SELECTED METHODS FOR THE MONITORING OF BENTHIC INVERTEBRATES IN ALBERTA RIVERS

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1.0 INTRODUCTION

The alteration of water quality by point source or non-point source discharges can result in structural and functional modifications in aquatic communities because of changes in distribution, abundance, or behaviour of certain taxa. Any biological community in an aquatic ecosystem is likely to respond measurably to changes in water quality and theoretically could be used to detect and assess pollution. It is not always possible to identify the most informative components of the ecosystem which should be given closest attention (Hellawell 1978). In general, however, the benthic invertebrate community has proven to be very useful in surveys. The two prime reasons are that benthic invertebrates are relatively sedentary in relation to flowing water (Alabaster 1977) and have relatively long life cycles (Hynes 1960) which make them useful as integrators of pollution effects (Hellawell 1978). Furthermore, they have been used for biomonitoring long enough that a considerable amount of literature is available to support their use and aid in data interpretation.

Benthic invertebrates and chemical and physical characteristics of the water have been used as complementary tools by Alberta Environment and by industries to monitor the effects of point source discharges on the water quality in Alberta rivers. Many industries are required by licence to monitor the effect of their effluent on the zoobenthos and to document their findings in written reports. These reports are reviewed by Alberta Environment.

The objective of this text is to provide general guidelines for the use of benthic invertebrates in the monitoring of water quality in Alberta rivers which receive effluents from industrial or municipal point sources.

Methods described herein are recommended because they have been used with success by the Water Quality Control Branch (Pollution Control Division) in Alberta rivers, but the exclusion of some other methods does not imply that they are deemed unsatisfactory.

The design of a sampling program depends on a variety of interacting factors and, consequently it is not possible to describe an "ideal study design" which would be applicable to all possible situations. Therefore, room has been left to allow for the inclusion of the expertise and scientific judgment of professional biologists involved in the monitoring of benthic invertebrates. Consultation with the Department on an individual basis is encouraged. Although detailed specifications applicable to all situations cannot be given there are some general rules which should be followed.

Green (1979) emphasized the need for a logical flow in environmental studies. The definition of the purpose of the study should be followed by the formulation of specific questions and hypotheses. The sampling should be designed keeping in mind study objectives and requirements for appropriate (statistical) data analysis which will allow hypothesis testing. Presentation and interpretation of results - in report form - are the final steps of most studies.

2.0 STUDY OBJECTIVES, FORMULATION OF QUESTIONS AND HYPOTHESES

The formulation of objectives, questions and hypotheses forms the basis of any type of scientific work. Benthic invertebrate monitoring should follow the same set of rules.

The first objective of most monitoring programs which utilize zoobenthic surveys to determine the effects of point source discharges on water quality, is to determine whether the effluent is responsible for differences in the invertebrate assemblies above and below the effluent outfall. A number of more specific questions, such as the following may be asked.

- How far downstream is the change measurable?
- Is there a difference in the benthic invertebrate associations along both banks?
- Are there seasonal differences in the effects of the effluent on the zoobenthos?
- Are zoobenthic assemblies below the effluent deteriorating (or improving) over the long term?
- Are all taxa affected in the same way?

In order to answer each question, different sampling regimes will have to be designed. A combination of two or more questions will greatly increase the complexity of the sampling program. Conversely, once a sampling program has been implemented it may provide data too limited to answer questions other than those proposed at the outset. To keep a sampling program reasonable in scope and intensity, it is imperative to establish priorities and formulate questions carefully and thoroughly as part of the planning process.

3.0 SAMPLING DESIGN

Hall et al. (1978) classified the basic approaches used for assessing the impact of water management practices on Oregon streams into four categories:

"before-after" data are gathered before and after treatment.

"post-treatment" data are gathered only after effluent release has started.

in "intensive" surveys only a small number of sites is monitored, usually over several years.

in "extensive" surveys many sites are monitored over a short time.

Regardless of which approach is used in monitoring aquatic invertebrates, the sampling design can be defined in terms of three components:

i. timing
ii. sites
iii. samples

3.1 Timing

The frequency and actual dates for collecting benthic invertebrate samples will be influenced by a number of factors, the most obvious of which are discussed below.

a. The study objectives.

The purpose of the study and the questions which are addressed are important in determining when and how often sampling should be carried out. For example, the assessment of seasonal differences in benthic invertebrate response to an effluent will require a much higher sampling frequency than the assessment of downstream effects of an effluent at low flow.

b. The benthic invertebrates.

The taxonomic composition of benthic invertebrate communities changes seasonally as a result of differences in life cycles among invertebrates. When planning a long term monitoring program it is important that the time of year at which sampling is conducted remains consistent throughout the length of the study.

The selection of the season is also important if one wishes to obtain a general indication of the diversity of the invertebrate fauna. Spring, when summer-emerging insects are well developed and easiest to identify is, according to Hellawell (1978) the optimum period if only one survey is planned each year. However, spring populations are often sparse and a higher number of sampling units per site will usually be required to obtain a desired level of accuracy in the density estimates of invertebrate groups. Additionally, differences between sites may become more difficult to measure when numbers are low. Fall samples usually have higher numbers of invertebrates but contain a higher fraction of immature insect larvae which are more difficult to identify.

Invertebrates are most likely to show the strongest response to effluents at high temperature and at low river flow (Cairns and Dickson 1971). Therefore, if time and funds limit the sampling frequency, at least one survey during this time will produce useful information.

c. River discharge, climatic conditions

Ice cover precludes winter and early spring sampling in most Alberta rivers. Consequently, invertebrate sampling may be restricted to the period from early May to late October. Other natural phenomena may limit the time during which benthic invertebrate sampling is feasible or desirable. Most Alberta rivers have dynamic hydraulic regimes which are marked by sudden and dramatic increases in river volume during spring runoff and following rain storm events. These climatic and hydraulic factors superimpose limitations on sampling programs of benthic invertebrates.

Benthic invertebrates are affected by sudden changes in river volume and flow rate (e.g. Elliott 1967, Fisher 1983, Allan 1987). In extreme situations dramatic increases in drift rate - referred to as catastrophic drift have been reported (Anderson and Lehmkuhl 1968, Pearson and Franklin 1968, Mackay and Kalff 1973). As the importance of river discharge and flow in determining the distribution pattern of aquatic invertebrates increases, the importance of the effects of point-source effluent discharges decreases. Consequently, benthic invertebrate distribution is a much less sensitive indicator of effluent induced patterns in water quality during periods of highly fluctuating discharge than during periods of nearly steady discharge. For examples of benthic invertebrate surveys in which this problem was encountered see Crossman et al. (1974) and Anderson (1986).

Substrates which were submerged before the water level rose may become inaccessible during periods of high water, particularly when samplers designed for shallow waters are used. Sampling substrates which have been inundated recently is not recommended: colonization by invertebrates may be too incomplete to be a reliable reflection of patterns in water quality. In general, if the purpose for sampling invertebrates is to monitor the effects of point source discharges, it is recommended to avoid periods of high water, i.e. to sample before spring runoff or several weeks after important increases in river discharge such as those induced by major storm events. Information on the daily discharge rates of many rivers in Alberta can be obtained from the River Forecast Centre (Alberta Environment) at 427-6278 in Edmonton.

d. The type of point source effluent discharge

Many point source effluents have a continuous discharge, but the quantity and/or the quality of the effluent may vary over time. When monitoring invertebrate populations to assess the effects of such effluents, points a to c will be the primary determinants for the timing of the sampling.

Some effluents discharge on an occasional basis depending on the plant activity; others discharge on a seasonal basis. In such cases, the plant activity is most likely to determine the timing of benthic invertebrate sampling. Accidental discharges such as those which occur during spills also fall under this category.

3.2 Sites

Location and number of sites are the key elements in any sampling program. Most biologists involved in environmental monitoring will agree with Cairns and Dickson (1971) that there is no set number of sampling sites that will be sufficient to monitor all the possible types of waste discharges. Some authors venture a few generalizations. Hellawell (1978) suggest that two sampling sites, one above and one below the effluent, are the absolute minimum; Green (1979) stresses the importance of controls (both spatial and temporal) and Cairns and Dickson (1971) believe that some general rules, if carefully followed will result in a sound survey design. Some of these rules are listed below.

1. Always have a reference site or sites above all possible discharge points. In practice, it is usually advisable to have at least two reference stations. One should be well upstream from the discharge and the other directly above the effluent discharge, but away from any possible discharge influence.

2. Have a station directly below each discharge.
3. If the discharge does not completely mix on entering the water-way, but channels on one side, stations must be subdivided into left-bank, mid-channel, and right-bank substations.
4. Have sites at various distances downstream from the last discharge to determine the linear extent of the impact to the river.
5. All sampling sites must be ecologically similar before the bottom fauna communities found at each sites can be compared.

For example, shallow, stony areas with continuous flow (riffles) provide the most useful sites for sampling as the fauna there is most likely to show a measurable response to changing conditions (Hynes 1960). Substrate type, current velocity, food substances, and chemical and physical characteristics of the water are thought to be the main factors which determine invertebrate micro-distribution (e.g. Cummins and Lauff 1969, Rabeni and Minshall 1977).

When the purpose for sampling benthic invertebrates is to obtain an integrative measure of chemical and physical characteristics of the water it is important to attempt to standardize other factors as much as possible. Because sampling locations are seldom identical in terms of substrate, current velocity, or food availability, some environmental biologists have found it helpful to describe the variability of these features among sites. Substrate composition has been evaluated using the Wentworth scale (Wentworth 1922, cited in Cummins 1962), by visual observation, by photographic records, or by obtaining a measure of the microprofile (Gore 1978). Some indication of food availability for primary consumers can be obtained from epilithic chlorophyll-a measurements.

6. Biological sampling stations should be located close to those sampling stations selected for chemical and physical analyses to enhance the validity of interpretations.
7. In order to make comparisons among sampling sites, it is essential that all sites be sampled at approximately the same time.

3.3 Samples

Four factors must be considered regarding sample collection in a benthic invertebrate monitoring program:

i. The sampling equipment to be used
ii. The number of samples to be collected
iii. The sample processing techniques to be employed
iv. The level of invertebrate identification to be used.

3.3.1 Sampling Equipment

An extensive array of apparatus has been developed for the sampling of benthic invertebrates (see Elliott and Tullett 1978). The most commonly used quantitative samplers in riffles are the Hess (Hess 1941) or the Neill (Neill 1938) cylinder samplers. This type of sampler is generally suitable for a wide range of substrate types in flowing waters and works well in sand, gravel, cobble and small boulders; its main limitations are the sampling depth and the need to sample in flowing water.

A fine mesh screen may be mounted on the upstream opening of the sampler to prevent contamination of the sample with drift organisms. It is also quite useful to fit the collection net with a sample bottle which can be replaced once a sample has been collected. A modified Neill cylinder sampler with a collection net with a mesh of 0.210 mm and a substrate contact area of 0.1 m^2 has been used by the Water Quality Control Branch, Alberta Environment, to sample benthic invertebrates in the major rivers of the Province. In order to standardize the sampling of erosional habitats, the use of a cylinder sampler with similar specifications is recommended.

It is important to press the cylinder securely into the substrate and to ensure that there are no gaps between cylinder and substrate by checking the base of the cylinder on the outside. Larger stones and stones covered with vegetation should be cleaned individually by gentle rubbing and rinsing inside the cylinder. A narrow shovel can be used to disturb the enclosed substrate for approximately one minute. Swift, well oxygenated rivers generally have rather coarse substrates with small amounts of fine particles filling interstices. Invertebrates tend to penetrate deep into such substrates and it is necessary to disturb the substrate for a longer time and to a greater depth than when sampling rivers with slow moving and poorly oxygenated waters. The latter tend to have more compact sediments with interstices filled with fine particulate material. Once the water within the cylinder is no more turbid than the water outside the cylinder, the sampler can be lifted and the collecting net cleaned.

Ponar or Birge-Ekman grabs are classic samplers for depositional areas. They are fairly efficient in mud or fine gravel, but are inadequate when larger stones (>16 mm) are present (Elliott and Drake 1981).

Airlift samplers are potentially the most useful for sampling deep rivers (e.g. see Pearson et al. 1973, Elliott et al. 1980, Drake and Elliott 1982, 1983). Airlift samples could also be used for the sampling of depositional areas where larger stones imbedded in silt make the use of grabs difficult.

Artificial substrates, especially basket-type artificial substrates, have become more popular in recent years (for a detailed overview see Cairns 1982). Rosenberg and Resh (1982) have discussed the advantages and disadvantages of using artificial substrates in the study of freshwater zoobenthos.

Generally, sampling with artificial substrates is more labour intensive than with conventional sampling techniques (e.g. sites must be visited twice to collect one sample). Substrates should be left in the water for a period ranging from several weeks to several months. The optimum colonization time is unknown and the user must decide arbitrarily when to retrieve samples. In theory, artificial substrates standardize the substrate colonized by invertebrates and should therefore reduce intra- and inter-site variability resulting from sampling heterogenous habitats. In practice, this advantage can be off-set by the accumulation of debris on the substrates and by the variable portion of the sample which is lost during the retrieval of the substrates.

Artificial substrates have been criticized for collecting a fauna which is not necessarily representative of local benthic invertebrate associations. Although this criticism is valid and would be a serious drawback if the purpose of the study were to describe local benthic invertebrate associations, it is of little consequence in studies where the zoobenthos is used as an integrative tool to compare water quality at different sites. Vandalism or substrate loss because of water level fluctuation are some other problems that the user of artificial substrates may have to face. However, assuming that artificial substrates are retrieved successfully after a trouble-free exposure time, samples should be cleaner and easier to process than those from natural substrates; variability among samples from the same site will often be smaller than among samples collected from natural substrates. Artificial substrates offer some advantages, but there are also some practical problems associated with their use. Therefore, it may be best to limit the use of artificial substrates to waters which are difficult to sample by other means, or to situations where natural substrate differences among sites are large and could account for much of the biological differences among sites.

3.3.2 The Number Of Samples: Degree of Precision

The degree of precision of invertebrate population density estimates depends on the combination of two things:

- i. the size of the replicate sample (i.e. sampling unit)
- ii. the number of replicate samples

a. Size of replicate sample

According to Green (1979) there are four main factors which define the appropriate sampling unit size:

- i. logistic considerations, such as the cost of sample collection, which may be proportional to the number of samples rather than to the area sampled.
- ii. the size of the organisms sampled. The ratio 'size of organism' to 'size of sampler' should be negligibly small. Green gives a ballpark figure of less than 0.05.
- iii. the avoidance movement of organisms. The ratio 'avoidance radius' to 'size of sampler' should be negligible.
- iv. the spatial distribution pattern of organisms. The sampling of populations with an aggregated spatial distribution requires sampling units which are as small as possible considering the limitations set by the three previous factors.

b. Number of replicate samples

The collection of replicate samples is the only way to obtain a measure of the magnitude of the variation in population densities. Green (1979) points out that most tests of hypotheses require an estimate of the variation within (sampling sites or times) and among (sampling sites or times) and warns against the pooling of sampling units or the collection of single large samples. The number of replicate samples required at each site is dependent upon the precision desired for the density estimates. Elliott (1977) states that a standard error equal to 20% of the mean is an acceptable error for most zoobenthic samples. In routine surveys, it is only reasonable to expect to reach this level of precision for variables such as the total number of invertebrates, numbers within major groups, or number for individual dominant taxa. The number of replicate samples required to reach this level of precision for all taxa could become unmanageably high.

The number of replicate samples needed in a random sample at each site for a standard error equal to 20% of the mean is derived from:

$$D = 0.2 = \frac{1}{\bar{x}}\sqrt{\frac{S^2}{n}}$$

D = accepted error
$\bar{x}$ = mean
S^2 = variance
n = number of sampling units required
T = t from Student's t-distribution ($T \approx 2.$)

or from $n = \frac{T^2}{D^2 \bar{x}^2}$

Elliott (1977) p. 129

In quantitative studies the approximate means and variances of the samples must be known in order to calculate the number of replicates necessary for a specific degree of precision. These are best derived from a small pilot survey or may be estimated from experience gained from previous investigations in the same area.

Usually, five cylinder samples are sufficient to obtain the desired level of precision (D = 0.2) for dominant taxa in productive rivers in Alberta (Alberta Environment, unpublished data). As a rule no fewer than five replicate samples should be collected at each site. Hamer and Soulsby (1980) indicate that in general water quality surveys, five replicate samples per site are sufficient to describe water quality as reflected by the biota and that, although this number is not sufficient to describe the fate of every taxon in the sample area, it reflects the changes in the most abundant taxa.

3.3.3 Sample Processing Techniques

Preservation of samples in 4% formaldehyde should be done as soon as possible after collection. Carnivores confined in the limited space of a sample container can prey heavily on some species, reducing their densities considerably.

Sorting live samples in the field or sorting preserved samples without the use of magnification produces a bias (e.g., Hilsenhoff 1982) and is unsatisfactory. Samples should be sorted under a dissecting microscope and the magnification(s) used during the sorting should be reported.

The use of dyes (e.g. Rose Bengal) increases the efficiency with which zoobenthic specimens are separated from the sample residue (Mason and Yevich 1967) and is strongly recommended. Approximately 5 mL of Rose Bengal solution (200 mg Rose Bengal dissolved in 1 L of water) for 500 mL preserved sample will give a deep pink color to invertebrates. The sample should be agitated at least once to ensure that all invertebrates have contacted the dye. The dye should be allowed to work for at least 24 hours. Samples should be rinsed with tap water before sorting.

The sorting of samples can also be facilitated by screening the samples through sieves with decreasing mesh sizes. The finest mesh size corresponds to the mesh size of the (Neill cylinder) collecting net. The residue on each sieve is then sorted separately.

Invertebrate densities can be very high in some samples, necessitating excessive sorting time. Sample fractioning or subsampling may be necessary. Several subsampling techniques have been discussed by Waters (1969) and Hickley (1975).

In most samples only a few taxa are very abundant, whereas the numbers in most other taxa are low. Subsampling should only by used for very abundant taxa. As a rule of thumb, the number of specimens in a given taxon should be at least 100 in the subsample. Subsampling methods generally assume random distribution of specimens in the sample and will be more accurate if the sample consists of particles of similar size (i.e. samples which have been screened.)

Wrona et al. (1982) describe a method which is reliable for the subsampling of finer sample fractions. The method involves the use of an Imhoff cone, an airstone and an air pump. The fine fraction of a sample is transferred to the cone and the sample volume adjusted to a convenient volume (e.g. one litre) by adding water. Air is bubbled through the sample for approximately five minutes to allow random distribution of particles. A few drops of household detergent may be added to the samples if particles tend to become trapped in the surface film. Up to 10 subsamples, each 50 ml large, are withdrawn from the Imhoff cone while air continues to bubble.

Several flotation techniques have been used to separate invertebrates from benthic samples (e.g. Anderson, R.O. 1959, Whitehouse and Lewis 1966, Lackey and May 1971, Jonge and Bauman 1977). Ludox AM, a commercial preparation of silica sol, can be used in the extraction of invertebrates from the finer fractions of relatively clean samples (i.e. sample with little flocculent or filamentous material). At least 95% of all invertebrate taxa (excluding case building caddisflies, mollusca and ostracods, which are not extracted satisfactorily) will float in a Ludox dilution with a specific gravity of 1.12. The fine fraction of a benthic sample is mixed in 8 times its volume of Ludox AM (specific gravity of 1.12) in a wide mouth container. The sample mixture is agitated thoroughly and allowed to settle for 15 minutes. Floating invertebrates are siphoned from the surface. The procedure is repeated twice after topping up the sample with Ludox. The residue remaining after the third extraction is examined for specimens which failed to float.

3.3.4 Level of benthic invertebrate identifications

Although detailed identifications (i.e. to species) provide a broader spectrum of information and are preferred, there are practical constraints which limit the degree of detail to which benthic invertebrates can be identified.

The number one constraint is the cost of sample processing. This is dependent upon the time involved in processing the samples and the degree of expertise which is required. Generally, riffles in Alberta rivers contain a wide assortment of benthic invertebrate groups, most of which can easily be identified to family or even genus level. The following list identifies the highest level of identifications recommended for routine monitoring studies (i.e. identifications should not be less detailed)

Phylum for Aschelminthes
Class for Coelenterata
Genus for Mollusca
Family for Annelida
Genus for Ephemeroptera
Genus for Trichoptera
Genus for Plecoptera
Subfamily for Chironomidae
Family for Diptera
Order for remaining Arthropoda

With reasonable training and a familiarity with Alberta river invertebrates, the levels of identification recommended above should only require the use of a good dissecting microscope. Taxonomic literature useful in the identification of invertebrates from Alberta rivers is listed under Section 7 of this report.

Some groups such as Nematoda, Oligochaeta, and Chironomidae have long been considered to be "difficult groups." Consequently, their identification in routine surveys has seldom been carried further than class (Nematoda and Oligochaeta) or family (Chironomidae). Recently published keys for Oligochaeta and Chironomidae (see Section 7) greatly facilitate the identification to genus or even species for these taxa, but the lack of suitable keys means that Nematoda are still a "difficult group." Unfortunately, the identification of both oligachaetes and chironomids to genus or species level still requires the mounting and clearing of specimens, and the subsequent examination of slides at high magnification (compound scope required). These procedures are time consuming and expensive, particularly when large numbers of samples and specimens must be examined. However, the detailed identification of oligochaetes and chironomids which are very diverse groups taxonomically and ecologically contributes valuable information which can facilitate and enhance the interpretation of the data.

The taxonomic references used to identify organisms should be noted in each report; personnel involved in the sorting, counting and identifications of benthic invertebrates should also be identified.

It is best to retain invertebrate specimens in the event that the results of routine surveys indicate the need for more detailed identifications.

4.0 DATA ANALYSIS

4.1 Condensation of Data

A recurring problem in biological surveys is that the data base soon takes on an overwhelming size making the condensation of the data a necessity despite the inevitable risk of loss of information. One of the most commonly used methods of condensing data is to describe the benthic invertebrate community at one site by a single numerical value, or index. Some of the most commonly used methods of condensing data have been given by Hellawell (1978) and Winterbourn (1981). They range from the use of basic data (e.g. abundance per taxon, total number of invertebrates, number of taxa), to pollution indices (e.g. biotic scores, Chandler 1970; saprobic index, Zelinka and Marvan 1966; trent biotic index, Woodwiss 1964; empirical biotic index, Chutter 1972; biotic index, Hilsenhoff 1977), diversity indices (e.g. sequential comparison index, Cairns et al. 1968; Simpson's index, Simpson 1949; information theory index, Shannon 1948) and comparative indices (e.g. distance measure, Sokal 1961; rank correlation coefficient, Spearman 1913). Simple taxa-numbers ordinations have also been employed (Hocutt 1975). All of these methods have been used in assessing pollution; all have been satisfactory to some degree and have advocates; all have received various amounts of criticism, underscoring the lack of unanimity in data condensation. In general it may be wise to follow Green's (1978) advice and keep any use of indices to simple, easily understood ones.

Basic data are the simplest to calculate and perhaps the most straightforward to interpret and compare. These should be used in the initial data analysis. The most relevant basic data are: the total number of invertebrates per sample, the total number of invertebrate taxa per sample, and the numbers for each major group, a major group being a taxonomic group which is numerically dominant in the sample series ("dominant" means comprising at least 10% of the total numbers).

If a detailed study precedes a routine survey program and shows that a particular index is a suitable descriptor of the impact, such an index may be considered for use in addition to basic data. However, caution must be exercised in the use of certain indices such as the information theory index which requires the same level of taxonomic identification throughout a sample series in order to yield valid comparisons.

4.2 Statistical Analysis

Green (1979) emphasizes that the choice of an appropriate statistical analysis should flow logically from the purpose, the hypothesis, and the sampling design. A variety of statistical tests may have to be used in the analysis of benthic invertebrate data. The reader is referred to basic references such as Elliott (1977), Siegal (1956), Sokal and Rohlf (1968), and Zar (1974) for an overview of statistical tests.

Statistical tests can be divided into two major groups: parametric and non-parametric tests. The normal distribution of data is a prerequisite for parametric tests, whereas non-parametric tests do not assume any underlying distribution of the data. Parametric tests are more powerful than non-parametric tests and should be used whenever data distribution is normal or whenever data can be transformed satisfactorily to achieve normality.

Many commonly used statistical tests are univariate (i.e. hypothesis testing is performed on a single variable). Multivariate tests are gaining popularity because they allow the simultaneous testing of more than once variable. Univariate tests are satisfactory for small data sets; multivariate tests or analysis techniques are more efficient for the analysis of extensive data sets.

The use of univariate tests requires the careful identification of dependent variables (tested variables). Examples of dependent variables are the total numbers of invertebrates, the total number of taxa, the numbers within each dominant taxon, and the numbers within individual taxonomic groups. The choice of test variables is largely determined by the study objectives, but the screening of data for patterns is an important aid in the selection.

The comparison of dependent variable means among sites (or dates) is probably one of the most common statistical problems in the zoobenthic component of water quality studies. The statistical tests applicable to this type of problem determine whether the variability within the sites is greater or less than the variability among sites.

The following tests are suggested for this type of problem.

1. Test for Normality. One basic assumption of several parametric tests is that the data for a particular dependent

variable are normally distributed. The Kolmogorov-Smirnov test (e.g. Siegal 1956, Hull and Nie 1981) may be used to check this assumption. If data are not normally distributed, transformation (e.g. Elliott 1977) will often improve the normality. If normality cannot be achieved through transformation, non-parametric tests should be used to analyze the data set (e.g. Mann-Whitney U-test, Kruskal-Wallis test (Elliott 1977, Siegal 1956, Hull and Nie 1981).

2. One-Way Analysis of Variance. Provided that transformed data for a particular dependent variable are normally distributed, a one-way analysis of variance (e.g. Sokal and Rohlf 1969, Elliott 1977, SPSS Inc. 1983) will indicate whether a significant difference exists among sites or among dates (independent variables for the mean value of that dependent variable).
3. Multiple Range Tests. (e.g. examples of such tests are Student-Newman-Keuls, Duncan, Schaffe, see Sokal and Rohlf 1969; SPSS Inc. 1983; Zar 1974) can be applied to determine which sites differ significantly in their means.

Analyses of variance and co-variance group a rather large number of tests with important applications in applied ecology. For example, in addition to the simple tests described above, one can examine the effects of more than one independent variable on one dependent variable (e.g. two-way analysis of variance or one can examine the effects of one or more independent variables on more than one dependent variable (multivariate analysis of variance).

Multiple regression analysis (Sokal and Rohlf 1968, SPSS Inc. 1983) examines the relationship between one dependent variable and two or more independent variables (e.g. environmental variables). The strength and quality of the relationship is measured by multiple correlation which can be tested for significance. Multiple regression analysis is a powerful tool in predictive modelling.

Classification and ordination techniques, such as cluster analysis, principal component analysis, factor analysis, discriminant analysis, and reciprocal averaging (e.g. Hill 1973, Wishart 1978, SPSS Inc. 1983, Romesburg 1984) have become increasingly popular in the assessment of water quality (e.g. Green 1979, Crossman _et al_. 1974, Sprules 1977, Culp and Davies 1980). These techniques are very useful in the exploratory stage of the data analysis of complex data sets (e.g. Sinha 1977). They offer the advantage of using each piece of information contained in a sample and of summarizing complex data matrices in graphical forms which are relatively easy to interpret.

Green (1979, p. 111) indicates that if one wishes to use many species as biotic variables, it is best to apply principal component analysis to log-transformed species abundance data or to binary data. Multiple discriminant analysis has been proposed by Green and Vascotto (1978) as a method for the analysis of environmental factors controlling patterns of species composition in aquatic communities. Hamer and Soulsby (1980) have suggested that the use of multivariate techniques in the analysis of survey data may lead to the development of more realistic biotic indices. One disadvantage of these techniques is that significance levels are frequently difficult to establish. However, the "bootstrap" method (Diaconis and Efron 1983) can be used to test the significance of cluster groups and principal component scores can be treated as ordinary variables on which significance tests can be performed (Frane and Hill 1976). Nonetheless, it is desirable to complement multivariate analysis techniques with simpler statistical tests (see above).

5.0 REPORT

Two important functions of a report are to document the work which was performed and to appraise the results that were obtained. Reports usually have five main components:

1. Introduction
2. Methods
3. Results
4. Discussion
5. Literature Cited

Sections entitled Conclusions, Summary, and or Executive Summary are frequently included as well. They do not add new information but repeat the information incorporated in the main body of the report in a somewhat different, more concise, perhaps more easily digestible form. An Acknowledgement section should be included in every report. Credit should be give to those who provide technical assistance in the field or in the laboratory, and to those who grant permission to access to sites, provide funds or advice, supervise the work, and assist in the preparation of the manuscript (e.g. typist, reviewers).

Valuable hints on how to assemble a report can be found in the CBE Style Manual published in 1978 by the American Institute of Biological Sciences and in Alberta Environment (1982). Sutcliffe (1979) points out some of the sources of inconsistencies and unintentional ambiguities in the published scientific literature. His editorial is a useful document to consult when preparing a report.

5.1 Introduction

The following questions should be addressed in this section:

Why was the study undertaken?

What were the study objectives?

What is the background knowledge on the subject?

e.g. - If benthic invertebrate surveys were conducted in the past to monitor the effects of a particular effluent, the results obtained and reports produced should be summarized or otherwise acknowledged.

Include a brief overview of the published literature on the effects of the particular effluent type on benthic invertebrates.

5.2 Methods

This section must give a full description of time, location, and procedures concerning the work undertaken:

- Include a map of the study area with the exact location of the sites relative to the point source discharge(s). Other sources or potential interferences should be indicated (e.g. potential runoff from the plant site, tributaries to the waterway under study, road crossing, ...) Coordinates (longitude and latitude) should be indicated on the map.
- A description of relevant features of the study area is also valuable.
- Specify sampling dates.

- Include a full description of techniques used in collecting and processing the samples. Refer to published literature when standard techniques are used. Duplication of methods utilized should be possible from the information presented here.

Occasionally the Results and Discussion sections are combined. However, unless the results are simple and the discussion short and straightforward, the combination of these sections is undesirable.

5.3 Results

This section should contain results which are pertinent to the study objectives. No interpretation should be included, nor should literature be cited in this section:

- It is important to include raw data (i.e. counts per replicate for each taxon identified) in the report. However, such information should be included as an appendix to the benthic invertebrate section of the report and not in the result section. The raw data sheets must indicate when and where samples were collected, who was involved in the collections, sorting, and identification of specimens, what sampler was used, and what the finest mesh size was in sample processing. This information is essential should the data be used as reference in years to come.
- A summary of basic data in the main body of the report is quite useful and it is a good idea to include descriptive statistics[1] for such data. It is essential to include

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[1] descriptive statistics: mean, variance, standard error, confidence limits, coefficient of variation

descriptive statistics for each variable which is used in hypothesis testing.

- Tables are the most concise form in which to include such data. However, graphs, (line graphs or bar graphs) may provide a better visual picture of trends. Although the inclusion of tables and graphs representing the same data is a duplication of information, it may be warranted in some cases where the writer wants to emphasize specific features of the data.
- When cluster or ordination techniques are used in the analysis of complex data bases, figures and tables representing the results of these techniques should be included.
- Statements about the significance of differences examined in statistical tests should be accompanied by a precise indication of the test used and the probability level chosen, or by a reference to a table which summarizes the results of such tests.

5.4 Discussion

This section should contain the interpretation of the data in relation to the original study objectives and hypotheses. As the section heading states, it is the discussion and interpretation of results that is intended here, not simply a repetition of results in a more generalized form. Pertinent literature (i.e. relevant, recent, and regional when it exists) should be used in the interpretation of the data. For example:

- Is anything known about the effects of a particular effluent type on benthic invertebrates in waters near, or of the type under investigation?
- What is known about the ecological requirements of a taxon which could explain its distribution pattern in the particular study undertaken? Results should be compared with those of published studies to identify similarities or discrepancies.

In ongoing monitoring studies, a general appraisal of past and present survey results should be included periodically (e.g. every five years or at the time the licence is renewed) to examine temporal trends in water quality and to identify the need to modify the study design.

5.5 Literature

This section contains an alphabetic list of all literature cited in the text. The formats outlined in the CBE Style Manual or Alberta Environment (1982) cited earlier are followed by most North American Journals and are recommended.

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