators based on a literature survey. From Hellawell (1977).

Table 2. Advantages and disadvantages of different taxa as indicators. From Hellawell (1977).

Taxon	Advantages	Disadvantages
Bacteria	Indicators of faecal pollution. Rapid response to environmental changes, including organic and heavy metal pollution. Samples relatively easy to obtain. Routine methodology well developed. Automatic methods for total counts.	Drifting cells--origin not clear, maximum numbers probably downstream of origin of organic pollution. Rapid recovery after intermittent pollution. Need for facilities for sterilizing equipment, plating, incubating, etc. Delay in obtaining results from cultures. Problem of distinguishing dead and living cells in direct counts without special techniques. Is bacteriology of "clean" waters well known? Variability of counts.
Protozoa	Supposed rapid response to changes. Saprobic valencies well documented even within genera, e.g., *Vorticella*. Gross collection of samples easy.	Taxonomic ability required and good facilities for examination. Some dispute over indicator values since present in "normal" environments. Studies must be quantitative. Drift problems. Variability of microhabitats.
Algae	Useful for eutrophication estimates and sensitive to turbidity effects (attached forms). Pollution tolerances well documented. Automation of counting total numbers.	Not directly useful for heavy organic pollution, nor as indicators of faecal contamination. Not as sensitive to pesticides and heavy metals as other groups. Taxonomic expertise needed. Drift problems with unattached forms. Quantitative sampling difficult for epilithic/epiphytic forms. Counting tedious. Possible rapid recovery of flora. Difficulty in distinguishing living and dead cells in some cases.

Continued...

Table 2. Concluded.

Taxon	Advantages	Disadvantages
Macroinvertebrates	Many sedentary forms--localized effects of pollution detectable. Good keys for most groups. Useful for integration of pollution effects, especially where life history is long. Periodic sampling may therefore be more valid. Qualitative sampling easy. Wide range of forms and habits--community as a whole likely to be sensitive indicator and application of numerical methods (species diversity indices) probably valid. Elaborate equipment not required.	Many species drift and may be found in regions where they cannot maintain themselves indefinitely. Groups expected in "poor", and especially lowland, conditions (chironomids and oligochaetes) not easily identified. Absence may be normal part of life cycle--results must be interpreted with care. Quantitative sampling difficult. Important to choose similar substrates when sampling.
Macrophytes	Fixed and relatively easily seen and identified. Good indicators of suspended solid loads. Good indicators of enrichment of soft water.	Response to pollution not well documented. Seasonal. Tolerant of intermittent pollution. Poor indicators of enrichment of chalk streams.
Fish	Integrators of responses of food chains as well as immediate effects on physiology. Methodology well developed. Identification easy. Monitored to some extent by anglers.	Mobile--avoidance behaviour. Collection of samples needs manpower.

Despite Hellawell's recommendation that preference in biomonitoring studies be given to benthic macroinvertebrates, good arguments can be made for a number of other groups. Hellawell's own literature survey (Figure 3) suggests that there is a great deal of support for the use of algae. Patrick (1973) recommends the use of periphytic algae, the diatom community in particular, to monitor environmental change in streams. She monitored the Savannah River over a 25-year period (Patrick 1977) and found that, despite the relatively short algal life cycle, changes in algal community structure were closely correlated with identifiable changes in the environmental status of the river (Figure 1). Diatoms, the group on which Patrick concentrated, dominate many algal communities, including, during much of the year, those of the Athabasca (McCart et al. 1977) and MacKay rivers (McCart et al. 1978), as well as small streams on Syncrude's Lease 17 (Tsui et al. 1978). As a group, they are well known, easily sampled from natural or artificial substrates, and readily identifiable through the characteristics of their siliceous frustules. Cairns et al. (1972) are very emphatic in their recommendation of the use of diatoms in identifying and quantifying certain biological effects of pollution. After discussing some of the advantages and disadvantages of the group, they (ibid.:91) concluded, "We feel it highly improbable that large numbers of algae belonging to other major groups (e.g., the blue-green and green algae) will prove as useful at the species level for pollution monitoring. While such species are probably as sensitive to pollutional stresses, the increasing difficulty for their accurate species identification which necessitates axenic cultures... all but eliminates these species for field bioassays. However, their usefulness at the higher taxa level (genera, family, etc.) or in studies of community structural and functional changes will remain important."

Cairns (1974a) also makes a case for the use of protozoans, the third most popular major group identified in Hellawell's (1977) literature survey (Figure 3), in biomonitoring and pollution

assessment. He lists a number of advantages to the group of which the following apply to the monitoring of natural communities:

1. They are small, easily handled, and require relatively small containers;
2. Since protozoans are unicellular, they are in very close contact with the environment and are thus exposed to unfavourable stresses of many kinds much more intimately and with less time lag than many higher organisms;
3. Despite the foregoing, the differences in tolerance to various waste materials among fish, invertebrates, and protozoan species are not as great as some suppose--sometimes more, sometimes similar, and sometimes less;
4. Since they have a cosmopolitan distribution and are thus likely to be found wherever ecological conditions are appropriate, one can use the same species on different continents and thereby eliminate questions concerning how much of the difference in results between investigators is due to methodology and to inherent differences in the test organisms;
5. Protozoans and other microbial species make up the largest portion of the total biomass of any aquatic system. Anything which affects them will have profound effects on aquatic ecosystems in general; and
6. A collecting permit is not required.

There are, however, a number of serious problems with using protozoans in routine biomonitoring. One is the problem of obtaining quantitative samples. It is difficult to obtain absolute counts of protozoans in samples and many of the standard community parameters (density, biomass, diversity indices) cannot, therefore, be calculated. A second is that samples are best examined live and there is a problem of identifying as many species as possible before the collection becomes distorted by death, encystment, reproduction, or predation. It is not advisable to examine

collections over 48 h old. A third difficulty is that the protozoans include a wide range of organisms, many of them only identifiable with difficulty. In fact, Cairns (1974a) recommends that survey teams include a protozoologist whose only responsibility is the identification of these organisms.

The use of bacteria, the fourth in order of preference of the major groups included in Hellawell's (1977) literature survey (Figure 3), has a long history in biomonitoring. The routine sampling of coliform bacteria in natural waters (which is a part of most public health programs)is, in fact, a kind of biomonitoring. The coliform count is used as an index of the presence of other, pathogenic bacteria. While most of the studies of bacteria in fresh waters have involved those in the water column, it is possible to obtain reasonably consistent samples of sessile bacteria from fine substrates in streams. Dr. W. Costerton (Professor of Biology, University of Calgary, telephone conversation, September 1979), for example, was able to obtain such samples in studies of hydrocarbon degrading bacteria in the Athabasca River. Sessile bacteria rather than those floating in the water column would probably be more useful in biomonitoring studies of point sources of effluents entering streams. The treatment of bacterial samples, once obtained, is well standardized as are the methods of data analysis. One of the possible difficulties in using bacteria in biomonitoring studies is their very short life cycle and rapid community response (e.g., within a few hours) to changing conditions. Sampling may, therefore, reflect only very localized and/or temporary conditions. Another potential problem is the high cost of sophisticated analytical facilities.

Fish, although they are most directly important to man, are very long lived and the effects of environmental change, especially those of low level pollution, may not become apparent for years. In addition, fish are highly mobile (many species are migratory) and may simply move out of areas where conditions become unsuitable, possibly useful as indication by absence but difficult to analyze using standard community parameters. While the latter

can be obtained, the techniques required are expensive, especially of manpower, and the results are often subject to such a high variability that it is difficult to demonstrate statistical significance to differences between samples. Another problem, of particular concern in Alberta, is that samples of fish, adequate for biomonitoring purposes, are difficult to obtain under winter ice.

Despite these disadvantages, there may be good reasons for including fish among the groups examined during routine biomonitoring studies:

1. Fish are an aquatic resource directly utilized by man and are, therefore, an object of often considerable public concern;
2. Providing that sufficient effort can be made, fish can provide an indication of changes in aquatic systems, particularly as they affect the highest trophic levels;
3. Because they are relatively long lived (some species in the AOSERP study area may live 20 years or more), trends in the characteristics of fish populations are well suited to assessing changes over periods of years; and
4. Some aspects of the life history of fish can also be used to assess environmental conditions over shorter periods; e.g., egg development and spawning success as well as the distribution, relative abundance, and growth of young-of-the-year and older juveniles.

Hellawell's (1977) assessment of the suitability of various groups applies particularly to streams and is based on a much larger body of information than is presently available regarding the suitability of various groups for biomonitoring in lakes. It is likely, however, that the groups most suitable for monitoring in streams would also prove most useful in lakes although one additional group, the plankton, which is important in lakes but generally rare in streams, must also be considered.

The plankton has the following advantages for biomonitoring studies:

1. It is relatively easy to sample quantitatively;
2. Sample sorting (i.e., from extraneous material) is rarely required;
3. Good keys to species are widely available for many zooplankton forms;
4. Precise, quick, easily used, and well-established techniques are available for measuring productivity of phytoplankton; and
5. Phytoplankton parameters such as chlorophyll-a quantities and primary productivity are good indicators of eutrophication.

Disadvantages are:

1. Identification of phytoplankters, particularly the small forms that often comprise most of the phytoplankton, requires considerable skill and often specialized, expensive equipment (e.g., inverted microscopes or an electron microscope); and
2. The plankton is highly mobile, making it difficult to localize impact sites.

The planktonic community may be particularly useful for monitoring lake acidification. Acidification reduces the number of species in limnetic crustacean plankton (Sprules 1974; Almer et al. 1974; Hendry et al. 1976), particularly below pH 5. The genus *Daphnia* appears to be particularly sensitive, rarely being found at pH values less than 6. At pH values of 5.6 to 5.9, phytoplankton diversity is frequently reduced (Hörnström et al. 1973; Almer et al. 1974; Braekke 1976; Kwiatkowski and Roff 1976), although plankton biomass is apparently quite insensitive to acidification (Yan and Stokes 1978; Hörnström et al. 1973) except possibly at very low pH (Conroy et al. 1976). Taxonomically, Chlorophyceae, Chrysophyceae, Bacillariophyceae, and Cyanophyceae are uncommon in, or absent from, acid lakes, but Dinophyceae predominate in them (Almer et al. 1974).

There is evidence that plankton may be a poor choice for monitoring for the effects of toxic metals. For example, Moore et al. (1979) found variations in plankton abundance to be poorly correlated with changes in the level of contamination of some subarctic lakes by cyanide, copper, lead, and arsenic. They suggested that the planktonic species had adapted to the pollution.

3. APPROACHES TO BIOMONITORING

There are four basic approaches to in-stream biomonitoring, including the use of:

1. Indicator species;
2. Indication by absence;
3. Community structure; and
4. Physiology, bioaccumulation, and behaviour.

Each of these approaches is discussed below.

3.1 INDICATOR SPECIES

Cairns (1974b: 338) points out that, "The idea that certain species can be used to indicate certain types of environmental conditions is well established ... The presence of a species indicates that the habitat is suitable and since some of the environmental requirements are known for many species, their presence indicates something about the nature of the environment in which they are found." Some species, termed "indicator species", are particularly useful in defining environmental conditions. The basic assumptions in their use are that:

1. Each organism has a particular set of environmental prerequisites essential to its survival;
2. At least the major environmental prerequisites of indicator species can be defined; and
3. The presence of the indicator species indicates that these environmental prerequisites have been met.

A good indicator organism should have the following characteristics (Harman 1974):

1. Be easily recognized by researchers who are not specialists;
2. Be abundant in their preferred habitats throughout a wide geographic range;
3. Exhibit approximately the same degree of tolerance, or be indicative of the same conditions, throughout their range;
4. Have a relatively long life span; and

5. Be comparatively sessile and unable to avoid temporarily stressed environments by rapid migration.

Indicator organisms are the basis of the '*Saprobian system*' which is widely used in monitoring aquatic ecosystems in Europe. The terminology used to indicate various degrees of pollution is given in Table 3. As an example of the use of the method, a list of organisms which have proved useful as indicators of pollution zones in Poland is presented in Table 4. The Saprobian approach, or ones like it, have found little favour in North America and, in fact, the whole question of the existence and utility of indicator organisms is a subject of continuing controversy (Cairns 1974b). One of the major problems is that new indicator organisms must be designated whenever new pollutants enter the environment. The organisms listed in Table 4 might be useful indicators of organic pollution (e.g., domestic sewage) while at the same time poor indicators of pollution resulting from industrial effluents.

The general view of the Saprobian approach and the use of indicator organisms, in general, is summarized by Cairns et al. (1972:82) who concluded that, "... knowledge of the environmental requirements and tolerances of species to toxicants limits the 'Saprobian system' to low predictive capability and requires constant readjustment in response to the appearance of information about new pollutants. The presence of organisms known to indicate certain conditions, such as, anaerobics, high salinity, high temperature, etc., can provide useful information if no assumptions are made regarding the characterization of the pollutional load by the presence of such indicator species. The '*Saprobian system*' approach, therefore is useful but less comprehensive or precise for assessing pollutional load than by examining changes in the structure and function of algal and protozoan communities. If a vast body of appropriate information accumulates, this situation may be reversed but this is not likely to happen in the next thirty years when many critical decisions must be made."

An approach using indicator organisms and/or the Saprobian system does not appear to be a very useful one in monitoring

Table 3. Relation between ecological groups, saprobic zones, and definition of degree of pollution. From Turoboyski (1977).

Predominant Ecological Group	Saprobic Zone	Degree of Pollution
1. Saprobiontic	Polysaprobic	Very polluted
2. Saprophilous	α-mesosaprobic	Heavily polluted
3. Saproxenous	β-mesosaprobic	Slightly polluted
4. Saprophobous	Oligosaprobic	Very slightly polluted (virtually pure)

Table 4. A selection of organisms, with the pollution zones with which they are frequently, but not necessarily exclusively, associated in Poland. From Turoboyski (1977).

Zone	Group	Organism
Polysaprobic	Bacteria	*Beggiotoa* sp.
	Ciliates	*Paramecium putrinum*
α-mesosaprobic	Bacteria	*Sphaerotilus natans*
	Fungi	*Leptomitus lacteus*
	Diatoms	*Navicula cuspidata var. ambigua*
		N. viridula
		Nitzschia acicularis
		N. palea
		N. sigmoidea
	Green algae	*Stigeoclonium tenua*
	Ciliates	*Chilodonella cucullus*
		Glaucoma scintillans
		Colpidium colpoda
		Paramecium aurelia
		P. caudatum
		Spirostomum ambiguum
		Prorodon teres
	Tubificid worms	
	Chironomid worms	*Tendipes plumosus*
β-mesosaprobic	Diatoms	*Ceratoneis arcus*
		Cymbella vetricosa
		Diatoma vulgare
		Fragilaria capucina
		Melosira varians
		Meridion circulare
		Navicula cryptocephala
		N. viridula
		Synedra ulna
	Green algae	*Scenedesmus quadricauda*
	Molluscs	*Ancylus fluviatilis*
		Limnaea stagnalis
	Ephemeroptera	*Baetis rhodani*
		Ecdyonurus fluminum
	Plecoptera	
	Crustacea	*Gammarus pulex*
Oligosaprobic	Turbellaria	
	Molluscs	*Ancylus fluviatilis*
		Limnaea ovata
	Ephemeroptera	*Ecdyonurus helveticus*
		Epeorus assimilis
		Heptagenia lateralis

Continued...

Table 4. Concluded.

Zone	Group	Organism
Oligosaprobic	Ephemeroptera	*H. sulfurea*
	Plecoptera	
	Trichoptera	*Rhyacophila vulgaris*
	Crustacea	*Gammarus pulex*
Extinction	Diatoms	*Navicula viridula*
	Tubificid worms	

aquatic ecosystems within the AOSERP study area. For most groups, neither their taxonomy nor their natural ecology are well known, at present, let alone their tolerance of changes in various components of their environment. As studies proceed, however, it is likely that certain organisms will be identified whose presence will provide at least a preliminary indication of environmental change.

3.2 INDICATION BY ABSENCE

Harman (1974) discusses this approach which is based on the notion that the absence of "clean water species" is a better indicator of environmental conditions than the presence of tolerant ones (for example, the absence of most species of stoneflies from stream reaches polluted by heavy organic loadings).

There are a number of difficulties with the method:

1. The ecology and physiology of many freshwater organisms is unknown so that no decision can be made concerning their tolerance or intolerance of polluted conditions. Indeed, tolerance may vary geographically or even seasonally within the same species;
2. Presence or absence data may vary greatly with the experience of the collector, sampling procedures, and the densities of species populations within the community being sampled;
3. The investigator must be very knowledgeable of the taxonomy and ecology of the group concerned in order to recognize the elements that are missing;
4. While the presence of a species indicates that at least certain minimal environmental conditions have been met, absence is more difficult to evaluate (Cairns 1974b). Aside from intolerance of existing environmental conditions, a species may be absent because:
   (a) it has not had the opportunity to invade the area under study but might survive if it did;
   (b) another species has assumed its niche (i.e., outcompeted it);

(c) there was insufficient sampling effort to discover rare species;
(d) the collections were made at the wrong time of year when the species was unavailable to the sampling gear or unidentifiable (for example, insects in the egg or very early juvenile stages); and
(e) chance.

Like the use of indicator species, indication by absence does not, at present, appear to be a very useful approach to biomonitoring in the AOSERP study area. However, as studies in the area proceed, it may be possible to designate certain species which, by their absence, would be indicative of certain kinds of environmental change. For example, there may be species which, though widely distributed, are intolerant of saline groundwater. Their absence in a particular stream reach, downstream of a source of saline effluent, could be used as at least a preliminary indication that the community is being stressed.

3.3 COMMUNITY STRUCTURE

The community approach to biomonitoring has been described by Cairns (1974b). The approach is based on the well recognized idea that, "... an aquatic community is an interlocking interdependent system of species in which an effect on one part will ultimately affect the whole..." (ibid.:340), and that communities as a whole respond to pollution stress. Cairns (1974b:343) points out that the use of community structure to assess pollution is conditioned by four assumptions:

"1. Given the opportunity to do so, natural systems will initially evolve toward greater and greater complexity of species, eventually stabilizing at some point where further fractionation has no selective advantage;
2. This process increases the number of cause-effect pathways for energy and nutrient translocation, thus increasing the functional complexity of the system;

3. Complex communities (highly diverse) are more stable than simple communities because the elimination of one species or the disruption of one cause-effect pathway will probably affect a greater proportion of the biomass of a simple community than of a complex system...
4. That pollutional stress will simplify a complex community by eliminating the more sensitive species and also increase the disproportion in numbers of individuals per species (by permitting some of the already abundant species to become more abundant, as a result of reduced competition for resources, if they can tolerate the pollutional load."

The typical responses of a community subjected to pollutional stress are first, a reduction in the total number of low density species and second, an increase in the numbers of individuals per species of at least some species especially favoured by the new conditions. These responses are illustrated by examples (Tables 5 and 6) provided by Roback (1974). The numbers of species in each of the major groups were reduced as the result of pollution (Table 6) but some less than others. For example, the number of species of Protozoa at damaged stations (34) was reduced to 65% of those at undamaged stations (52) but the overall representation of protozoan species, as a percentage of all species in the major groups, increased from 36 to 55%. Fish, on the other extreme, were reduced from 18 to 5 species, only 28% of their abundance at undamaged stations. Their overall representation also declined, from 13% of all species at undamaged stations to 7% of those at damaged stations. Clearly, the Protozoa, as a group, though adversely affected, were more tolerant of pollution than were fish.

Similar responses can be demonstrated within major groups. For example, among the insects (Table 5), the Odonata were clearly more tolerant of pollution than were the Ephemeroptera.

Table 5. Comparison of mean numbers of species within orders of aquatic insects at undamaged (unpolluted) and damaged (polluted) stream stations. Bracketed values are numbers of species at damaged stations as a percentage of those at undamaged stations. Data from Roback (1974).

	13 Undamaged Stations		10 Damaged Stations		
	Species	%	Species	%	%
Odonata (Dragonflies, etc.)	8	16	4	29	(50)
Ephemeroptera (Mayflies)	8	15	2	9	(25)
Plecoptera (Stoneflies)	3	5	<1	1	(<33)
Hemiptera (Bugs)	5	9	1	5	(20)
Megaloptera (Dobsonflies)	2	3	<1	2	(<50)
Coleoptera (Beetles)	11	20	3	20	(27)
Trichoptera (Caddisflies)	5	9	2	9	(40)
Diptera (Midges, etc.)	12	23	4	25	(33)

Table 6. Comparison of mean numbers of species within major groups at undamaged (unpolluted) and damaged (polluted) stream stations. Bracketed values are numbers of species at damaged stations as a percentage of those at undamaged stations. Data from Roback (1974).

	13 Undamaged Stations		10 Damaged Stations		
	Species	%	Species	%	%
Protozoa	52	36	34	55	(65)
Insects	52	38	15	22	(29)
Other Invertebrates	20	13	10	16	(50)
Fish	18	13	5	7	(28)

Figure 4 (Patrick 1949) illustrates the same phenomena with the organisms distinguished in terms of their tolerance as well as their taxonomic status. As the level of pollution increases, tolerant species become more abundant as the intolerant species are progressively eliminated. At the very highest levels of pollution, however, the tolerance limits of almost all species are exceeded and few survive.

In the samples presented thus far, only a single parameter, number of species (taxonomic diversity), has been presented. Other aspects of community structure may also change, including the relative abundance of species, density (the number of individuals per unit area or unit volume), biomass (the weight or volume of organisms per unit area or unit volume), and species diversity (as indicated, for example, by the Shannon-Weaver Diversity Index or by the Equitability Index). All of these can be affected by pollutional stress and have been used in assessing the effects of such stress on community structure. Hellawell (1977) described ways in which several of these parameters describing community structure might change in relation to one another (Figure 5). Relative abundance, density, and biomass are easily understood parameters. Diversity and equitability indices are, however, more complex concepts and deserve more detailed discussion.

A variety of species diversity indices has been developed but the one most commonly used is the Shannon-Weaver Species Diversity Index (Shannon and Weaver 1963). This is one of several diversity indices based on information theory (Margalef 1958) and used to analyze natural communities. Cairns (1977:174) notes that, "This technique equates diversity with information. Maximum diversity, and thus maximum information, exists in a community of organisms when each individual belongs to a different species. Minimum diversity (or high redundancy) exists when all individuals belong to the same species. Thus, mathematical expressions can be used for diversity and redundancy that describe community structure.

"As pointed out by Wilhm and Dorris (1968) and Patrick et al. (1954), natural biotic communities typically are characterized

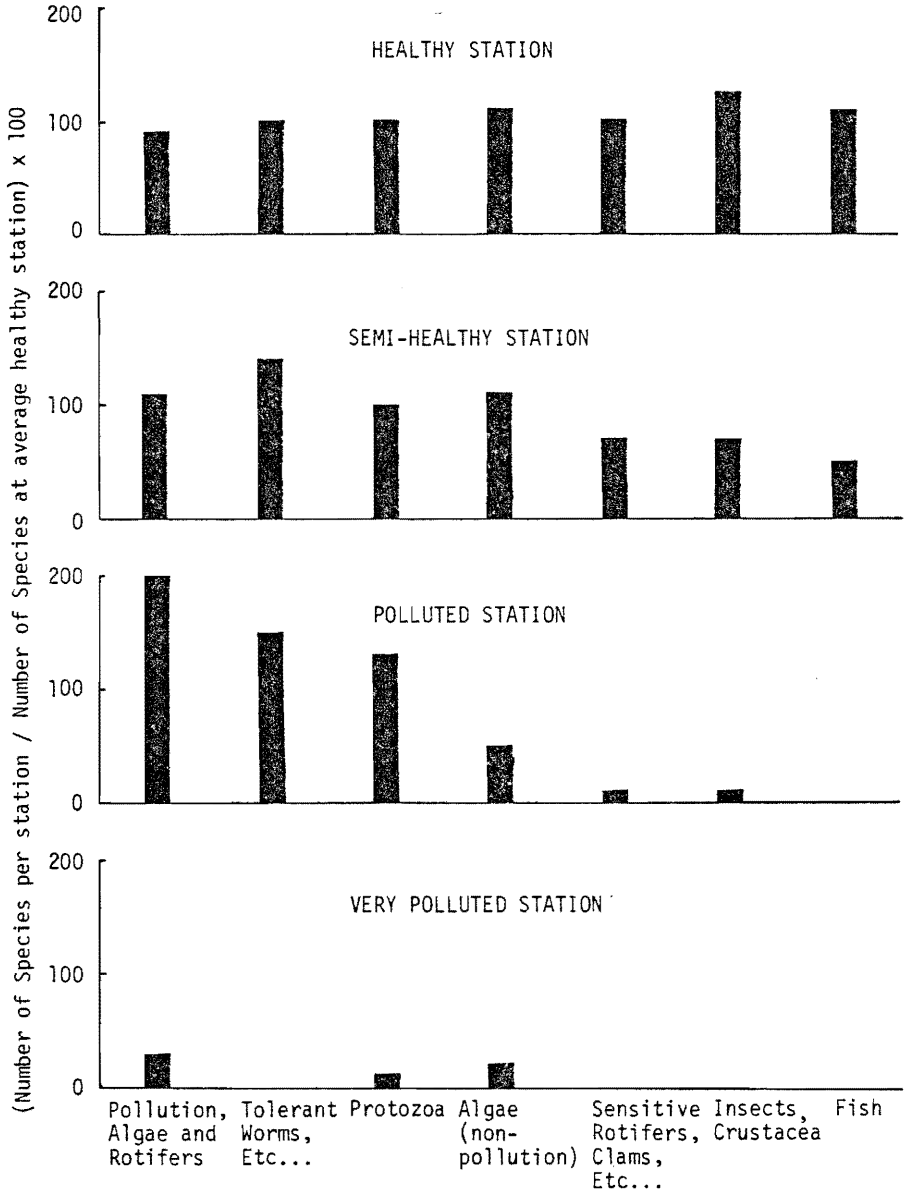

Figure 4. Effects of increasing pollution on numbers of species in streams. From Patrick (1949).

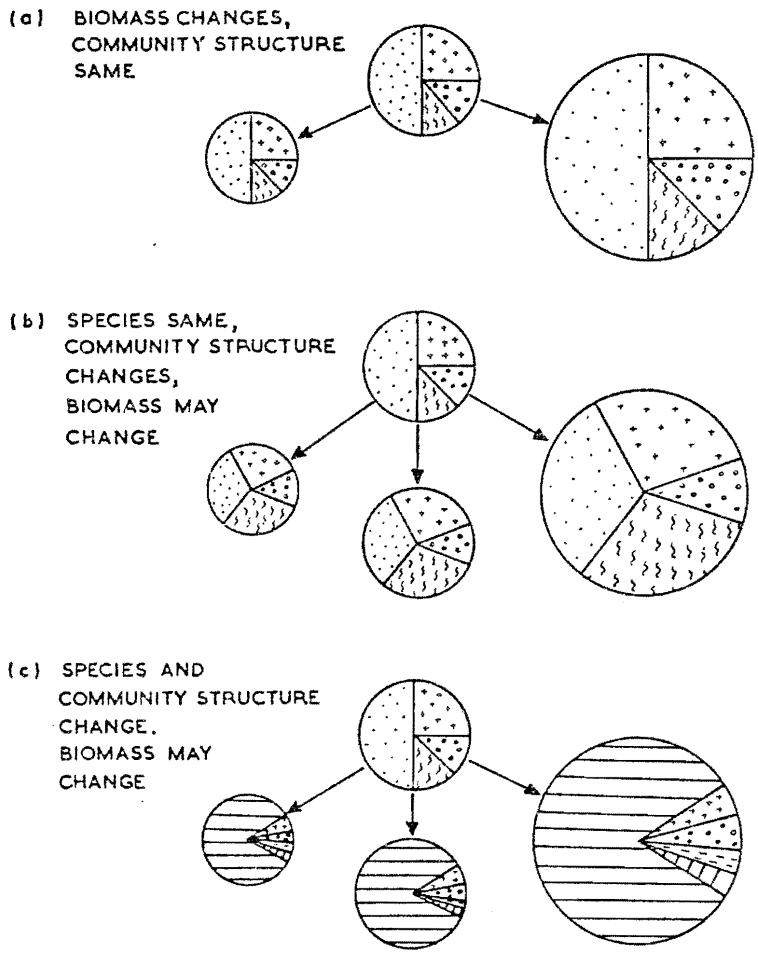

Figure 5. Diagram of possible changes in community structure (relative abundance of species numbers) and biomass. From Hellawell (1977).

by the presence of a few species with many individuals and many species with a few individuals. An unfavorable limiting factor such as pollution results in detectable changes in community structure. As it relates to information theory, more information (diversity) is contained in a natural community than in a polluted community. A polluted system is simplified and those species that survive encounter less competition and therefore may increase in numbers. Redundancy in this case is high, because the probability that an individual belongs to a species previously recognized is increased and the amount of information per individual is reduced...

"...Diversity indices that permit the summarization of large amounts of information about the numbers and kinds of organisms have begun to replace the long descriptive lists common to early pollution survey work. These diversity indices result in a numerical expression that can be used to make comparisons between communities or organisms. Some of these have been developed to express the relationships of numbers of species in various communities and overlap of species between communities."

The Shannon-Weaver Diversity Index is defined as (Poole 1974:392-393):

$$\bar{d} = - \sum_{i=1}^{s} P_i \text{ Log } P_i$$

where s is the number of species and P_i is the proportion of the total number of individuals consisting of the ith species. Logarithms to the base e (natural logs, or Ln) are usually used in the calculation. This formula is a biased estimator of $\bar{d}$, and the expected value of $\bar{d}$ is more correctly found from the series:

$$\bar{d} = \left[- \sum_{i=1}^{s} P_i \text{ Ln } P_i \right] - \left[\frac{s-1}{2N} \right] + \left[\frac{1 - \Sigma P_i^{-1}}{12 N^2} \right] + \left[\frac{\Sigma (P_i^{-1} - P_i^{-2})}{12 N^3} \right] + \ldots$$

where N is the total number of organisms in the sample. Usually only the first two terms are worth calculating, the remainder being so small as to be insignificant. The variance of the corrected estimate may be calculated from the formula:

$$\text{var}(\bar{d}) = \frac{\sum_{i=1}^{s} P_i \text{Ln}^2 P_i - (\sum_{i=1}^{s} P_i \text{Ln} P_i)^2}{N} + \frac{s-1}{2N^2} + \ldots$$

It is usually sufficient with large samples to calculate only the first term in the series.

Assumptions in the calculation of $\bar{d}$ are that the figures used are from a random sample of an idefinitely large statistical population, that the number in the species pool is known, and that all species are represented in the sample (Poole 1974:391,393). If one of the species is not represented in the sample, then $P_i=0$, $\text{Ln}P_i=-\infty$, and $\bar{d}$ cannot be calculated. As long as the sample contains most of the species in the total statistical population, the error will be small if the calculation is made as if all species are represented. When many species in the sample are represented by only a few individuals, however, it is likely that the number of species in the sample is substantially less than the number in the statistical population, therefore $\bar{d}$ cannot be accurately calculated. The latter condition is common in aquatic invertebrate samples in our experience, and might pose a serious impediment to the use of the Shannon-Weaver Diversity Index as a monitoring tool.

Shannon and Weaver's measure of species diversity depends on the number of species (richness) and the distribution of individuals among the species (evenness). It is considered to be a measure of mean species diversity per individual ($\bar{d}$), and is sensitive to, and increases with, both species richness and evenness. The value of $\bar{d}$ is proportional to the uncertainty of identification of an individual selected at random from a multi-species population. In general, $\bar{d}$ values range from zero to any positive number, but are seldom greater than 5. The $\bar{d}$ value is at a minimum when all individuals belong to the same species, whereas $\bar{d}$ is at a maximum value when each species contains the same number of individuals. Most benthic freshwater communities in streams which are not severely polluted have diversities ranging from 2 to 4 (Wilhm 1970).

Diversity indices ($\bar{d}$) obtained as described above can be compared with a hypothetical maximum based on MacArthur's broken

stick model (MacArthur 1957) of natural populations (population with a few relatively abundant species and increasing numbers of species with only a few individuals). Such comparisons result in an index termed "equitability" or "e" by Lloyd and Ghelardi (1964). Equitability values are computed from the following formula:

$$e = \frac{S'}{S}$$

where: S = number of species (forms) in the sample

S'= the tabulated number of species for MacArthur's model of equal diversity (Lloyd and Ghelardi 1964).

Values of "e" usually range from 0 to 1; however, higher values are possible (Goodman 1975). Environmental Protection Agency biologists in the U.S. have found the equitability index to be very sensitive to even slight levels of environmental degradation. In natural streams, "e" values range between 0.6 and 0.8, while in stressed streams values are usually below 0.5 (Weber 1973).

While diversity indices may be useful in assessing changes in the structure of aquatic communities, they must be used intelligently, in combination with other kinds of evidence. Cairns (1977: 174) claims: "The diversity index is probably the best single means of assessing biological integrity in freshwater streams and rivers. It is less effective and may even be inappropriate in lakes and oceans. As a screening method for locating trouble spots in most flowing systems, it is superb! Unfortunately, many investigators looking for a single all-purpose method, use it alone when an array of evidence is required. Beware of the investigator who tries to use a single line of evidence of any type instead of multiple lines of evidence to assess biological integrity."

Table 7, which summarizes information on the generalized responses of benthic macroinvertebrate communities to various categories of stress, emphasizes Cairns's point that no single community parameter can be expected to respond in an unequivocal way to all potential stresses.

Aside from the Shannon-Weaver and Equitability indices, other diversity measures are described in the ecological and

Table 7. Responses of benthic macroinvertebrate communities to various categories of stress. (Dr. P.T.P. Tsui, Mobil Oil Canada Limited, Calgary. Notes presented to the Petroleum Industry Training Service, Edmonton).

Stress	No. of Taxa	Standing Crop (Numbers/ Biomass)	Species Diversity	Drift Rate
Toxic Substance	Reduced	Reduced	Reduced	Increased
Non-Toxic/ Inert Substance	Reduced or Variable	Reduced	Variable (Often no change, or increase)	Increased
Thermal Variations	Reduced	Variable	Reduced	Increased
Inorganic Nutrients	Variable (Often no detectable change)	Increased	Reduced	Variable
Organic Nutrients (High O_2 Demand)	Reduced	Increased	Reduced	Variable
Sludge Deposits (Non-Toxic)	Reduced	Increased	Reduced	Variable

biomonitoring literature. Any of these might be appropriate in particular circumstances. The sequential comparison method (Cairns et al. 1968) is of particular interest because it was especially designed for use by individuals without any special training in the taxonomy of aquatic organisms. Cairns et al. (1972:92) state that, "The primary weakness of the species diversity method relates to the considerable amount of taxonomic training required for accurate data collection. Were these methods to be utilized universally even on a moderate scale, the paucity of properly trained people would render the approach ineffective. The development of the sequential comparison index (Cairns et al. 1968 and Cairns and Dickson 1971), a [diversity] method depending on changes in the number of morphologically different macro-invertebrates, attempted to alleviate this problem. The method does not require formal species identification, but merely the ability to distinguish structurally different organisms. Admittedly, this method lacks the precision of the diatometer and related methods, but requires less time and virtually no experience. Thus, the problem of a sufficient labor force for employing this technique on a wide scale is resolved. In theory, the method should also have application to microorganisms.

"The sequential comparison index has an operator error in that the level of discrimination of some operators is more precise than that of others, that is there are 'splitters' and 'lumpers'. However, the same difficulties exist for biological assessment techniques involving formal systems of classification since 'splitters' and 'lumpers' are found at all educational levels."

The major disadvantage of the method is that, because individual species are not identified, some potentially useful information is inevitably lost. This disadvantage could be partially offset by training analysts to identify individual species (or groups) of particular interest (e.g., indicator species).

During the past decade, the concept of species diversity, and especially the Shannon-Weaver Diversity Index ($\bar{d}$) often used to measure it, has been seriously questioned. For example, Hurlbert

(1971) argued that species diversity had no generally agreed-upon definition. Sometimes it has been considered as a function of species richness and equitability, but at other times it has been equated with species richness alone. Hurlbert pointed out that species diversity, defined as in the former case, can increase in response to environmental change at the same time that species richness is decreasing. He further noted that indices of diversity based on information theory, such as $\bar{d}$, appeared to have no obvious biological meaning.

Goodman (1975:242) took up this latter point, describing the interpretation of $\bar{d}$ as "bizarre". In his words, the precise interpretation of $\bar{d}$ is "... the negative logarithm of the geometric mean of the probability per individual of correctly guessing, in sequence, the species identity of each individual in a random ordering of an assortment of individuals whose relative species frequencies are given by $\{P_i\}$, when the 'guess' is carried out by picking some arbitrary ordering of this assortment of individuals" (Ibid 1975:242). He goes on to note that no ecological process appears to correspond to this process of ordering.

Recently, Green (1979:96-102) marshalled several lines of evidence from the literature to support an argument against the use of diversity indices in environmental impact studies. He concluded that the use of diversity indices could not be justified on theoretical grounds alone, in part because of the problems noted by Hurlbert (1971) and Goodman (1975), discussed above. The claim that diversity indices might be justified empirically for specific purposes is weakened by the fact that estimates of diversity calculated from samples are likely to suffer from uncorrectable bias. Furthermore, the common assumption that high diversity indicates high environmental quality is apparently not generally valid, according to evidence in the literature. Even when the use of diversity indices might be justified, Green (1979) argued that simple indices (such as the number of species) are more biologically meaningful and often more informative than $\bar{d}$. Finally, Green (1979) contended that the strongest argument against the use of diversity

indices in impact studies is that other methods retain more of the biological information, while reducing it to a more ecologically meaningful form. He supported multivariate statistical approaches instead, followed by well-designed visual displays to aid in interpreting the data to the non-specialist. He illustrated his point with a comparison of diversity index and cluster analysis approaches to the same data on benthic invertebrate communities near a pulp-mill effluent. The comparison convincingly shows the cluster analysis approach to be more sensitive and informative about the nature and extent of the pollutant impact.

There is no question that diversity indices can be used to demonstrate pollution effects in aquatic environments. There is some question, however, whether they are the best parameters to use. Hurlbert's (1971) and Goodman's (1975) complaint that the Shannon-Weaver index has no clear biological meaning seems justified, despite the explanation by Cairns (1977), that $\bar{d}$ equates species diversity with information. Equating diversity with information is merely an analogy, not a functional description. Where resources, in terms of money and highly-trained manpower, are adequate, Green's (1979) arguments for a multivariate statistical approach on species abundance data are convincing. At the very least, biologists should heed Cairns's (1977) caveat that diversity indices must not be used alone, without reference to other biological data.

3.4 BEHAVIOUR, PHYSIOLOGY, AND BIOACCUMULATION

The three approaches discussed so far are well established, traditional types that have been taken in a large number of impact studies. They are not the only possible approaches, however. Practically any biological response to environmental variables could be used for biomonitoring, the number being limited only by the imagination and analytical skills of the investigators. Some of the more promising and innovative of the many other possible approaches are discussed below.

Recently, a number of authors have investigated the use of various physiological responses of fish as a method of monitoring

effluent quality. These include assessments of changes in swimming activity, breathing rates (opercular rhythms), rheotaxis, and surfacing rates (Besch et al. 1977; Brungs 1973; Cairns et al. 1973b, 1973c, 1973d, 1974; Kleerekoper 1977). Some of the proposed methods are highly automated involving automatic monitoring using electronics (Morgan 1977; Westlake and van der Schalie 1977). They are, however, better suited to in-plant monitoring of effluents rather than to the biomonitoring of natural populations which is the subject of this discussion.

Procedures more suitable for monitoring natural populations include taste tests, physiological and histological parameters which can be measured on samples taken in the field, and bioaccumulation studies.

Tainted fish flesh does not necessarily imply a toxic or harmful condition either for the fish or the consumer. It does, however, certainly affect the human uses to which fish can be put and is therefore an important consideration. It is a common problem where refinery wastes and other effluents originating from the petroleum-related industries enter natural waters. Table 8 lists compounds which are known to impart off-flavour to fish flesh. Some of these, particularly phenols and other hydrocarbons, are likely to occur in effluents entering streams in the AOSERP study area. As an example, Thomas (1973) described a study of tainting of catfish *(Ictalurus punctatus)* flesh in the Ohio River. A taste panel was able to distinguish the taste of fish held in cages upstream and downstream of a wastewater discharge. Caged fish exposed for 3 d acquired 70% of the tainted flavour of native fish.

Recent studies, sponsored by AOSERP (Tsui et al. in prep.), assessed the effects of long-term (more than 90 d) exposure of fish and invertebrates to saline mine depressurization water, one of the major potential pollutants resulting from oil sands development. Briefly, they found that:

1. Among fish, there was:
   (a) an elevation of opercular pumping frequency and

Table 8 . List of compounds which are known to impart off-flavour to fish flesh. From Thomas (1973).

Compound	Threshold Odor Concentration mg/L
Acetophenone	0.5
Benzkatechin	2.5
o-*sec* butylphenol	0.3
p-*tert* butylphenol	0.03
p-Chloride Phenol	0.06
Chlorophenol	0.01
o-Chlorophenol	0.015
p-Chlorophenol	0.05
Coal-Coking Wastes	0.02
Coal-Tar Wastes	0.1
Cresylic Acid ("meta para")	0.2
Cresols	10.0
Cresol	10.0
"Cutting" Oil (Emulsifiable)	15.0
o-dichlorobenzene	0.25
B,B-dichlorodiethyl Ether	1.0
2,4-dichlorophenol	0.01
2,4-dichlorophenol	0.005
Diphenyl Oxide	0.05
Ethylbenzene	0.25
Gasoline	0.005
Simple petroleum hydrocarbon	1.0
"Insecticide" Oil (Heavy Aromatic Naphtha)	0.1
Isopropylbenzene	0.25
Kerosene	0.1
Kerosene	0.5
Kraft Mill Effluent--raw	1.0 (% by volume)
Alpha-methylstyrene	0.25
Naphthaliene	1.0
α-Naphthol	0.5
B-Naphthol	1.0
α-Naphthylamine	3.0
Outboard Motor Exhaust Wastes	0.5
Petroleum Refinery Effluents	0.25 (threshold odour number)
Aromatic, neutral substances of phenols	2.6-3.4
Phenols in Polluted River	0.02-0.15
Phenol	0.02-0.1
Phenol	1.0
Phenol	15-25
Phenol	25.0
o-Phenyl phenol	1.0
Phloroglucin	100.0
Pyridine	5.0

Continued...

Table 8 . Concluded.

Compound	Threshold Odor Concentration mg/L
Pyrogallol	20-30
p-quinone	0.5
Quinoline	0.5-1.0
Resorcin	30.0
Sewage containing phenols	0.1
Styrene	0.25
Toluene	0.25
p-Toluidine	20.0
Xylenols	1-5
Type of Industry	**Off Flavor**
Petroleum	none
Paper	present and toxic
Municipal Sewage	severe
Chemical - general	severe and toxic
Type of Manufacture	
Synthetic detergents	none
Acetylene	none
Carlude	none
Glycol	present
Synthetic elastomer	present
Fluoridated hydrocarbons	present
Methylene	present
Chloronal	present
Chloroform	present
Hydrogenchloride	present
Synthetic Rubber	severe
Metal	present and toxic

coughing rate during initial exposure but a depression during chronic exposure,
(b) an increase in red cell volume and blood hematocrit, and
(c) an increase in gill mucus production and buildup of mucus on the gills;

2. Among invertebrates, there was:
(a) a decline in the number and density of chloride cells on gill lamellae (Figure 6),
(b) a reduction in moulting frequency, and
(c) a delay in emergence;

3. Among both fish and invertebrates, there were increases in the tissue concentrations of Na and K ions as well as in the heavy metal ions Cu and Zn (Table 9).

Several of these parameters might be useful in monitoring natural populations, notably red cell volume and blood hematocrit, chloride cell density, and tissue concentrations of selected ions.

Although petroleum is an important potential contaminant in many areas of the world, Walton et al. (1978) claim that there is no concensus on any single, satisfactory method of chemical measurement for it (see also Kananaskis Centre for Environmental Research in prep.). Walton et al. (1978) have described a biological technique that could be used to monitor for petroleum contamination. They note that exposure to even low concentrations of petroleum induces the production of aryl hydrocarbon hydroxylase (AHH) in several fish species, and that AHH persists for up to 2 wk after exposure ceases. The induction appears to be highly specific for petroleum. Once a laboratory is equipped to do the AHH analysis, biomonitoring of fish for AHH as an indicator of petroleum contamination would be simple, fast, economical, and sensitive.

One of the weaknesses of biological monitoring methods is that organisms may become resistant to a toxicant and therefore show little or no effects. This mechanism has been involved by Moore et al. (1979) to explain the apparent lack of effect of toxic mine wastes on plankton in some subarctic lakes. Luoma (1977) has

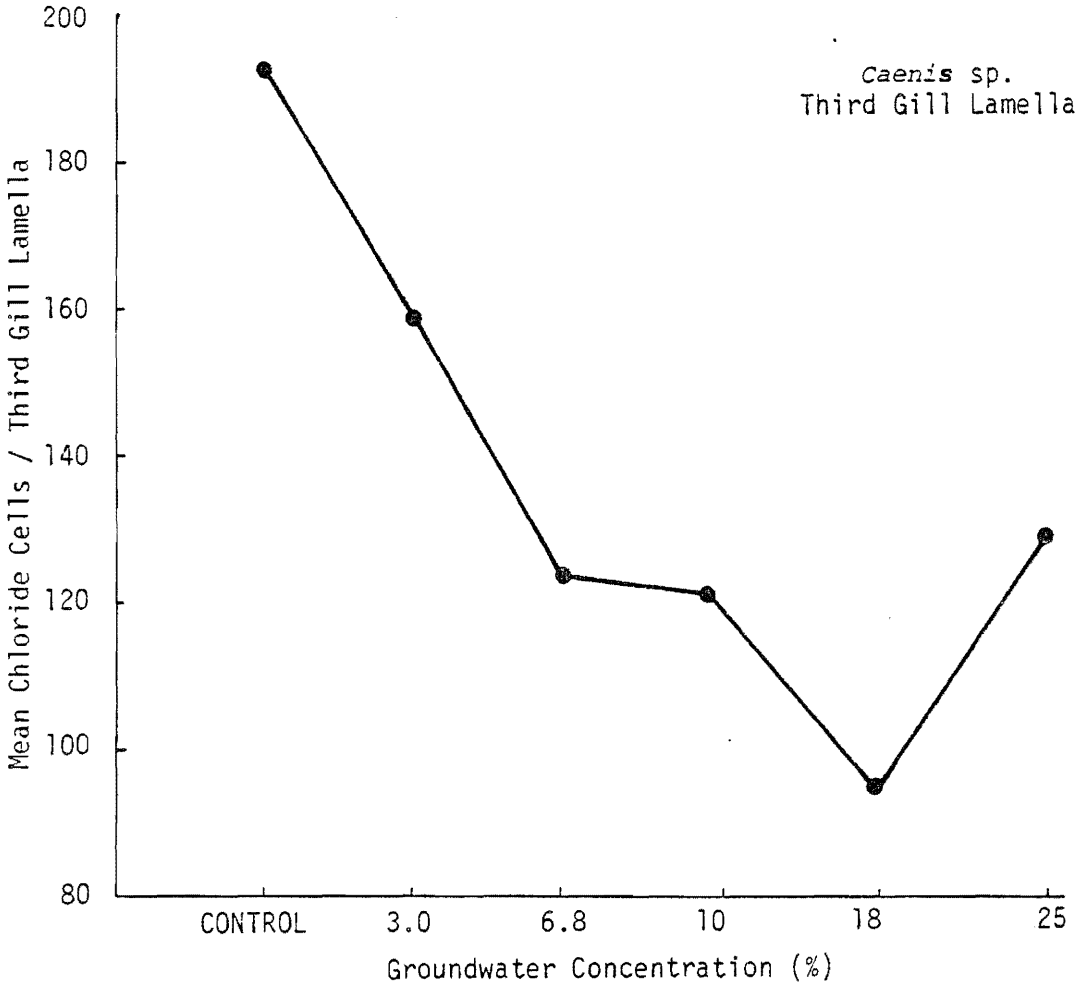

Figure 6 . Effect of varying groundwater concentration on number of chloride cells on third gill lamella of *Caenis* sp. From Tsui et al. (1980).

Table 9. Accumulation of copper and zinc in tissues and in the exuviae of two invertebrates exposed to groundwater. From Tsui et al. (in prep.).

	Cu^{++}		Zn	
	Tissues	Exuviae	Tissues	Exuviae
Caenis				
Control	13	5	7	4.4
3%	13	4	7.2	5.2
6.8%	17	3	16.4	5.0
10%	12	7	15.5	3.4
18%	10	4	16.5	5.2
25%	14	4	19	5.9
Hyalella				
Control	15.4	6.5	6.5	<2
3%	14.6	7.5	8.6	2.5
5%	16.5	6.5	7.1	1.5
10%	21	6.5	6.3	2.5
18%	24	10	15.1	2
25%	23	10.5	19.5	<2

suggested an approach that might help to solve this sort of problem. He argued that, "If one population of a species is more resistant to a toxicant than are other populations, it is direct evidence that the concentration of the toxicant in the environment of the resistant population is sufficient to elicit biological effects. The presence of a toxicant-resistant population of one species in an ecosystem further suggests that other species may have been affected by the resistance-eliciting substance" (ibid.:436). To test for resistance, organisms from an unaffected environment and the impacted habitat could be subjected to the suspected toxicant and their responses compared in a bioassay procedure. This method would appear to be ideally suited for detecting certain sublethal effects, such as changes in behaviour or physiology, which may be undetectable by routine biomonitoring methods based on comparisons of field collections.

Measurement of primary productivity of phytoplankton and periphytic algae by the well-known radioactive carbon or dissolved oxygen techniques is a physiological method that is particularly attractive for monitoring eutrophication. Vollenweider (1974) described the phytoplankton techniques in detail; Rodgers et al. (1978) and Wetzel (1974) described representative techniques for periphytic algae.

The radioactive carbon methods in particular are highly sensitive and capable of measuring even low primary productivity levels. Although sample processing requires expensive and complicated equipment, an international laboratory, the Carbon-14 Centralen in Denmark, exists solely to process C^{14} samples. It offers highly efficient, low-cost, and expert analysis, supplying C^{14} solutions, filters, and analysis as a package.

4. SAMPLING METHODS, SAMPLING DESIGN, AND DATA ANALYSIS

4.1 PRINCIPLES

The degree to which a biomonitoring program is successful depends directly on the adequacy of the sampling design and data analysis. Green (1979:inside front cover) suggested that the following 10 principles should be followed in field studies of environmental impact:

"1. Be able to state concisely to someone else what question you are asking. Your results will be as coherent and as comprehensible as your initial conception of the problem.

2. Take replicate samples within each combination of time, location, and any other controlled variable. Differences among can only be demonstrated by comparison to differences within.

3. Take an equal number of randomly allocated replicate samples for each combination of controlled variables. Putting samples in 'representative' or 'typical' places is not random sampling.

4. To test whether a condition has an effect, collect samples both where the condition is present and where the condition is absent but all else is the same. An effect can only be demonstrated by comparison with a control.

5. Carry out some preliminary sampling to provide a basis for evaluation of sampling design and statistical analysis options. Those who skip this step because they do not have enough time usually end up losing time.

6. Verify that your sampling device or method is sampling the population you think you are sampling, and with equal and adequate efficiency over the entire range of sampling conditions to be encountered. Variation in efficiency of sampling from area to area biases among-area comparisons.

7. If the area to be sampled has a large-scale environmental pattern, break the area up into relatively homogeneous subareas and allocate samples to each in proportion to the size of the subarea. If it is an estimate of total abundance over the entire area that is desired, make an allocation proportional to the number of organisms in the subarea.
8. Verify that your sample unit size is appropriate to the size, densities, and spatial distributions of the organisms you are sampling. Then estimate the number of replicate samples required to obtain the precision you want.
9. Test your data to determine whether the error variation is homogeneous, normally distributed, and independent of the mean. If it is not, as will be the case for most field data, then (a) appropriately transform the data, (b) use a distribution-free (nonparametric) procedure, (c) use an appropriate sequential sampling design, or (d) test against simulated H_0 data.
10. Having chosen the best statistical method to test your hypothesis, stick with the result. An unexpected or undesired result is not a valid reason for rejecting the method and hunting for a 'better' one."

4.2 SAMPLING METHODS

The enormous number of sampling devices and methods for collecting freshwater organisms have been thoroughly reviewed in readily accessible books (e.g., Vollenweider 1974; Edmondson and Winberg 1971; Bagenal 1978; Hynes 1970; Brinkhurst 1974) and will not be described here. The present discussion is primarily limited to an evaluation of two general classes of sampling methods (artificial substrate and natural substrate sampling), with particular emphasis on their applicability in the AOSERP study area.

There has been a continuing debate among biologists concerning the use of artificial substrates to determine the characteristics of aquatic communities. Natural substrate sampling provides samples of the natural communities present at the sampling site, although most such methods are selective to some extent (e.g., Flannagan 1970). Variances in samples taken from natural substrates are usually very great (Elliott 1971). One method cannot be used for all types of substrates, and some substrate types are extremely difficult to sample at all. Artificial substrates were developed to overcome these problems (Beak et al. 1973) by standardizing substrate type and improving the retrievability of samples. Artificial substrate sampling has several disadvantages, however, including the obvious one that the communities sampled are artificial. Artificial substrates are subject to vandalism because they must be incubated in place to permit colonization, different orientations of the samplers can produce different colonizing communities, and many organisms could be lost during retrieval of certain types. The following discussion, largely based on studies in the AOSERP study area, indicates the kinds of differences to be expected between natural and artificial substrates and circumstances where the latter might be preferable.

4.2.1 Periphyton

McCart et al. (1978) compared samples of natural periphyton (attached algae) communities removed from rock surfaces with a brush sampler (Stockner and Armstrong 1971) with those colonizing glass and plexiglass artificial substrates. Their results, for three stations in the MacKay River in the AOSERP study area, are summarized in Tables 10 and 11. Briefly, they found that, in general, the mean values for natural substrates exceeded those of artificial substrates in number of species (t), species diversity ($\bar{d}$), equitability (e), density, and biomass. The greatest discrepancies were in density and biomass which were, respectively, five to six and seven to 14 times greater on natural than artificial substrates. On natural substrates, the ratio of diatoms to blue-green algae,

Table 10. Variations in the means and ranges of the taxonomic diversity (t), Shannon-Weaver Species Diversity Index ($\bar{d}$), and equitability (e) of the periphyton communities on various substrate types in the MacKay River (May to September 1977). Data from McCart et al. (1978).

Substrate Type	Parameter	STATIONS					
		Upper		Middle		Lower	
		Mean	Range	Mean	Range	Mean	Range
Natural	t	31.67	22-37	24.33	19-31	22.67	19-28
	$\bar{d}$	2.36	2.03-2.73	2.15	1.73-2.4	2.29	2.20-2.45
	e	0.23	0.19-0.27	0.25	0.21-0.3	0.29	0.25-0.32
Plexiglass	t	22.33	15-36	17.25	14-21	27.50	19-36
	$\bar{d}$	1.39	0.63-2.17	1.54	1.10-1.98	2.40	2.00-2.80
	e	0.17	0.13-0.20	0.22	0.20-0.24	0.30	0.28-0.32
Glass	t	21.25	13-30	21.75	16-31	21.33	16-30
	$\bar{d}$	1.51	1.26-1.94	1.70	1.27-2.34	1.85	1.46-2.10
	e	0.18	0.13-0.23	0.21	0.16-0.23	0.22	0.19-0.28

Table 11. Comparison of mean values and ranges for various parameters describing periphyton on natural, plexiglass, and glass substrates (N=8 in every case). From McCart et al. (1978).

	Natural		Plexiglass		Glass	
	Mean	Range	Mean	Range	Mean	Range
Species (t)	26.9	19-39	22.2	14-36	23.9	18-31
Diversity ($\bar{d}$)	2.31	1.73-2.73	1.71	0.63-2.80	1.74	1.30-2.34
Equitability (e)	0.25	0.19-0.32	0.22	0.13-0.32	0.19	0.13-0.28
Density (1000 cells/cm^2)	485.6	10.8-1356.4	90.1	12.0-275.8	79.9	13.8-124.9
Biomass (mg/m^2)	7757.8	579.7-29565.2	1125.6	379.0-3484.8	550.7	273.9-1206.7
Diatoms/Blue-Greens	1.01	0.33-1.79	2.22	0.14-8.82	2.24	0.36-5.59

the two most important groups in all samples, averaged only half that on artificial substrates. The only major difference between the two artificial substrates was in biomass. The mean biomass on plexiglass was approximately twice that for glass.

The authors suggested that the differences in the communities on natural and artificial substrates were the result of two factors: habitat diversity and length of exposure. Rock surfaces are much less uniform and provide a greater range of habitat than either of the artificial substrates. Regarding length of exposure, they recommended further studies to determine optimum exposure time for artificial substrates. The 30 d exposure time recommended by Weber (1973) for streams in the continental United States may not be sufficient to allow the establishment of a stable community at higher latitudes.

Lock and Wallace (1979) noted that attached algae are by far the most important primary producers in stream systems but that, due to variation in sampling techniques, direct comparisons between various studies are virtually impossible.

It is well known that artificial substrates are selective to varying degrees and samples therefore yield results which differ from those obtained by sampling natural algal communities. Despite these data, describing algal communities developing on artificial substrates have proved extremely useful in biomonitoring studies (e.g., Patrick 1977). The diatoms (Bacillariophyceae), in particular, have been widely used in the eastern United States.

4.2.2 Benthic Invertebrates

McCart et al. (1977) compared a rock-filled basket sampler (Mason et al. 1967) with a modified, weighted Ekman grab (Burton and Flannagan 1973) in sampling benthic macroinvertebrates in the Athabasca River in the vicinity of Syncrude's Lease 17. The modified Ekman was selected because it appeared to be the best method of sampling the generally fine substrates of the river.

The authors found that:

1. The basket sampler consistently collected more invertebrate taxa than the Ekman grab (Figure 7);
2. The basket sampler collected significantly greater numbers of riffle insects such as caddisflies, mayflies, and stoneflies (Figure 8); and
3. The diversity indices ($\bar{d}$) for the basket samplers were consistently higher than those for the grab samples (Figure 9).

On the basis of these studies, McCart et al. (1977) concluded that the basket sampler was a more suitable device than the Ekman grab for routine biomonitoring in the Athabasca River. They gave the following reasons (ibid.:118-122):

"1. The basket sampler collects larger number of invertebrate taxa and there is a more even distribution of individuals among the collected species. These characteristics are particularly desirable in water quality monitoring. In comparing the efficiencies of the basket sampler and the Peterson dredge, Anderson and Mason (1968) concluded that in water quality monitoring studies, it is best to have as many different organisms as possible on which to base conclusions. Dickson et al. (1971) also indicated that, in a comparison of biological water quality between selected stations, the number of taxa collected and the community structure of the macroinvertebrates sampled are more reliable indices of water quality than the number of specimens obtained.
2. The basket sampler selectively collects more of the 'pollution sensitive' macroinvertebrates such as the nymphs of mayflies and stoneflies. The basket sampler is therefore a more sensitive biomonitoring tool.
3. The basket samplers can remain in the river throughout the study period, and are therefore continuously assessing the biological response of the benthic fauna to water quality.

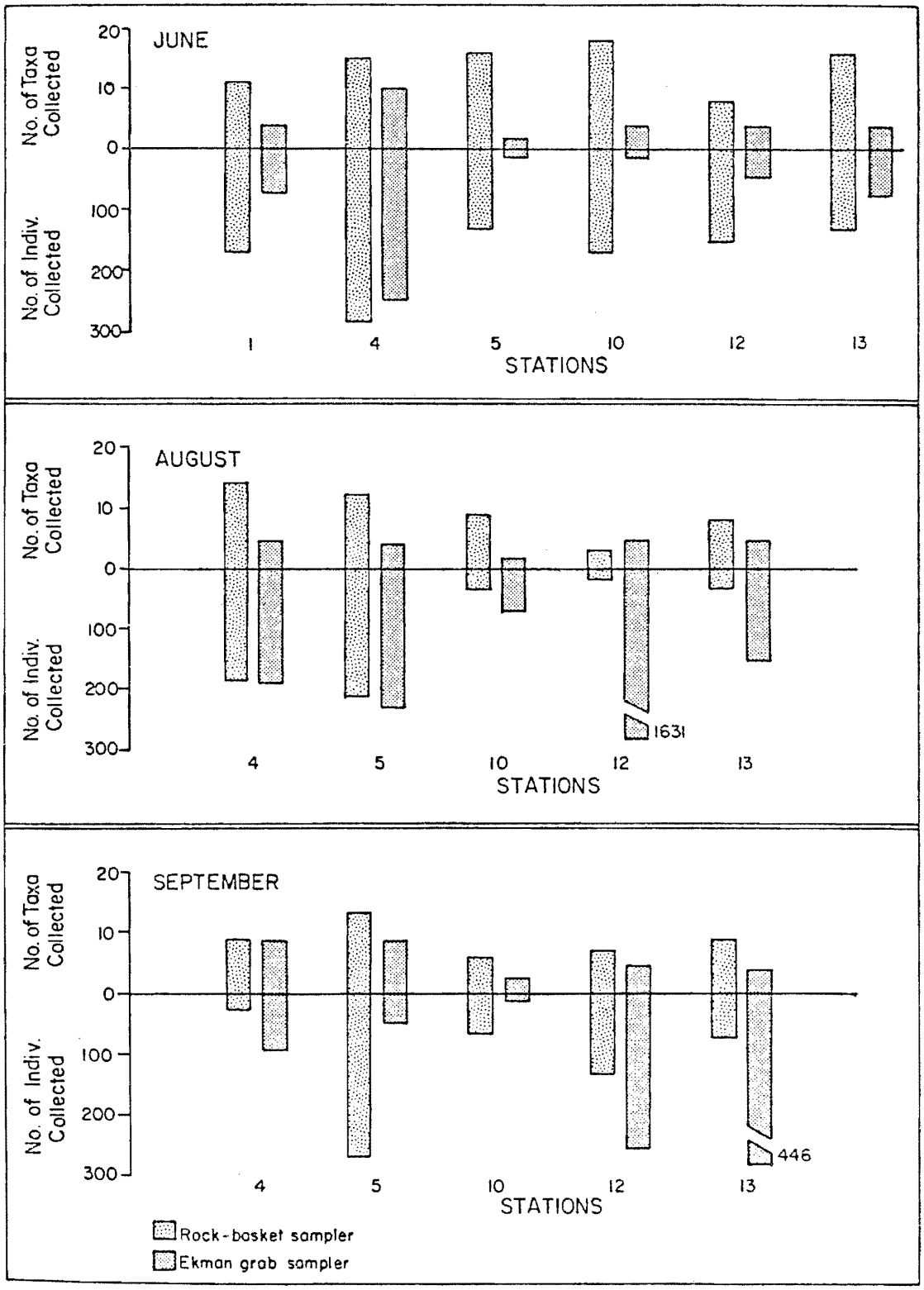

Figure 7. Comparison of the number of invertebrate taxa and individuals collected by the rock-basket and Ekman grab samples, Athabasca River. From McCart et al. (1977).

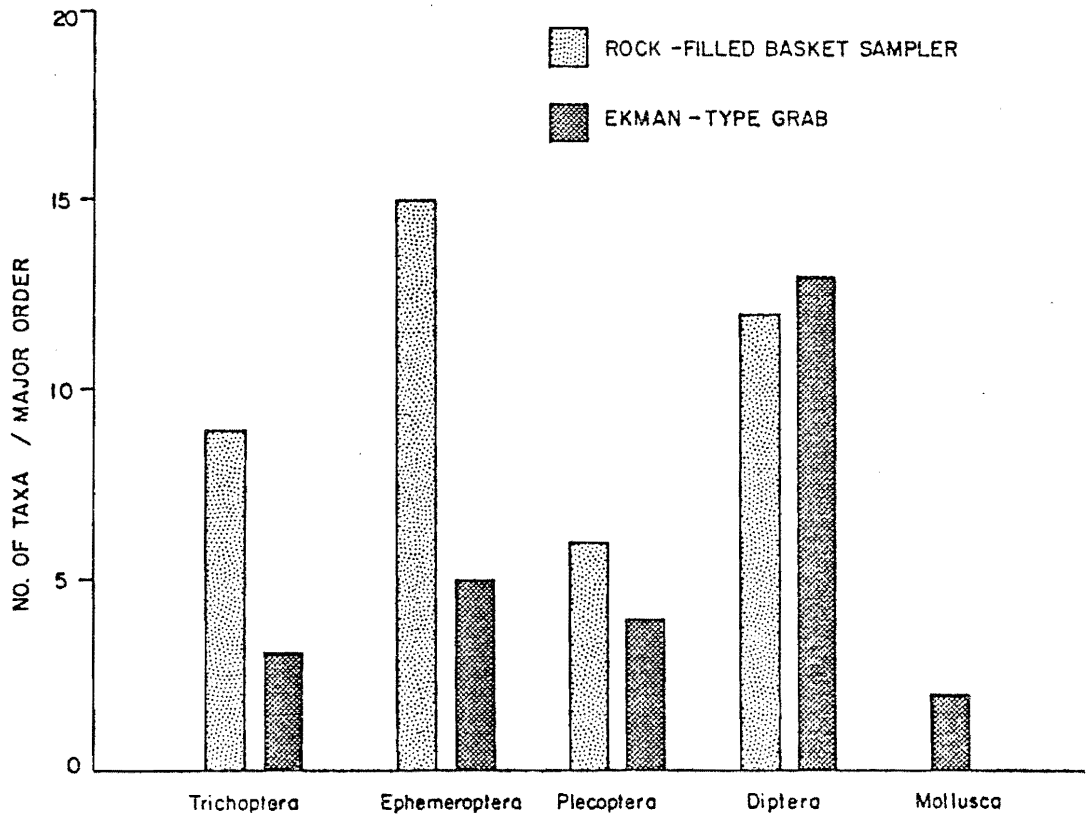

Figure 8. Comparison of the number of taxa per major invertebrate group collected by the rock-basket sampler and the Ekman grab, Athabasca River. From McCart et al. (1977).

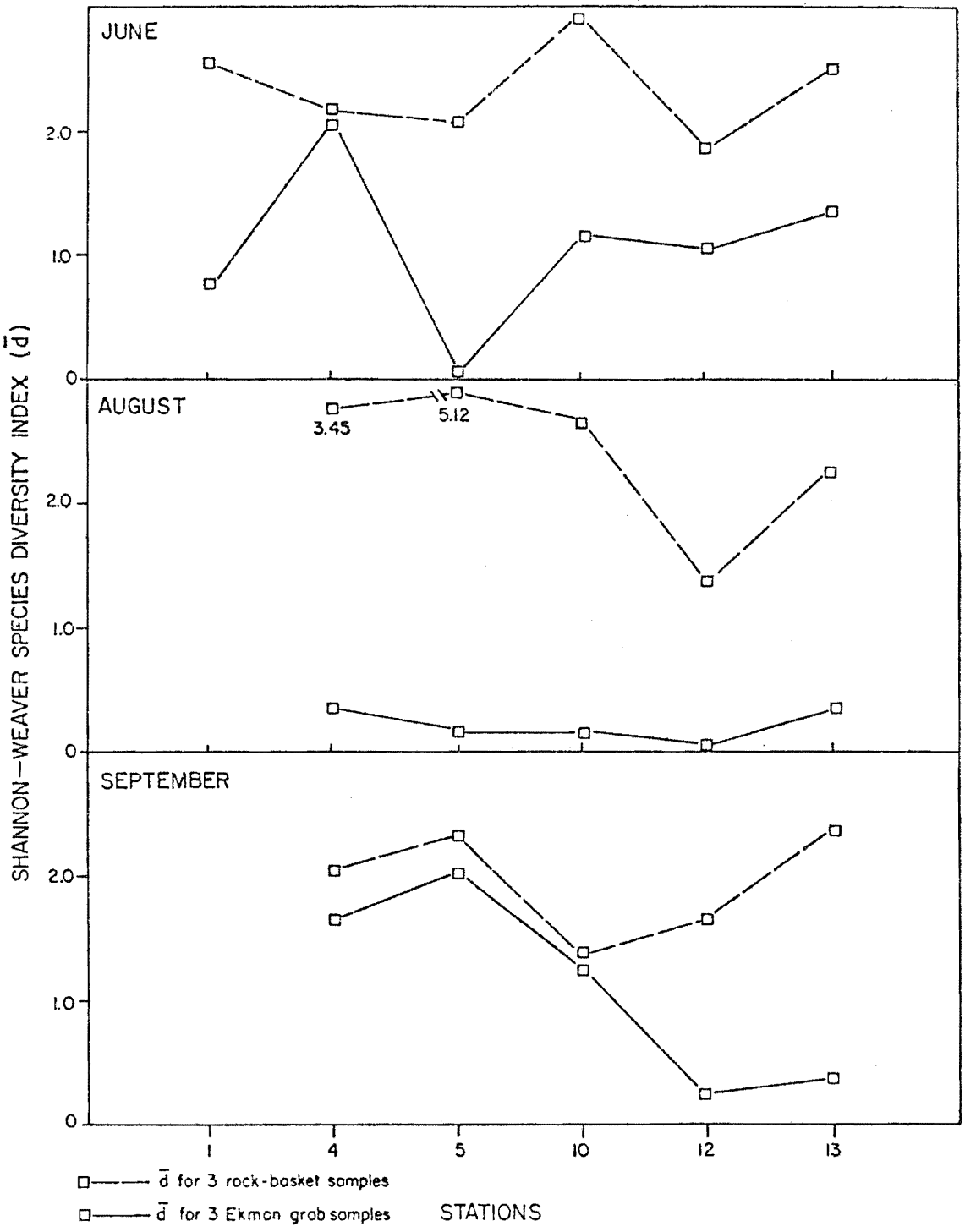

Figure 9. Comparison of the species diversity of benthic samples collected by the basket sampler and the Ekman grab, Athabasca River. From McCart et al. (1977).

4. The artificial substrates provided by the basket sampler reduce sample variation due to substrate difference and therefore can collect comparable samples at different times and places (Beak et al. 1973).
5. Because of substrate variability, a large number of bottom grab samples would be needed to give an adequate estimate of the benthic composition. Recent studies (Dickson et al. 1971; Mason et al. 1973) have indicated that a relatively small number of basket samplers are needed to obtain reliable quantitative data. For a large river, Mason et al. (1973) demonstrated that three replicate baskets can be expected (p=0.95) to provide an estimate of the true mean number of macroinvertebrates within $\pm$ 20% of the sample mean."

McCart et al. (1978) compared the sampling performance of basket samplers (both rock and tar sand substrates) and a Surber sampler at three stations on the MacKay River within the AOSERP study area. The authors found little difference between the communities developing on the rock and tar sand substrates suggesting that the latter is non-toxic and inert. Though there was some variation between stations, the basket samplers generally collected more taxa and more individuals than the Surber samplers (Figure 10). On the other hand, both diversity and equitability indices were generally lower for the artificial substrate samples than for the Surber samples (Table 12). The authors suggested that this may have been the result of insufficient colonization time and recommended that any future studies involving the use of artificial substrates should include trials to determine the optimum exposure time for colonization by the benthos characteristic of the study streams, i.e., the time required to establish a dynamically stable benthic community on the artificial substrates.

The two studies, together, suggest that artificial substrates have an additional advantage, that they can provide consistent results in any stream, large or small, over coarse or

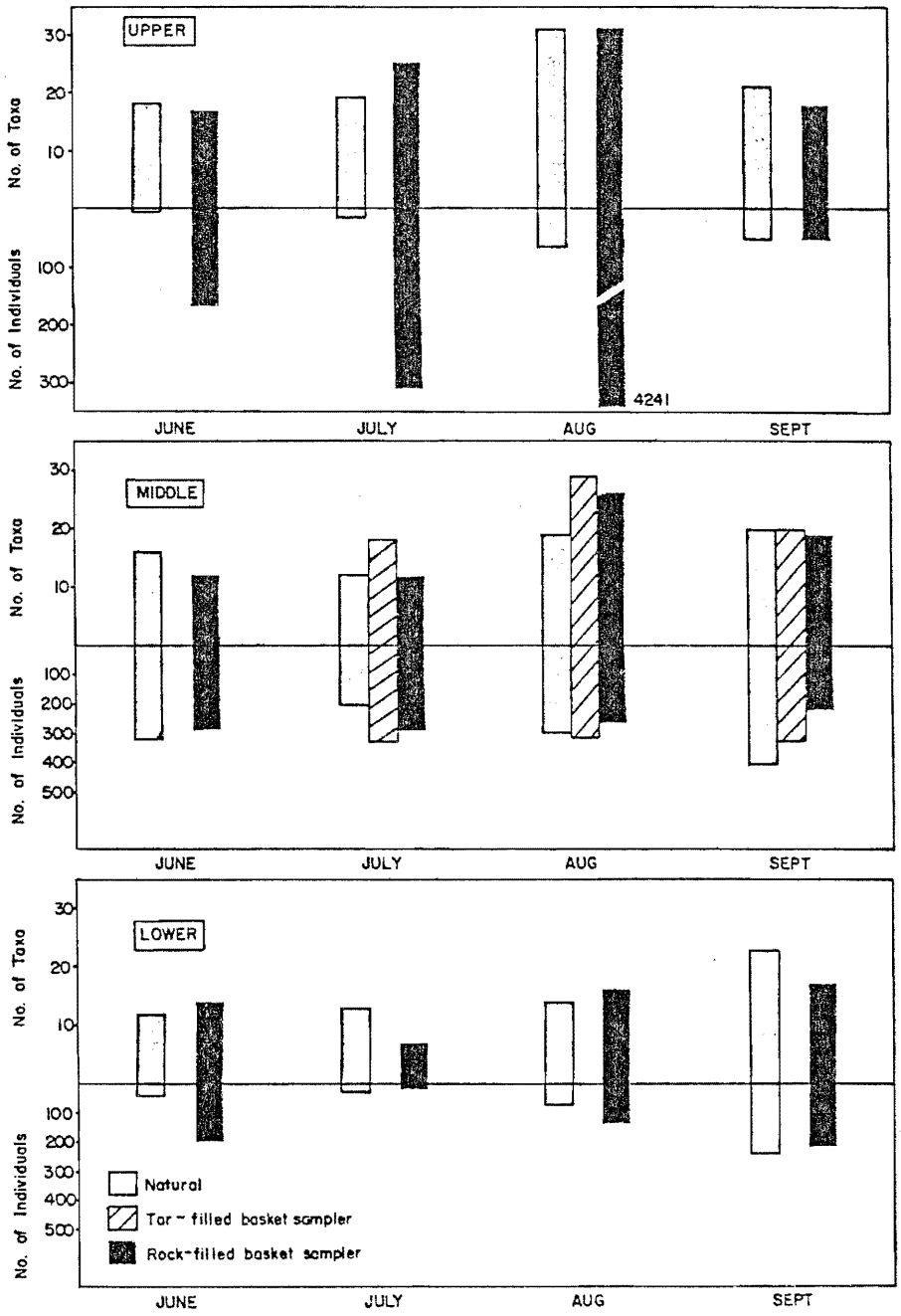

Figure 10. Comparison of the number of invertebrate taxa and individuals collected by the basket and Surber samplers in the MacKay River, 1977. From McCart et al. (1978).

Table 12. A comparison of the Shannon-Weaver Species Diversity Indices ($\bar{d}$) and MacArthur's Equitability Indices (e) for the rock-basket samples (B) and the Surber samples (S) at the Upper, Middle, and Lower Stations of the MacKay River, June to September 1977. From McCart et al. (1978).

Station	Parameters	June		July		August		September		Means	
		B	S	B	S	B	S	B	S	B	S
Upper	$\bar{d}$	1.90	3.60	3.16	3.05	3.54	3.83	2.99	3.21	2.90	3.42
	e	0.29	0.94	0.52	0.63	0.54	0.68	0.61	0.62	0.49	0.72
Middle	$\bar{d}$	3.14	2.90	3.11	2.70	3.76	3.09	3.14	3.23	3.29	2.98
	e	1.00	0.63	0.63	0.75	0.77	0.63	0.68	0.65	0.77	0.67
Lower	$\bar{d}$	2.03	3.36	1.93	3.20	2.14	2.80	3.09	3.07	2.30	3.11
	e	0.43	1.25	0.71	1.00	0.38	0.71	0.71	0.52	0.56	0.87

fine substrates, at any time of year where there is sufficient depth of water to accommodate the sampler, whereas the standard techniques for sampling natural substrates all have severe limitations with respect to depth and substrate type. This is an important consideration in an area where future monitoring efforts are likely to include streams ranging in size from very large to very small.

While artificial substrates seem to be the preferred method of indexing the benthos during biomonitoring studies, the basket sampler which was used in the studies described above (McCart et al. 1977, 1978) is not necessarily the best. Crowe (1973) compared four types of samplers and found that they varied considerably in their sampling effectiveness in a Manitoba stream. They were, from most to least effective: the steel tray sampler, the Hester-Dendy multiple-plate sampler, the basket sampler (identical to the one used in the studies described above), and a pipe sampler. Though most effective, the tray samplers were expensive to construct and difficult to handle and the author preferred the second of the four methods, the Hester-Dendy sampler. She found this sampler to be inexpensive, easy to set and retrieve, and nearly as effective as the tray sampler in sampling macroinvertebrates. A comparison of the effectiveness of various sampling devices under conditions in the AOSERP study area should be carried out as part of the preliminary field studies, before an artificial substrate, if any, is selected for routine biomonitoring in the future.

4.3 SAMPLING LOCATIONS

4.3.1 Streams

One approach in sampling stream habitats is to establish a number of stations, more or less equidistant along the study reach, though their locations may be shifted somewhat in order to assess the impacts of major sources of pollution such as cities and large industrial developments. This approach is best suited to assessing longitudinal variation within study reaches as well as the general

health of the river rather than pinpointing and assessing the effects of particular sources. It is this approach which has been taken by the Westwater Research Centre in their routine monitoring of the Fraser River, B.C. The centre has established 10 monitoring stations along the approximately 160 km of river from Hope to the mouth of the North Arm of the river.

A more detailed approach, which assesses individual, point sources of pollutants, has been outlined by Cairns and Dickson (1971). They list the following criteria for the location of sampling sites:

1. Always have a reference station or stations above all possible discharge points. Because the purpose of a monitoring program is to determine the effects of development on aquatic life, there must be some basis for comparison between areas above and below the point or points of discharge. In practice, it is usually advisable to have at least two reference stations. One should be well upstream of the discharge and one directly above the effluent discharge, but out of any possible influence from the discharge.
2. Have a station directly below each discharge.
3. If the discharge does not completely mix on entering the waterway but channels to one side, stations must be subdivided into left-bank, midchannel, and right-bank substations. All data collected--biological, chemical, and physical--should be kept separate by substations.
4. Have stations at various distances downstream from the last discharge to determine the linear extent of damage to the river.
5. All sampling stations must be ecologically similar before the communities found at each station can be compared. For example, the stations should be similar with respect to bottom substrate (sand, gravel, rock,

or mud), depth, presence of riffles and pools, stream width, flow velocity, and bank cover.

6. Biological sampling stations should be located close to those sampling stations selected for chemical and physical analyses to assure the correlation of findings.
7. Sampling stations for bottom fauna organisms should be located in an area of the stream that is not influenced by atypical habitats, such as those created by road bridges.

A sampling pattern based on these criteria would require from five to 10 or more sampling stations (including substations where there are required to assess variation across the width of the stream) for each point-source under consideration, depending on the size of the stream, the volume of the effluent in relation to stream discharge, and the extent of the downstream effects.

It is worthwhile emphasizing Cairns and Dickson's criterion six (above), that biological sampling stations should be located close to those for chemical and physical sampling. Cairns et al. (1973a) point out that biological monitoring does not replace chemical and physical monitoring. They all provide converging lines of information and supplement each other but are not mutually exclusive. They emphasize, however, that a biological monitoring program is essential in determining the synergistic and antagonistic interactions of waste discharges and the receiving system. Bio-monitoring can indicate when pollution is occurring and even pinpoint the source. It is extremely useful as a guide to the location of situations where more detailed physical and chemical monitoring should take place.

4.3.2 Lakes

It is more difficult to define criteria for the location of sampling sites in lakes than in streams. The former are much more complex and any sampling distribution must take into account not only the distribution of pollution sources but also depth, the possibility of thermal stratification, and zones of oxygen depletion,

as well as habitat variability (substrate type, presence of aquatic vegetation, shoreline exposure, etc.) and season.

Where there are point sources of pollution, the following general criteria can be applied to the location of sampling sites in lakes selected for detailed study:

1. Potential point sources should be identified and sampling stations arranged in an array about them. Those closest to the point source would be considered the affected stations, those further away would provide varying degrees of control;
2. In thermally stratified lakes, sampling sites and/or the sampling procedure should ensure that some samples are taken above and some below the depth stratum within which the thermocline is likely to occur; and
3. Insofar as possible, depth and substrate type at sampling sites should be kept uniform.

4.4 NUMBER OF SAMPLES

The problem of determining sample number (degree of replication) in the design of biological sampling programs has been treated by Elliott (1971), Southwood (1966), Cassie (1971), and, most recently, by Green (1979). Examples are presented by some of these authors using their suggested methods for determining the number of samples required to achieve commonly accepted degrees of precision in the estimation of mean values. Inevitably the results lead to one conclusion: that the number of samples required far surpasses the number that could realistically be collected and processed in any extensive biomonitoring study. For example, Green (1979) demonstrated that to estimate the mean density of organisms with a precision such that 0.95 confidence limits are $\pm$ 20% of the mean, then approximately 100 samples must be taken. If $\pm$ 40% is adequate, then 25 are required. In the case of benthic invertebrate monitoring, a sampling intensity of 25 replications could easily cost more than $2500 per station per date for collection, sorting, identification, counting, and data analysis. Furthermore,

such intensive sampling in some habitats could produce a short-term impact of its own.

While a high degree of replication may be necessary and possible in certain types of impact study, in a monitoring program, intensive replication is usually not possible and only a small number of replicates, with the resulting imprecision, must be tolerated. Where "... a number must be pulled out of a hat, three replicates per treatment combination is a good round number" (Green 1979:40), but should be considered the minimum.

4.5 TIMING OF SAMPLING

Where there is marked seasonal variability in natural populations of organisms, differences in samples taken at different times can make data interpretation difficult. Cairns and Dickson (1971) stated that, in order to make meaningful comparisons among a series of sampling stations, all stations should be sampled at approximately the same time and that no more than 2 wk should lapse between sampling at the first and last stations. There is still the question, however, of how often and at what times of the year sampling should be carried out.

Patrick (1977) concluded that continuous monitoring involving selected groups (she recommended diatoms colonizing glass substrates) and intermittent monitoring of other major groups, provided the best combination of facts concerning environmental conditions and water quality.

Continuous monitoring is not always possible, however, and a more practical alternative might involve monitoring, at short intervals, of selected groups and/or stations, combined with less frequent, intermittent monitoring of other groups and sampling sites.

The intermittent monitoring can be done as seldom as once a year. If so, mid-summer (July/August) would appear to be best, when temperatures are relatively high and the overall productivity of aquatic systems is probably close to a maximum. It is also a period when sampling for all of the major groups, especially fish, can be carried out most efficiently. If intermittent sampling is

carried out twice a year, a second sampling period in late winter (March/April), just prior to spring breakup, would seem to be an appropriate time. This is a period when many organisms are likely to be under unusual stress due to low temperatures, low oxygen conditions, and when, because of low discharge and flushing rates, effluents form a relatively high proportion of the total volume of many waterbodies. More frequent intermittent sampling is of course possible, semi-monthly, quarterly, etc., or seasonally, for example, late winter, spring, mid-summer, and fall.

4.6 METHODS OF DATA ANALYSIS

Methods of data analysis must be tailored to a specific project, but some general recommendations can be made as to approach. The following points should be considered (Green 1979):

1. The choice of statistical analysis should flow logically from the purpose, the hypothesis, and the sampling design.
2. All statistical methods are based on certain assumptions about the data. There must be a trade-off between using methods that have few assumptions but are less powerful tests of an hypothesis (non-parametric), and those that have several assumptions but are powerful tests of an hypothesis (parametric). In general, the latter are preferable because violations of the assumptions can usually be corrected.
3. The analysis used should be the most efficient one appropriate to the hypothesis model, despite its complexity. Results can later be explained to lay audiences in non-technical terms. Simple, easy-to-use methods may fail to efficiently test hypotheses.
4. Efficient methods of statistical analysis are conservative, powerful, and robust. Conservative tests have a low probability of concluding that there were biological effects when in fact there were none. Powerful tests have a low probability of concluding

that there were no biological effects when in fact there were. Robust methods are not seriously affected by violations of their basic assumptions.

5. Results of analyses should be presented whenever possible in effective visual displays.

Univariate techniques, such as t-tests, and one-way analysis of variance are satisfactory for testing, for example, differences in abundance of an indicator organism between or among control and impact stations. Good handbooks on the simpler methods are Elliott (1971), Sokal and Rohlf (1969), and most standard statistical textbooks.

Comparisons which involve many species, stations, and time periods, as are commonly required in impact studies, are best handled by more elaborate multivariate techniques. For example, multifactorial analysis of variance is a useful technique for determining whether stations differ in species abundance and composition. Discriminant analysis can then be used to determine which of the species contribute most to the differences between stations. Cluster analysis is an effective method of showing relationships in species composition among stations in a visually striking way. Examples of the use of these and other multivariate methods are provided by Green (1979). Crossman et al. (1974) provide a good example of the use of a cluster analysis technique to detect the effects of several co-occurring environmental effects on the benthic invertebrate community of a river.

4.7 DATA STORAGE AND RETRIEVAL

A biomonitoring program continuous over a long period of time will generate a large volume of detailed information requiring a computerized data storage and retrieval system. One of the tasks to be completed during the first year of a biomonitoring program should be the development of an appropriate system. This would include the following:

1. Forms suitable for keypunching on which raw data are recorded;

2. Codes for non-numeric information (e.g., for taxonomic groups);
3. Data Verification Programs to check for unlikely or impossible values and to verify taxonomic codes;
4. File Creation Programs to rearrange the original keypunch data in a form suitable for further analysis;
5. Data Summary Programs to search data files for requested information which is then presented as a printout, either in the form of tables or as computer plotted graphics; and
6. Components for Standardized Statistical Analysis Packages (e.g., Biomedical Computer Program Package, Statistical Package for the Social Sciences) suitable for the analysis of biomonitoring data.

5. POTENTIAL SOURCES OF MAJOR IMPACTS ON AQUATIC COMMUNITIES WITHIN THE AOSERP STUDY AREA

There are a number of sources of materials which might affect aquatic communities within the AOSERP study area, including:

1. Domestic sewage;
2. Mine depressurization water;
3. Sediments;
4. Hydrocarbons;
5. Pesticides; and
6. Airborne contaminants.

Each of these is discussed briefly below.

5.1 DOMESTIC SEWAGE

The principal existing source of domestic sewage within the AOSERP study area is the Town of Fort McMurray.

The town has recently established a system of continuous discharge from aerated lagoons. Discharge is approximately 2 to 3 million gallons per day with no consistent seasonal variability (Andreychuk in prep.). This flow is only a very small proportion of even the minimum flows in the Athabasca River; for example, only only about 0.06 to 0.09% of mean monthly discharge below Fort McMurray even during the minimum flow month of March.

A second major source of domestic sewage effluents would be associated with the development of the New Town which is being considered in relation to the proposed Alsands Oil Sands Mining Project. Other potential sources of sewage effluents in the AOSERP study area are either small or temporary (e.g., construction camps).

While domestic sewage can have major adverse effects on aquatic communities, at the level of development anticipated for the AOSERP study area in the near future, it is unlikely that sewage effluents along will have any more than limited, local effects on the Athabasca River. Smaller tributaries might, however, be adversely affected if they are used to channel sewage from camps, plant sites, and towns to the river.

5.2 MINE DEPRESSURIZATION WATER

Mine depressurization groundwater is saline, with high concentrations of some heavy metals and organic compounds. Lake and Rogers (1979) and Strosher and Peake (1978) include detailed profiles of the chemical composition of mine depressurization water from Syncrude's Lease 17 (Tables 13 and 14). Further data describing chemical composition are in McMahon et al. (1977), including an analysis of a precipitate which sometimes forms (Table 15).

Machniak (1977) reviewed the general literature concerning the impact of saline waters upon freshwater biota. McMahon et al. (1977) found that groundwater from Syncrude's Lease 17 was acutely toxic to fish and aquatic invertebrates (Figure 11). Lake and Rogers (1979) reached similar conclusions. Sprague et al. (1978) and Anderson et al. (1979) have examined the toxicity of individual components of saline groundwater (vanadium, nickel, and phenol) but, as yet, there is no obvious correlation between the toxicity of mine depressurization groundwater and any particular constituent.

At present, the Syncrude operation is the most important potential source of saline mine depressurization groundwater to the Athabasca River. Water from the project area enters the river via Poplar Creek, Ruth Lake, and the Beaver Creek reservoir. Carmack and Killworth (1979) concluded that, at least in 1977 and 1978, there was very little saline groundwater leaving the Beaver Creek diversion system, suggesting that the reservoir was fairly effective in diluting saline groundwater. They cautioned, however, that they could not rule out the possibility of an increase in saline discharge from the system in the long term. Saline groundwater will also be contributed to the Athabasca and Muskeg rivers by the Alsands Project for a period of several years (Alsands Project Group 1978).

5.3 SEDIMENTS

Increased sediment loads can have a variety of adverse effects on aquatic systems. Increased turbidity can reduce primary production while sedimentation of substrates can reduce populations

Table 13. A profile of the chemical composition of mine depressurization water from Syncrude Canada Limited, Lease 17. From Lake and Rogers (1979).

Parameter	Mean	Range
Inorganics		
Ammonia	7.65	2.2-13.65
Bicarbonate	3150.29	1828.5-5427
Calcium	71.86	1.0[a]-318
Carbonate	27.18	0.0-396
Chloride	7615.47	2250-10500
Conductivity	25658.5	9400-48000
Fluroide *(sic)*	0.71	0.48-1.25
Hardness, Total	741.58	295-1377
Magnesium	133.89	47-253
Nitrite	0.1[a]	0.1[a]-ND
Nitrite	0.037	0.1[a]-0.061
pH	7.55	6.9-9.1
Phosphorus, Ortho	0.06	0.005[a]-0.242
Phosphorus, Total	0.17	0.0005[a]-1.74
Potassium	45.44	19-65
Silica	4.39	2.0-18.0
Soldium *(sic)*	5622.63	2150-7900
Sulphate	10.22	0.5[a]-78
Sulphide	0.051	0.02[a]-0.11
Total Inorganic Carbon	479.19	250-820
Organics		
Alkalinity, Total	2628.78	1524-4452
Biochemical Oxygen Demand	3.67	2.0-8.0
Carbon, Total	757.31	474-1130
Chemical Oxygen Demand	231.4	10-1282
Humic Acid	1.17	1.08[a]-1.36
Hydrocarbon, Total	15.01	0.001[a]-324
Nitrogen, Total Kjeldahl	11.37	4.8-22.9
Oil and Grease	2.43	0.1[a]-36.3
Phenol	0.0055	0.0001[a]-0.029
Polychlorinated Biphenyls	0.00015	0.0001[a]-0.0006
Surfactants	0.32	1.02[a]-188
Tannin and Lignin	0.62	0.1[a]-2.0
Total Organic Carbon	189.42	1.0[a]-9319
Physical		
Color	25.31	5-98
Color T_2	98.62	98-99
Color T_3	96.62	96-97
Odor	17.31	2-100
Total Dissolved Solids	15561.16	9319-19245
Total Residue	15479.32	8810-19330
Total Filterable Residue	14688.68	5768-19240

Continued...

Table 13. Concluded.

Parameter	Mean	Range
Total Filterable Residue Fixed	16 268.39	5376-19140
Total Non-Filterable Residue	55.84	0.4[a]-436
Total Non-Filterable Residue Fixed	47.33	0.4[a]-394
Turbidity	32.16	0.01[a]-298
Metals		
Aluminum	0.16	0.005[a]-2.3
Arsenic	0.004	0.0002-0.02
Boron	2.60	0.48-7.08
Cadmium	0.013	0.001[a]-0.053
Chromium *(sic)*	0.008	0.002[a]-0.036
Cobalt	0.05	0.002[a]-0.165
Copper	0.015	0.001[a]-0.032
Iron	1.51	0.04[a]-7.45
Lead	0.028	0.002[a]-0.142
Manganese	0.194	0.65-1.2
Mercury	0.0034	0.0001[a]-0.07
Nickel	0.059	0.002[a]-0.32
Selenium	0.0014	0.005[a]-0.0037
Silver	0.011	0.001[a]-0.05
Vanadium	0.004	0.001[a]-0.02
Zinc	0.024	0.001[a]-0.2

Alkalinity and Hardness expressed as Calcium Carbonate
Conductivity in µS/cm
Metals as Totals mg/L
Nitrite, Nitrite & Nitrate, Ammonia expressed as N
pH in pH units
Phosphorus, Ortho expressed as P
Phosphorus, Total expressed as P_4
Turbidity in J.T.U.

[a]Less than

Table 14. Organic analysis of mine depressurization water from five wells from Syncrude Canada Limited Lease 17. From Strosher and Peake (1978).

Parameters (All values as mg/L)	Sample Dates	
	14 Sept 1976	30 Nov 1976
Original Carbon	35	24
Extractable Carbon	21	16
Residual Carbon	14	8
Percentage of Carbon Extracted	60	64
Asphaltenes	3.41	1.80
Alkanes and Alkenes	0.11	0.01
Aromatics	0.60	0.02
Polar Compounds	0.03	0.02
Sulphur Compounds	2.7	3.3
Elemental Sulphur	0.001	0.001
Phosphorous Compounds	0.0001	0.0001
Chlorinated Hydrocarbons	0.01	0.006
Organic Nitrogen Compound	0.014	0.029
Aldehydes	0.48	0.54
Amides	0.36	0.27
Ketones	0.01	0.01
Quinones	0.45	0.55
Esters	1.15	1.34
Phenols Colorimetric	0.003	0.001
Phenols by GC	0.19	0.19
Organic Acids	1.1	2.6

Table 15. Chemical constituents of groundwater precipitate from an unidentified well on the Syncrude Lease. From McMahon et al. (1977).

	Lower[a] Concentration Limit (ppm)	Number 144-03-03
Antimony	50	bcl
Arsenic	50	bcl
Barium	5	2000
Beryllium	5	bcl
Bismuth	5	bcl
Boron	20	2000
Cadmium	20	bcl
Calcium[b]	0.05%	0.5%
Chromium	10	bcl
Cobalt	10	10
Copper	1	50
Gallium	2	5
Germanium	20	bcl
Iron[b]	0.05%	1.0%
Lead	5	50
Magnesium[b]	0.02%	0.2%
Manganese	5	200
Molybdenum	10	bcl
Nickel	5	50
Niobium	50	bcl
Silver	1	2
Strontium	20	1000
Tantalum	200	bcl
Tellurium	200	bcl
Thorium	100	bcl
Tin	10	20
Titanium	5	500
Vanadium	10	20
Zinc	50	2000
Zirconium	20	bcl

[a] Concentration Range

>5 000 ppm =	>5 000 ppm	50 ppm =	25 - 100 ppm
5 000 ppm =	2 500 - 10 000 ppm	20 ppm =	10 - 50 ppm
2 000 ppm =	1 000 - 4 000 ppm	10 ppm =	5 - 20 ppm
1 000 ppm =	500 - 2 000 ppm	5 ppm =	2 - 10 ppm
500 ppm =	250 - 1 000 ppm	2 ppm =	1 - 4 ppm
200 ppm =	100 - 400 ppm	1 ppm =	0.5 - 2 ppm
100 ppm =	50 - 200 ppm	bcl =	below concentration limit

[b] Ranges for Iron, Calcium, and Magnesium are reported in %.

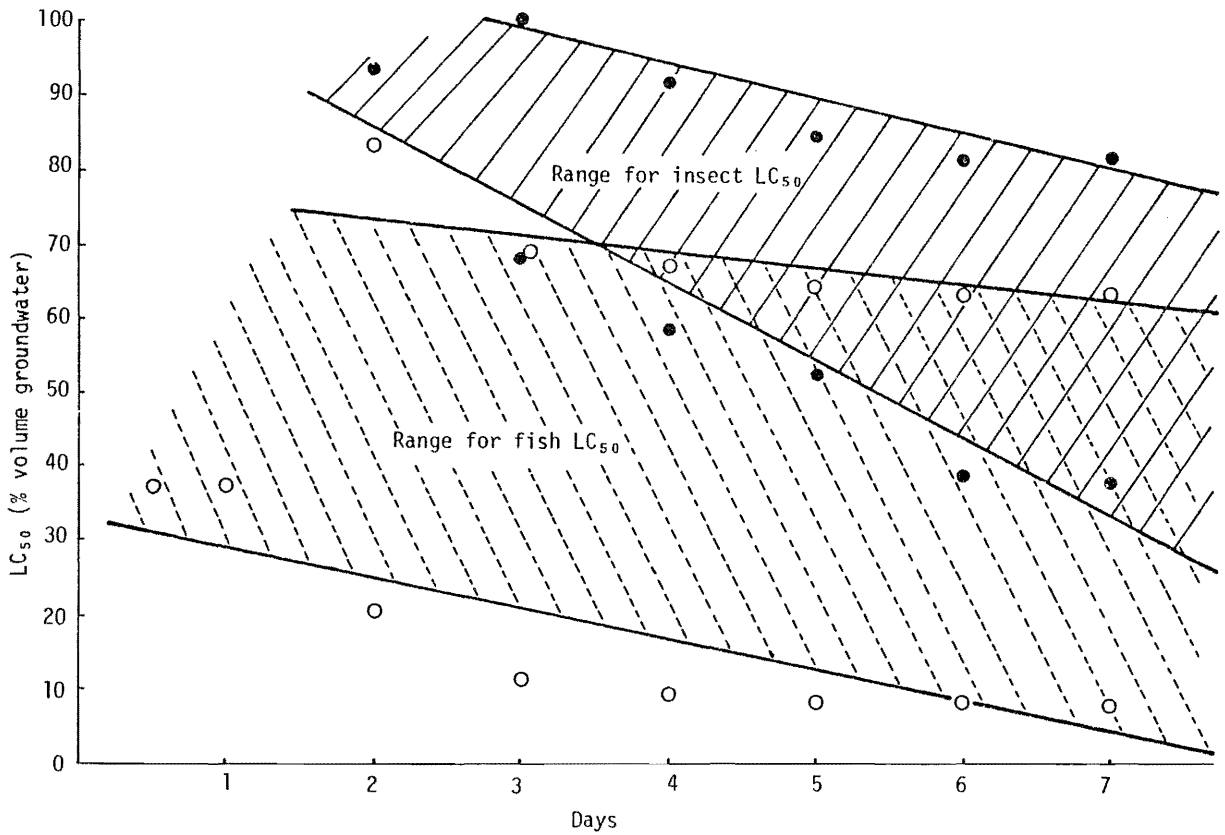

Figure 11. Approximate range of values for LC50 for fish and benthic invertebrates tested in saline groundwater at 5°C. Black circles indicate extreme values for insect species and open circles indicate extreme values for fish species (not including eggs). From McMahon et al. (1977).

of invertebrates and fish. In streams, these effects are likely to be short-term, as long as sediment production is not continuous and sediments can be removed by natural processes such as scouring during freshets. In lakes, they may be longer term and potentially more serious.

In the AOSERP study area, sedimentation is most likely to affect smaller tributaries in the immediate vicinity of mining operations and other developments (ea the Beaver River in the vicinity of the Syncrude operation and, potentially, Hartley Creek and the Muskeg River in the vicinity of the proposed Alsands Project). These small streams are in the immediate vicinity of major construction projects and have only a limited sediment transport capacity. The Athabasca River, which has a much larger sediment transport capacity and is already turbid throughout the ice-free season, is less likely to be adversely affected by sedimentation arising from mining developments. One major development has been proposed, however, which could produce sufficient sediment during construction to have a measurable impact on the Athabasca River. This is the ice-control weir which has been proposed for the river between Fort McMurray and Mountain Rapids, a major spawning area for lake whitefish from Lake Athabasca.

5.4 HYDROCARBONS

Streams have cut through oil sands in many places within the AOSERP study area. As a consequence, concentrations of naturally occurring hydrocarbons are relatively high (Table 16) and bitumen is a common constituent of stream substrates, for example, in the Athabasca River (McCart et al. 1977) and MacKay River (McCart et al. 1978). There is evidence of major groundwater intrusions into the Athabasca River, for example, along the east bank upstream of the Muskeg River (McCart et al. 1977), which probably include some organic constituents. Other potential sources include mine depressurization water which has a significant hydrocarbon component (Table 13), refinery effluents which escape

Table 16. Organic analysis of Athabasca River water: upstream sample. From Lake and Rogers (1979).

Parameters (All values as mg/L)	Sample Dates		
	15 Sept 1976	15 Dec 1976	17 Feb 1977
Original Carbon	15	15	9
Extractable Carbon	3	3	3
Residual Carbon	12	12	6
Percentage of Carbon Extracted	20	20	33
Asphaltenes	ND[a]	ND	ND
Alkanes and Alkenes	0.004	0.001	0.001
Aromatics	0.009	0.002	0.001
Polar Compounds	0.030	0.010	0.002
Sulphur Compounds	0.06	0.04	0.01
Elemental Sulphur	0.003	0.001	0.001
Phosphorous Compounds	0.00001	0.00002	0.00006
Chlorinated Hydrocarbons	0.001	0.001	0.001
Organic Nitrogen Compounds	0.001	0.001	0.001
Aldehydes	0.19	0.26	0.48
Amides	0.50	0.42	0.22
Ketones	0.01	0.01	0.01
Quinones	0.10	0.12	0.10
Esters	0.001	0.029	0.027
Phenols Colorimetric	0.001	0.001	0.001
Phenols by GC	0.01	0.01	0.01
Organic Acids	0.01	0.01	0.01

[a]ND - no data

the recycling process, domestic sewage from Fort McMurray and other sources, as well as spills of fuel and crude.

The responses of aquatic communities to hydrocarbon contamination vary depending on the nature of the contaminant. The following observations have been made within the AOSERP study area:

1. Barton and Wallace (1979a) found that a portion of the Steepbank River which cuts through the Athabasca Oil Sands supported a less diverse benthic invertebrate community, especially Plecoptera and Trichoptera, than did upstream areas. As a substrate for benthic invertebrates, oil sand appears to be analogous to bedrock, supporting about 60% as many animals as adjacent rubble substrates.
2. McCart et al. (1978) found that rock and oil sand substrates immersed in rock basket samplers in the MacKay River were colonized by benthic invertebrate populations which were very similar in terms of density, taxonomic and species diversity, and equitability. The only major difference in the two substrates was among the chironomids. Of the 10 species found in July, four were restricted to the oil sand substrate.
3. Barton and Wallace (1979b) introduced a small instantaneous spillage of oil sands tailings sludge (from Suncor) containing fine silt, heavy sticky oils, and heavy metals into the Muskeg River. In the immediate vicinity of the spill there was a 60% reduction in the standing stock of benthic invertebrates throughout a four week period. Sensitive organisms decreased in abundance as much as 30 m downstream.
4. Lock and Wallace (1979) found evidence that refined, light oils (synthetic crude oil) stimulated the growth of microflora. They suggested that while massive oil spills to running fresh waters would have an initial detrimental effect on fishes and benthos, the subsequent degradation or erosion of the oil by

natural processes may allow for a more rapid recovery than was previously thought, at least for light oils.

5.5 AIRBORNE CONTAMINANTS

Air emissions from oil sands upgrading plants contain several substances which might affect aquatic communities. The most important of these are sulphur compounds and heavy metals, especially vanadium and nickel (Alsands Project Group 1978). Hesslein (1979) concluded that, because of the high alkalinity, most lakes in the AOSERP study area were highly resistant to pH change. Exceptions were the Gardiner-Namur lakes. The Alsands Project Group assessed the available evidence regarding the toxicity of nickel and vanadium and concluded that the concentrations of these two metals in the Athabasca River would have to increase, respectively, 25 and 27 times to exceed the acceptable levels for fish.

5.6 PESTICIDES

Between 1974 and 1977, methoxychlor was released in the Athabasca River in an experimental program to control blackflies within Athabasca County, upstream of the AOSERP study area. The program is being reviewed and treatment of the river might resume on a continuing basis in future. Its effects must be considered in assessing the results of biomonitoring.

6. SUGGESTIONS FOR BIOMONITORING IN THE AOSERP STUDY AREA

6.1 APPROACH AND RATIONALE

From the foregoing, it is apparent that there is a wide variety of potential impacts on aquatic systems within the AOSERP study area, each with its own range of effects, many of them acting simultaneously. In some instances, for example, where a major outfall of sewage effluents or where mine depressurization groundwater enters a stream, there are likely to be significant local effects, assignable to a specific source. In others, such as the airborne contaminants, the effects are likely to be more generally distributed and unassignable to any specific source.

The question arises: What is the best way to detect these effects, if present, and reduce them to a minimum? In discussions with various interested parties during this study, some essentially have taken the view of Gaufin (1973:97):

"The assessment of water pollution is principally a biological problem in that its primary effect is on living organisms. Nevertheless, despite this biological relationship, most studies of water pollution have been directed toward obtaining primarily chemical and physical measurements such as dissolved oxygen, BOD, suspended solids, and other such parameters. Since chemical studies give information on physical-chemical conditions only at the time of sampling, and pollution surveys frequently cannot be made during the period of the most critical conditions, there is need for additional methods that can be used throughout the year for determining the extent and severity of brief critical or limiting environmental factors. The qualitative and quantitative composition of an aquatic population is determined by recurring critical conditions, even though of short duration, as well as the more stable or long-term environmental factors. Therefore, the complex of organisms which develops in a given area is, in turn, indicative of environmental conditions which have occurred during its development. Organisms having life histories of a year or more will thus serve to indicate unfavorable or limiting

conditions that have occurred several months previously. Because aquatic populations are a result of past environmental conditions, they serve as a means for determining such conditions in a stream. They are especially valuable because they can be used during fall, winter, or spring months, when flows may be large, dilution is at a maximum, dissolved oxygen is near saturation, and visual evidence of pollution at a minimum, to delineate former septic areas or to indicate critical conditions of short duration."

Others have taken the view that chemical and physical monitoring would permit polluted conditions to be detected before they cause any biological damage, or that once biological effects are detected, it will still be necessary to conduct studies linking the effects with specific pollutants.

These various viewpoints are not mutually exclusive. Biological monitoring of aquatic habitats in the AOSERP study area must be undertaken, if for no other reason than to determine whether the numerous and expensive procedures implemented to safeguard aquatic biota in the area are, in fact, working. Furthermore, aquatic biomonitoring should be conducted because it is practically never possible to predict, on the basis of chemical and physical analyses alone, the enormous number of synergistic or antagonistic interactive effects that pollutant mixtures will have on biological communities. Physical and chemical monitoring must be done because degradation of water quality has direct effects on people, on uses to which water can be put, and because any biological effects detected in the biomonitoring program will have to be related to water quality information. Because of this latter need, the two types of monitoring should be closely coordinated. Finally, both types of monitoring should be a part of a sound, integrated, environmental research and management program for the AOSERP study area that seeks to identify potential or present environmental problems and provide solutions to them.

The role of the aquatic biomonitoring program *per se* could only be to detect impacts, if any, on the biota and, if possible, to identify sources of impact. It would remain for the

overall research and management program to identify specific pollutants, modes of action, and solutions to specific problems. A discussion of the water quality monitoring program, and the overall research and management function, is beyond the terms of reference for this study, and all further comments are limited to a discussion of the aquatic biomonitoring program only.

6.2 OBJECTIVES

An aquatic biomonitoring program for the AOSERP study area should have the following two general objectives:

1. To assess the impact on aquatic systems of known, point sources of potentially damaging materials; and
2. To provide a continuing assessment of the general health of aquatic systems in waterbodies within the study area.

These two functions are not mutually exclusive and a system can be devised which serves both simultaneously.

To be effective, the program should be based on methods which:

1. Are quantitative and objective, rigorous and repeatable;
2. Are appropriate to northern boreal seasonal conditions;
3. Can provide quick feedback to government and industry representatives, charged with making pollution abatement decisions;
4. Are routinely workable by a small staff;
5. Measure both short and long term changes in water quality and habitat; and
6. Are cost effective.

While much is known of the baseline ecology, within the AOSERP study area, of some groups of aquatic organisms (particularly benthic invertebrates and fish and, to a lesser extent, the periphyton), no studies have been directed specifically at the problem of biomonitoring. Without such studies, it is not possible to design a detailed, continuous biomonitoring program for the area. (One of the recommendations of this report is that preliminary

studies be carried out for at least a year before there is a committment to any particular methodology or sampling strategy). What follows are, therefore, general suggestions concerning the conduct of a biomonitoring program in the AOSERP study area.

6.3 PRELIMINARY STUDIES

The basic purpose of preliminary studies would be to test a variety of sampling and analytical methods, both in the field and in the office, to arrive at a practical combination which is both sensitive to the kinds of impacts likely to occur within the study area and, at the same time, cost effective.

The results of these studies would include:

1. A detailed sampling strategy which would outline:
   (a) the locations of sampling stations;
   (b) a sampling timetable indicating the kinds of samples to be taken at various times during the year;
2. An identification of the groups which should be included in the sampling program along with a rationale for their inclusion;
3. An outline of the precise methodology which should be used in sampling each of the selected groups;
4. Details of the methods to be used in analysing the resultant data;
5. Design of a data storage and retrieval system; and
6. Recommendations concerning the organization of a continuing biomonitoring group.

6.4 SAMPLING STRATEGY

There is a variety of possible sampling techniques which can be used within the AOSERP study area, ranging from very general to very detailed. The choice between these depends on the purposes for which biomonitoring is conducted, whether is is simply an assessment of the continuing "health" of waterbodies or a more detailed monitoring of the effects of individual sources of potential pollution.

6.4.1 Sampling Locations

At a minimum, sampling stations for aquatic biomonitoring should be located to assess the major sources of potential impact on streams in the study area. These major sources include Fort McMurray, Syncrude, Suncor, and the proposed Alsands plant; the streams involved being the Athabasca, Hangingstone, and Muskeg rivers, and Poplar, Beaver, and Hartley creeks. As development within the area proceeds, other stream and lake stations should be added as applicable.

In general, stations should be located according to the criteria of Cairns and Dickson (1971), discussed in Section 4.3.1. Because such a sampling pattern might require from five to 10 or more stations for each point-source under consideration, however, this level of effort might become prohibitive. For example, a scheme designed to assess the impact of saline water from the Syncrude operation, entering the Athabasca River via the Poplar River, might require the following:

1. Two control stations on the Athabasca above the Poplar confluence;
2. One station (with three substations) immediately below the confluence of the Polar and Athabasca rivers; and
3. Four stations (each with three substations) at approximately equal intervals to the downstream limit of effects.

This would be a total of seven stations or 17 sampling locations if substations are considered individually. A scheme to assess the individual effects of four major sources (Fort McMurray, Syncrude, Suncor, and the proposed Alsands plant) of effluents entering the Athabasca River, based on Cairns and Dickson's (1971) criteria, might require 50 to 60 individual sampling locations (stations assessing the downstream effect of one source could act as control stations for one further downstream).

It seems likely that a reasonable sampling program for the Athabasca River can be designed which includes 15 to 20 stations

(some including several substations) to provide a general assessment of conditions within the AOSERP area, from above Cascade Rapids downstream to the Delta, as well as more detailed information on conditions in the stream reach which is most likely to be heavily impacted, from Fort McMurray to a point downstream of the proposed Alsands Project. As previously indicated, the preparation of a detailed scheme for sampling locations in the Athabasca River should be one of the objectives of the preliminary biomonitoring studies which have been recommended. The scheme should be based on the results of a field reconnaissance and of the survey of effluent sources which is currently being prepared for AOSERP by Andreychuk (in prep.). The details of sampling distribution on other streams should be an objective of the recommended preliminary biomonitoring studies.

Before leaving the subject of sampling locations, it is worth emphasizing that, in waterbodies such as those in the AOSERP study area, where there may be marked seasonal (Figures 12, 13, and 14) and spatial (Figure 15) differences in physical and chemical conditions, a correlated physical and chemical monitoring program is essential in order to avoid erroneous interpretations of purely biological data. The details of such a program should be one of the objectives of the preliminary biomonitoring studies which have been recommended but should include, as a minimum, such biologically relevant parameters as concentrations of major ions, salinity and conductivity, nutrient (P and N) concentrations, pH, O_2 concentration, suspended sediments and turbidity, and concentrations of selected organic compounds, as well as the concentrations of metals (e.g., Cu and Zn) which might be subject to bioaccumulation.

6.4.2 Timing of Sampling

Studies of aquatic communities in streams in the AOSERP study area (e.g., McCart et al. 1977, McCart et al. 1978) indicate that there is considerable seasonal variability in each of the major biotic groups. As Cairns and Dickson (1971) recommended,

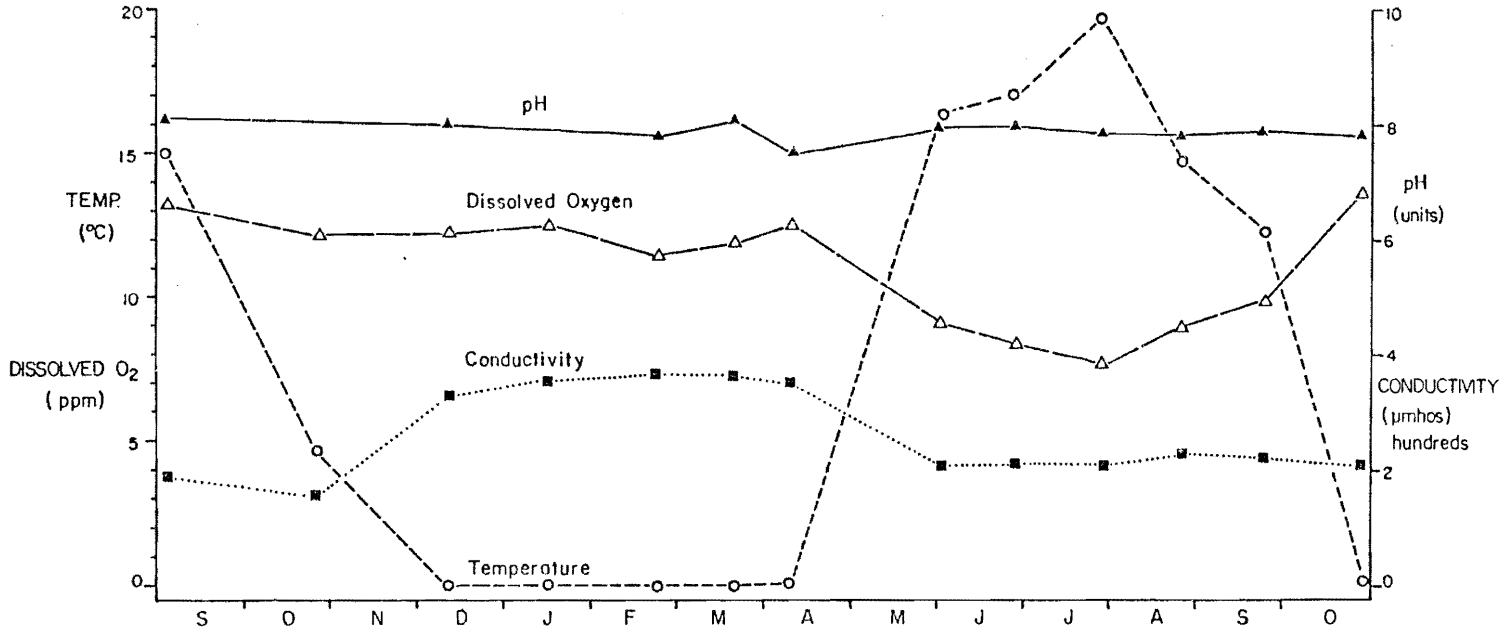

Figure 12. Seasonal variation in mean values for pH, dissolved oxygen, conductivity, and temperature in Athabasca River, 1974 and 1975. From McCart et al. (1977).

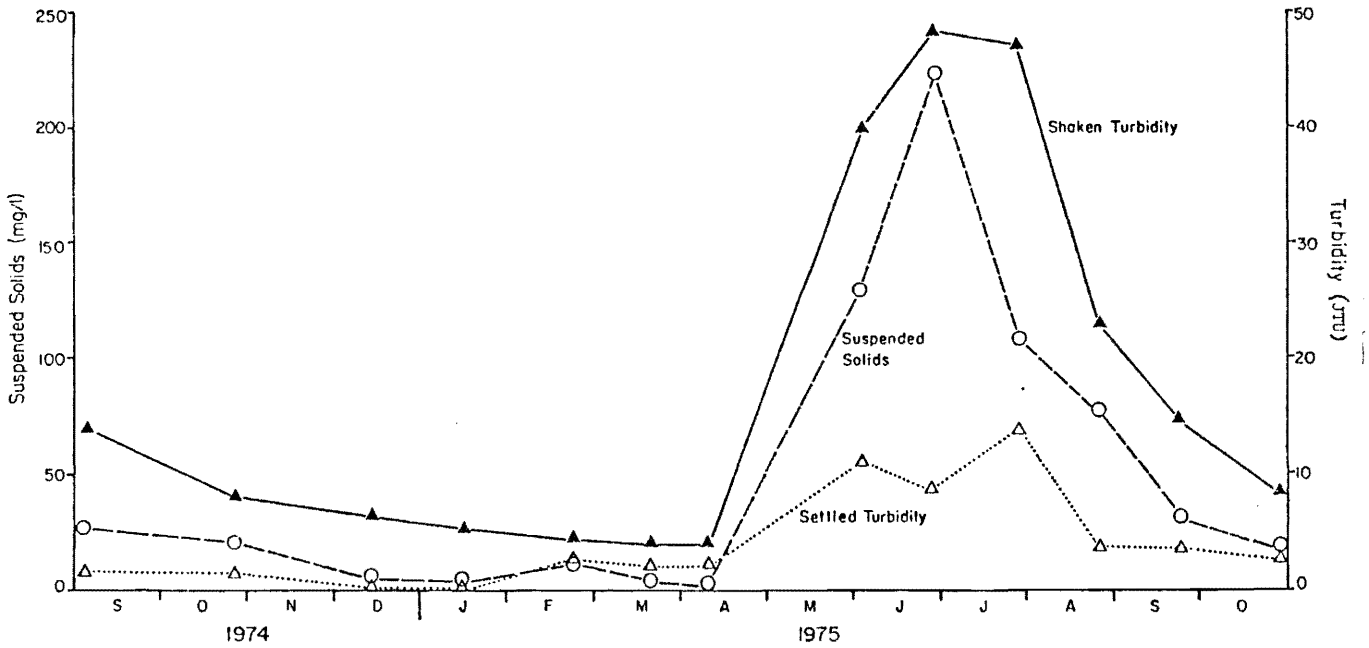

Figure 13. Seasonal variation in mean values for turbidity (shaken and settled) and suspended sediments in the Athabasca River, 1974 and 1975. From McCart et al. (1977).

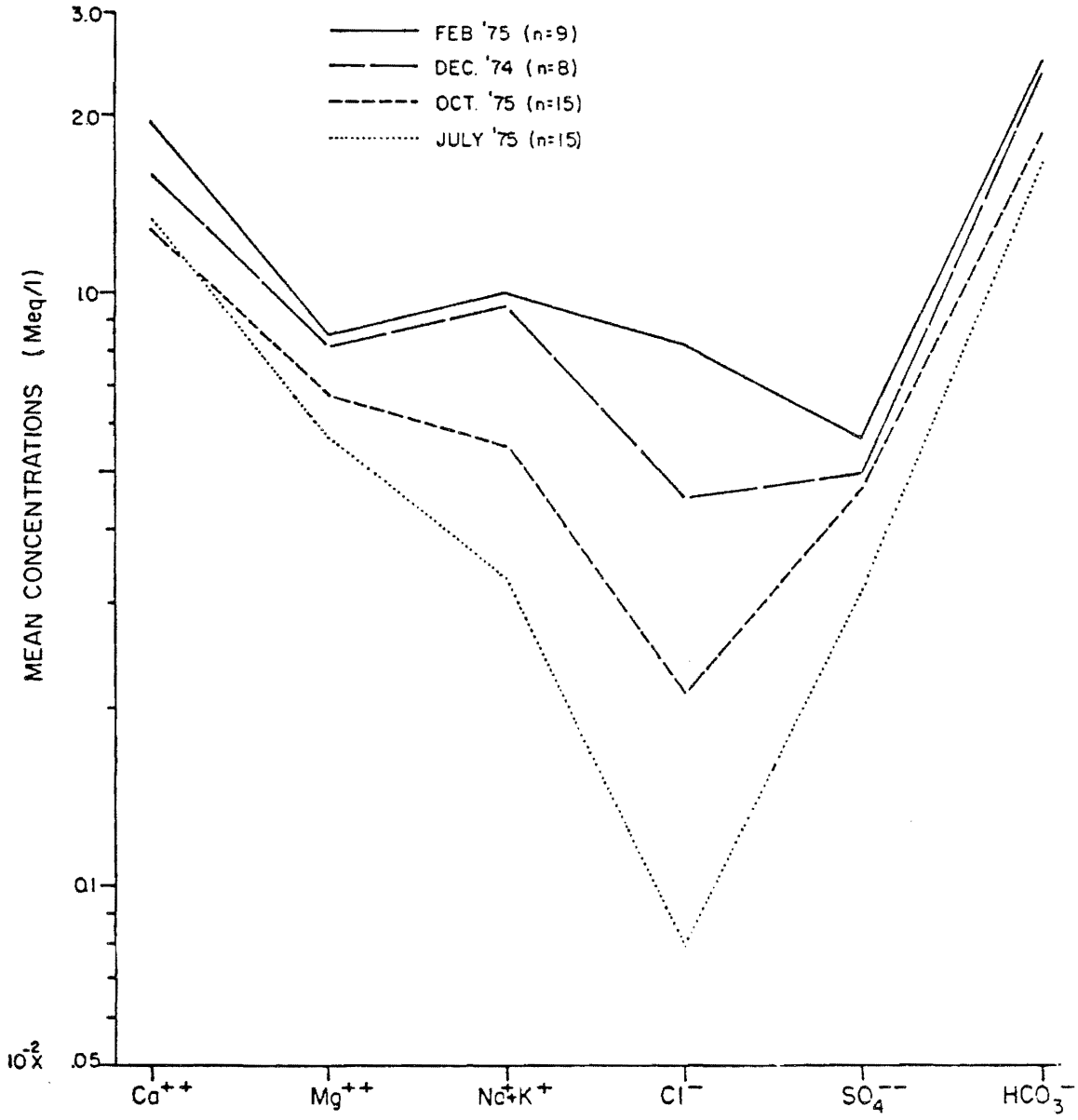

Figure 14. Proportions of major ions in water samples taken from the Athabasca River on various dates. From McCart et al. (1977).

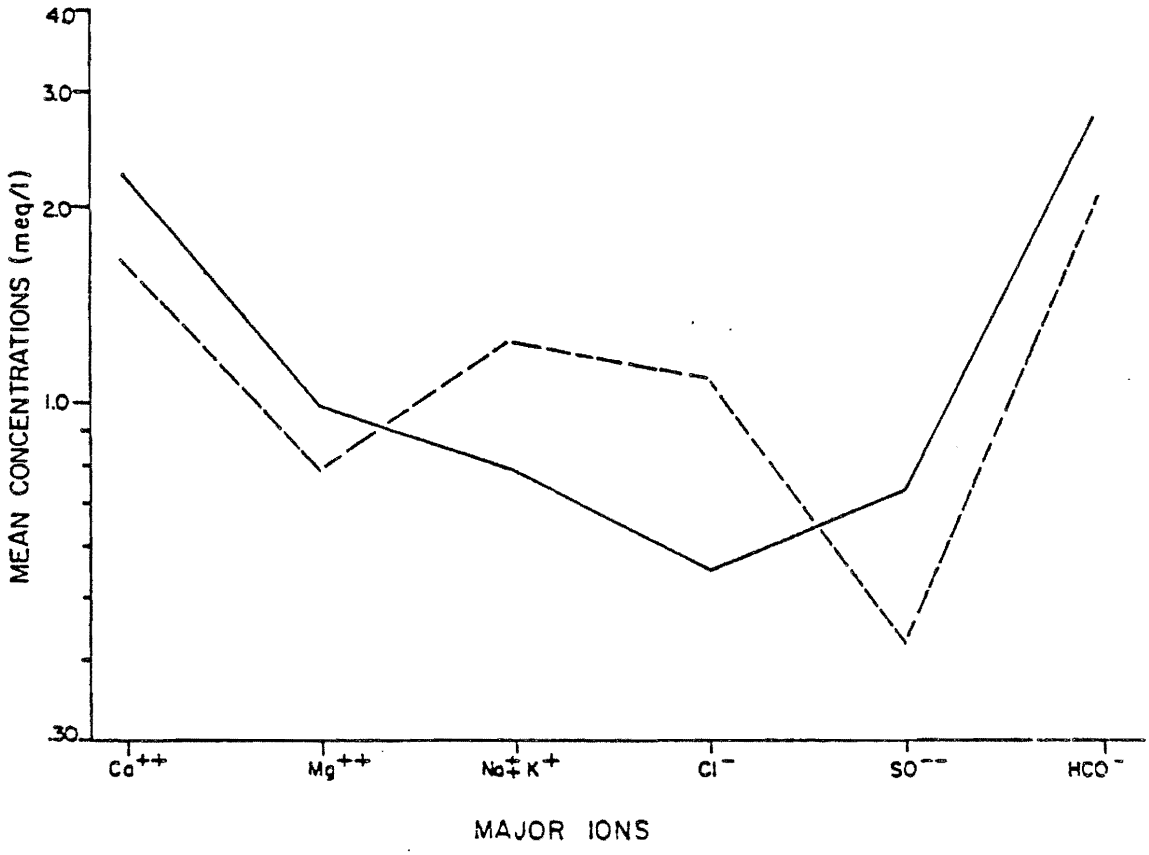

Figure 15. Mean values for major ions from four sites along the east (dashed line) and west (solid line) banks of the Athabasca River, 22 February 1975. From McCart et al. (1977).

therefore, all sampling stations should be sampled within no more than a 2 wk period at each sampling time.

Sampling should be frequent enough to indicate the range of conditions likely to be encountered throughout the year. At a minimum, this would mean sampling once in spring, autumn, and winter, and twice in summer, for most groups of aquatic organisms. Usually, monthly sampling during the open-water period and sampling twice in winter would be preferable and realistic.

If it is deemed necessary to reduce sampling frequency for some reason, a reasonable solution would be to establish a series of primary and secondary stations, sometimes referred to as "master" and "slave" stations (e.g., Carmack and Killworth 1979). The primary, or master, stations would be strategically located to be most sensitive to any potential impact. For example, control and impact primary stations could be located immediately upstream and downstream of an effluent pipe. The primary stations would be sampled frequently. If an impact was detected, secondary, or slave, stations would be sampled at various distances from the impact point to delineate the extent of the impact. This strategy could greatly reduce the response time of the biomonitoring program, because effort could be concentrated on obtaining quick results from a reduced number of key stations. Critical information would not be lost from a failure to frequently sample the secondary stations, particularly if the entire array of stations were subjected to an annual "check-up" sampling. The check-up would preferably be done in mid-summer, when temperatures and productivity are relatively high, and when sampling for most organisms could be done most efficiently.

6.5 SELECTION OF ORGANISMS

The object in selecting groups of organisms for inclusion in a routine biomonitoring program is to provide a range of organisms at least some of which will be sensitive to the anticipated impacts. One must be careful, on one hand, not to include an excessive number of groups so that the effort and expense required became excessive nor, on the other, to narrow the range (e.g., to a few

indicator species) to the extent that there is a likelihood that some impacts, to which none of the selected organisms is sensitive, will be missed.

Hellawell (1977) found, as a result of a literature review, that among groups, the benthic invertebrates, algae, and bacteria were, in order, the three groups most frequently favoured in biomonitoring studies (Figure 3 and Table 2). These three groups, particularly the first two, would also appear to be among the best candidates for biomonitoring in the AOSERP study area. A fourth group, which seems a worthwhile candidate for biomonitoring in the study area, is fish, although Hellawell found that these were not generally favoured, largely because of high sampling costs and the fact that fish are highly mobile and therefore less likely than more sessile organisms to reflect local conditions. Despite these disadvantages, there are good reasons for monitoring fish, outlined in Section 2.4 above.

Each of the candidate groups is discussed briefly below.

6.5.1 Benthic Invertebrates

Benthic invertebrates have been widely studied in the AOSERP study area so that a broad baseline of data is already available (Table 17), second only to that for fish. Sampling can be conducted throughout the year and the group seems well-suited to continuous monitoring.

As mentioned in Section 4.2.2, artificial substrate sampling shows promise for sampling benthic invertebrates in the AOSERP study area, particularly in the Athabasca River. It will be important, however, to be able to relate findings based on artificial substrate samples to what is actually going on in natural habitats in the river. There should therefore be a comparative study of natural versus artificial substrate samples as part of the preliminary studies. Furthermore, various types of artificial substrates (e.g., rock basket, multiple-plate, steel tray) should be tested to determine the best method for use year-round, during the open-water season and while the rivers are frozen.

Table 17. Summary of studies of fish, benthic invertebrates (benthos), bacteria, and algae for waterbodies in the AOSERP study area. References are given by number and are appended to this table.

Waterbody	Fish	Benthos	Algae	Bacteria
MAJOR STREAMS				
Athabasca River	6,7,8,16,21,22, 28,38,39	2,15,17,38	28	43,44,45
Beaver River	16,31,32,35,37	16,37		45
Christina River	16,40	16,40		
Clearwater River	16,21,22,38,39	16		44,45
Dover River	11,16	16		45
Ells River	16,30,48	16,30,48		45
Firebag River	16,30,48	16,30,48		45
Gregoire River	16,40	16,40		45
Hangingstone River	16,40	16,40	19,20	45
Hartley Creek	9,10,16,33	16,18		45
Horse River	16,40	16,40		45
MacKay River	11,16,29	16,29	19,20,29	45
Marguerite River	16,30,48	16,30,48		
Melvor River	16	16		
Muskeg River	9,10,16,33	1,2,16	19,20,26	45
Peace-Athabasca Delta	3,4,5,6,7,13, 14,23,24,25,36			
Steepbank River	16,27,30,48	1,2,16,30,48	19,20,26	45
Surmont Creek	16,40	16,40		45
MINOR STREAMS				
Algar River	16,40	40		
Cameron Creek	40	40		
Clark Creek	16			
Conn Creek	16			Continued...

Table 17. Continued.

	Fish	Benthos	Algae	Bacteria
Eymundson Creek	16			
Poplar River	34,35	34,35		45
Prairie Creek	40	40		
Redclay Creek	16			
Saline Creek	40	40		
Saprae Creek	40	40		
Tar River	16			45
West Interceptor Ditch, Syncrude Lease Site	37	37	37	
LAKES				
Algar Lake	40	40		
Birch Mountain Lakes	16,41	16,41		
Gardiner Lake	16	16		
Namur Lake	16	16		
Fort Chipywan Area Lakes	36			
Gregoire Lake	12,16,40	12,16,40		45
Pearson Lake	12,16	12,16		
Richardson Lake	3,4,24			
Ruth Lake	34,35	34,35	34,35	
Richardson Tower Lakes	46			

1. Barton and Wallace 1979c
2. Barton and Wallace 1979d
3. Bidgood 1968
4. Bidgood 1971
5. Bidgood 1973
6. Bond and Berry 1980a
7. Bond 1980
8. Bond and Berry 1980b

Continued...

Table 17. Concluded.

9.	Bond and Machniak 1977
10.	Bond and Machniak 1979
11.	Bond et al. in prep.
12.	Bradley 1969
13.	Dietz 1973
14.	Donald and Kooyman 1974
15.	Flannagan 1977
16.	Griffiths 1973
17.	Griffiths and Walton 1978
18.	Hartland-Rowe et al. 1979
19.	Hickman et al. 1980
20.	Hickman et al. in prep.
21.	Jones et al. 1979
22.	Jones et al. 1978
23.	Kooyman 1973
24.	Kristensen et al. 1976
25.	Kristensen and Pidge 1977
26.	Lock and Wallace 1979
27.	Machniak and Bond 1979
28.	McCart et al. 1977
29.	McCart et al. 1978
30.	Walder in prep.
31.	Renewable Resources Consulting Services Ltd. 1971
32.	Renewable Resources Consulting Services Ltd. 1973
33.	Renewable Resources Consulting Services Ltd. 1974
34.	Syncrude Canada Ltd. 1975
35.	Renewable Resources Consulting Services Ltd. 1977
36.	Rhude 1976
37.	Tsui et al. 1978
38.	Tripp and McCart 1979a
39.	Tripp and McCart 1979b
40.	Tripp and Tsui in prep.
41.	Turner 1968a
42.	Turner 1968b
43.	Costerton and Geesey 1979
44.	Nix et al. 1979
45.	Routine W.Q. monitoring
46.	Ash and Noton in prep.
47.	Herbert 1979
48.	Walder et al. in prep.

The tests of the various types of samplers should include attempts to evaluate losses of fauna during retrieval; for example, by using divers to make direct observations.

In addition to artificial substrate sampling, the following two simple "natural" sampling methods could be evaluated for use in the AOSERP study area as part of the preliminary studies. Donald and Mutch (in press) successfully used bankside collections of stoneflies, usually a pollution-sensitive group, to detect impacts on the Bow River, Alberta, from dams and urban effluents. Adult stoneflies do not usually move far from their bankside emergence sites; therefore, streamside collections of adults are indicative of conditions in the adjacent stream. Advantages of the method are:

1. There is very little sorting of insects from debris required; and
2. The adults are identifiable to species, not just genus, as is the case for the aquatic stages of these and many other stream insects.

Dr. Hans Boerger (Department of Biology, University of Calgary) suggested in a review of a draft of this report, that drift, particularly the pupal exuviae of emergent chironomids, could be monitored to detect impacts on the Athabasca River. Variants of the general method were suggested and successfully used by Larimore (1974) and Wilson and McGill (1977). The advantages of drift sampling are similar to those cited above for bankside collections: sorting is usually relatively quick, and (in the case of chironomid exuviae) species identifications can often be made.

Some of the more promising of the physiological approaches tested in the lab by Tsui et al. (in prep.), or suggested by Luoma (1977), and discussed in Section 3.4, should be field-tested. Baseline data on chloride cell density in mayfly gills and tissue concentrations of selected metal ions should be obtained. Comparative field bioassays of selected invertebrate populations from impacted and non-impacted (e.g., by saline groundwater) habitats should be run to test for resistance in impacted populations as a means of detecting sublethal effects.

Another question that should be considered during biomonitoring is the contribution of various kinds of benthic invertebrates to the sensitivity of the monitoring program. It is possible, for example, that a program based on Chironomidae alone (or a grouping such as the Trichoptera, Plecoptera, and Ephemeroptera combined) would provide nearly as much information as one based on a consideration of all benthic invertebrates including such difficult ones as the Oligochaeta. The aim should be simplicity and the fewer animals that must be examined, identified, and enumerated, the better.

6.5.2 Algae

Among the algae, the periphyton, sessile forms which grow on exposed surfaces, seem best suited to a biomonitoring program in streams. Some data are already available for the AOSERP study area (Table 17). Periphyton can be sampled year-round using either natural or artificial substrates (McCart et al. 1977, 1978) and would appear to be an excellent candidate for continuous monitoring. As with benthic invertebrates, the contribution of various subgroups to the sensitivity of the biomonitoring program should be assessed. In other areas, diatoms alone have proved to be very useful in biomonitoring programs (Patrick 1977). Again, like the benthic invertebrates, there should be a comparison of various sampling methods, involving both natural and various artificial substrates (e.g., glass, plexiglass, polished stone) to determine those best suited to a continuous biomonitoring program.

Serious consideration should be given to the physiological monitoring approach of using C^{14} or oxygen techniques on periphyton to monitor for changes in aquatic primary productivity. If the methods could be adequately standardized, they could be used as a highly-informative supplement to, or replacement for, the community structure approach.

6.5.3 Bacteria

Dr. J.W. Costerton (Department of Biology, University of Calgary, telephone conversation, September 1979) feels that sessile bacterial populations might prove useful as part of a biomonitoring program for the AOSERP study area. Studies in the Athabasca River (Costerton and Geesey 1979) indicated that consistent samples of sessile bacteria can be taken from natural silt substrates. Artificial substrates can also be used; Lock and Wallace (1979), for example, used standardized granitic substrates to obtain samples of bacteria from the Muskeg River. Certainly, the possibility of using sessile bacteria in a continuing biomonitoring program should be investigated further, during the Preliminary Biomonitoring Studies which have been recommended.

6.5.4 Fish

Fish have been more widely studied within the AOSERP study area than any other group (Table 17). As previously discussed (Section 2.4), the group, because of the effort required in sampling (especially in winter), the general mobility, and long life of its members, is not well suited to continuous biomonitoring or to situations where immediate feedback is required. Monitored on a seasonal basis, however, fish could provide a great deal of useful information regarding long term changes. Seasonal sampling should probably be confined to the ice-free season. It might take one of several forms:

1. A monitoring of populations of relatively stable species (i.e., those which do not undertake extensive seasonal migrations). These include a variety of forage species (e.g., lake chub, flathead chub, trout-perch, longnose dace, emerald and spottail shiners, brook stickleback, fathead minnow) as well as one sports species, the pike. Comparisons between years could be made on the basis of the relative abundance of species and, within species, between life history stages and catch per unit effort (seconds electro-

fished, area seined, hours of gillnetting). Some life history parameters (length/age and length/weight relationships, fecundity, etc.) might also be compared between years. These species are probably best sampled during the height of the productive season, in July or August.

2. A monitoring of those species which undertake major migrations within the AOSERP study area, either in the Athabasca River itself or in its tributaries (white and longnose suckers, goldeye, walleye, and lake whitefish). While migrations in some of the tributaries (e.g., the Steepbank and Muskeg rivers) can be monitored using weirs, the gillnet seems to be the most universally applicable technique. Standard gillnets could be set at selected localities to span the anticipated migration period for each species. Comparisons between years would be based on catch per unit effort and on various life history parameters.
3. A monitoring of the production of young-of-the-year of selected species, using drift nets and small mesh minnow seines. Longnose sucker, lake whitefish, and walleye in the Athabasca River would be monitored in this way, providing information on spawning and incubation success. Again, comparisons between years would be based on catch per unit effort.

Among physiological approaches that would be worth further evaluation, baseline data on red blood cell volume and blood hematocrit of fish should be obtained. This would be a field test of the promising method lab-tested by Tsui et al. (in prep.) for detecting chronic effects of saline groundwater on fish. The stationary species mentioned under item 1, above, could be tested in the field and the lab for their utility in monitoring for petroleum contamination using the aryl hydrocarbon hydroxylase (AHH) method, as outlined by Walton et al. (1978) and discussed in Section 3.4.

Finally, taste tests on selected fish species, and monitoring of heavy metal and pesticide residues, should be conducted annually.

6.6 DATA ANALYSIS

The details of analytical methods used in data analysis will depend on what is measured in the monitoring program, and on what specific hypotheses are to be tested. Some general suggestions, only, can be made here.

The most useful approaches to analyzing the field data are likely to be through multivariate statistical techniques as suggested by Green (1979) and Green and Vascotto (1978). For example, the species abundance data could be analysed by cluster analysis to define associations, and these could be plotted on maps to see if certain assemblages are characteristic of impacted areas. Multiple discriminant analysis could be used to relate the clusters (species assemblages) to environmental variables such as suspected pollutants. Cluster and discriminant analysis methods should be extended to detect temporal, as well as spatial, changes. Diversity indices, particularly those based on information theory such as the Shannon-Weaver Diversity Index, are unlikely to be of as much value as multivariate techniques for reasons discussed extensively in Section 3.3.

6.7 DATA STORAGE AND RETRIEVAL

A data storage and retrieval system, as briefly outlined in Section 4.7, should be developed as part of the preliminary studies. It is recommend that:

1. The programs be prepared in Fortran, the most widely used computer language for scientific purposes;
2. That a detailed manual be prepared describing all data file procedures, including programs and codes; and
3. That duplicate copies, on magnetic tape, be prepared of all data, one copy to remain with the users, the other to be stored in a separate and safe location.

The latter two recommendations will ensure AOSERP continuing access to data over the years.

In the final analysis, the samples themselves are the most complete storehouse of information on the monitored biota. If at all possible, they should be carefully curated, catalogued, and retained in a safe location. This will permit backchecking and reanalysis in case reported results are questioned, or more powerful processing and analysis techniques are developed that could improve the monitoring system. It is possible that certain museums would be willing to undertake this task, because long series of rigorously-collected and statistically-valid samples of aquatic biota are extremely rare, and constitute a scientific resource of considerable value.

6.8 MANAGEMENT

It is recommended that any long-term biomonitoring program for the AOSERP study area be the responsibility of a group whose sole function is the conduct of such studies. This will be necessary to ensure continuity of the project, and comparatibility of methods and results. Aside from its own studies, one of the functions of the monitoring group would be to cooperate with independent groups (for example, industrial developers) conducting similar studies in the area to ensure that sampling methods and analytical techniques are comparable, as far as possible.

While much of the work may be routine, the interpretation of the results may involve a considerable degree of sophistication. The project manager should, therefore, be someone with wide training in the ecology of aquatic organisms.

7. CONCLUSIONS

It is the major conclusion of this report that an aquatic biomonitoring program should be instituted as soon as possible within the AOSERP study area. The area is now, and will be increasingly in the future, subject to a variety of impacts resulting directly or indirectly from oil sands development. Biomonitoring shows considerable promise as method for effectively assessing the combined effects of a range of impacts. Furthermore, aquatic biomonitoring must be undertaken to determine whether measures taken to safeguard the aquatic system are working, so that modifications in these measures may be made, if necessary. The ongoing results of the biomonitoring program will also help to give direction to the overall research and management program in the AOSERP study area, by identifying and locating problems and areas of concern in the rivers and lakes themselves.

8. RECOMMENDATIONS

An aquatic biomonitoring program should be initiated in the AOSERP study area to:

1. Assess the impact on aquatic systems of known point sources of potentially damaging materials; and
2. Provide a continuing assessment of the general health of aquatic systems in waterbodies within the study area.

To be effective, the program should be based on methods which:

1. Are quantitative and objective, rigorous and repeatable;
2. Are appropriate to northern boreal seasonal conditions;
3. Can provide quick feedback to government and industry representatives, charged with making pollution abatement decisions;
4. Are routinely workable by a small staff;
5. Measure both short and long term changes in water quality and habitat; and
6. Are cost effective.

Prior to initiation of a routine biomonitoring program, there should be at least one year of preliminary studies, the results of which would include:

1. A detailed sampling strategy which would outline the locations of sampling stations and a sampling timetable indicating the kinds of samples to be taken at various times during the year;
2. An identification of the groups which should be included in the sampling program along with a rationale for their inclusion;
3. An outline of the precise methodology which should be used in sampling each of the selected groups;
4. Details of the methods to be used in analysing the resultant data; and

5. Recommendations concerning the organization of a continuing biomonitoring group.

Four groups of aquatic organisms are recommended as candidates for detailed assessment during the preliminary studies. These include benthic invertebrates, periphyton, sessile bacteria, and fish.

During the preliminary studies, considerable effort should be expended in defining preferred methods of data analysis which are simple and can be routinely performed by relatively unskilled personnel.

Routine biomonitoring in the AOSERP study area should be the responsibility of a group whose sole function is to conduct such studies. The project manager should be someone with wide training in the ecology of aquatic organisms.

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