MAINE

BIOLOGICAL MONITORING AND BIOCRITERIA DEVELOPMENT PROGRAM

SUSAN P. DAVIES
Maine Department of Environmental Protection

LEONIDAS TSOMIDES
Maine Department of Environmental Protection

DAVID L. COURTEMANCH
Maine Department of Environmental Protection

FRANCIS DRUMMOND
Department of Entomology
University of Maine, Orono, Maine

Maine Department of Environmental Protection
Bureau of Land and Water Quality
Division of Environmental Assessment
Augusta, Maine 04333

March, 1993

Revised January, 1995

Printed under account:013 06A 1441 132 4929

CONTENTS

TABLES

APPENDICES

FIGURES

STATE OF MAINE

DEPARTMENT OF ENVIRONMENTAL PROTECTION

BIOLOGICAL MONITORING

AND BIOCRITERIA DEVELOPMENT

PROGRAM

MAY, 1993

INTRODUCTION

In April, 1990 the United States Environmental Protection Agency (USEPA) issued a state guidance document titled "BIOLOGICAL CRITERIA: National Program Guidance for Surface Waters" (USEPA, 1990). This document directs states to pursue the development of narrative biological criteria to be used to assess the biological integrity of aquatic communities, a goal not addressed by the physical and chemical water quality assessment approaches that have been practiced for decades. The State of Maine had already recognized this information gap in the early 1980's and, by 1986, had passed a revised water quality classification law that included consideration of the condition of aquatic life when assigning water quality classifications. Consequently, Maine is in a position to formally incorporate biological information into water quality management practices, and Maine's approach to refining aquatic life use classifications and developing biological criteria has been discussed in several US EPA documents (Courtemanch and Davies, 1987; United States Environmental Protection Agency ,1988a; USEPA, 1988b; USEPA, 1990).

USEPA has defined the term "biological assessment" to mean "an evaluation of the biological condition of a waterbody using biological surveys and other direct measurements of resident biota in surface waters" (USEPA 1990, pg.v). The assessment may be performed on any of a number of components of the overall biological community, for example, on a taxonomic group (e.g. algae, invertebrates or fish, etc.), or on an organizational level (individual, population, community etc.), or a functional unit (primary producers, secondary producers, decomposers, etc). It is not practically feasible to assess every component of the aquatic community so most investigators choose one or several components to assess. The chosen component is then used to indicate the condition of the entire, interacting community of aquatic life in the system, with conclusions regarding the well-being of that component being generalized to conclusions about the health of the entire aquatic community. The work of many water pollution scientists from all over the world, since the early part of this century, has contributed to the present day sophistication with which the different community components are understood. For most investigators, the focus has settled on either the assessment of the fish community or the assessment of the benthic macroinvertebrate community, or a combination of the two. Benthic (i.e., living on the bottom) macroinvertebrates are animals without backbones, which are visible to the eye. Examples are early life stages of aquatic insects, snails, clams, leeches, worms, crayfish, etc., which live on, under and around rocks, gravel and mud on the bottoms of lakes, rivers and streams. Maine has initiated it's biological assessment of rivers and streams using information from the benthic macroinvertebrate community, but recognizes the importance and usefulness of fish community data as well. The following are some points which contributed to Maine's choice of benthic macroinvertebrates as the community component to be used to assess the condition of the State's river and stream life:

1. Benthic macroinvertebrates are generally limited in mobility and are therefore less able to avoid the effects of pollutants. Fish, on the other hand, often have the ability to swim away from the effects of a pollutant and so may not be as reliable at indicating local environmental conditions.

2. Within the macroinvertebrate group there is a very wide range of pollution tolerances of different species. Some sensitive species may be killed or excluded by very low levels of pollutants, while other types may actually thrive in huge numbers only in the presence of certain types of pollution. There is a great deal of information contained in a single sample of macroinvertebrates.

3. Benthic macroinvertebrates are an extremely diverse group, having a greater number of different types of taxonomic components and feeding and energy use strategies than Maine's fish communities, which are relatively low in diversity. This feature makes benthic macroinvertebrates the community component with the greatest information content regarding energy transfer and functional well-being in the whole system.

4. Benthic macroinvertebrates have longer, more complex life cycles than algae or bacteria, frequently living one or more years in the aquatic environment, and therefore may integrate water quality effects over time.

5. Fish, which are a valuable State resource, are largely dependent on the macroinvertebrate community as a food source. Since the pollution tolerances of certain types of insects and other invertebrate organisms are broadly comparable to those of certain types of fish, assessment of macroinvertebrates is an indirect method of gaining information about the potential of a fishery in the area without directly assessing the fish community.

6. Some form of benthic macroinvertebrate life can be found in all but the most severely poisoned or disturbed habitats, unlike fish which may be absent due to natural causes like obstructions to passage.

7. Methods of sample collection and analysis of results are well established. Availability and ease of capture of macroinvertebrates make them a cost effective group to sample.

MAINE'S AQUATIC LIFE STANDARDS:

The biological classification of Maine's inland waters was authorized by the Maine State Legislature with the passage of M.R.S.A. 38 Public Law Chapter 698: The Classification System for Maine Waters (April, 1986). This law states that it is the State's objective "to restore and maintain the chemical, physical and biological integrity" of it's waters, and establishes a water quality classification system to enable the State to manage it's waters so as to protect their quality. The classification system further establishes minimum standards for dissolved oxygen , bacteria and aquatic life for each class as well as related charactistics necessary to preserve the designated uses assigned to each classification. This can be illustrated with the Class A standards as an example. The law states that the designated uses for Class A waters are: "drinking water after disinfection; fishing; recreation in and on the water; industrial process and cooling water supply; hydroelectric power generation; navigation; and as habitat for fish and other aquatic life". It is further specified that the "habitat shall be characterized as natural". The dissolved oxygen standard of "not less than 7 ppm or 75% of saturation" is set to protect the designated uses of fishing and natural habitat for fish and other aquatic life. The bacteria standard and the aquatic life standard are both "as naturally occurs" protecting the designated use of "habitat for fish and other aquatic life", and preserving the characteristic of "natural" habitat. See Table 1 for a description of the aquatic life standards, and the management perspective for each class.

The State's aquatic life standards establish, in narrative form, the characteristics of the aquatic community that are required to exist in order for a waterbody to attain a given classification, and these characteristics are specific and different for each water quality classification (Table 1). These standards are further refined in the statute by defining many technical and specific use terms, allowing a clear conceptualization of the general differences in aquatic life between classes.

The aquatic life standards are fundamentally quite different from the conventional dissolved oxygen and bacteria standards. Rather than specifying numeric criteria in statute for one discrete parameter, e.g. "dissolved oxygen shall be not less than 7 ppm", or bacteria "may not exceed a geometric mean of 64 per 100 ml", the aquatic life standard is a narrative standard which can potentially be assessed with a vast suite of measurable parameters. Numeric criteria, and decision rules that precisely define the way in which aquatic life classes are assessed, are specified in the Water Bureau's Aquatic Life regulations (06-096 Chapter 530, Section 12). Examples of quantitative measures used to assess attainment of aquatic life standards include the abundance of specific types of organisms (e.g. number of plecopteran individuals), numbers of different types of organisms (e.g. taxonomic richness), and indices which summarize large amounts of quantitative biological information into one number (e.g. diversity, similarity, etc.).

Development of numeric criteria in support of the aquatic life standards has been a time-consuming process for several reasons. The State has had to gather and analyze all of the data required to set numeric criteria for Maine. This has required the collection and statistical analysis of a baseline dataset of sufficient size and covering an adequate time frame to afford a high degree of certainty that valid generalizations could be drawn from the data. This is because significant differences exist in the distribution and abundances of macroinvertebrate fauna from one region to another, making it necessary to establish the baseline to strictly reflect the faunal distributions of the State of Maine. By contrast, the dissolved oxygen standard, was developed many years ago to protect fish life, by researchers from all over the United States. It is broadly applicable anywhere in the country and Maine was able to develop its oxygen standards without extensive data collection or analysis.

DATA COLLECTION METHODS

In 1983 the Department of Environmental Protection began a standardized benthic macroinvertebrate sampling program (MDEP,1987) to build a database to be used to establish the criteria that would allow the Department to classify a waterbody according to the State's aquatic life standards. The Department has collected aquatic life samples (benthic macroinvertebrates) from upstream and downstream of all major licensed wastewater discharges in the State, as well as from a large number of relatively undisturbed and unpolluted waterbodies. These sampling locations were chosen to represent the range of water quality conditions in Maine. Data collection upstream of a source of pollution allows for the establishment of a clean reference station which can be used to identify expected biological conditions in the absence of the pollution source. The pollution-impacted locations provide information on the presumed "worst-case" conditions and recovery zones of the rivers and streams sampled because the locations chosen and the season sampled represent times and places of maximal stress to the aquatic system.

Statistical analysis was performed on a 145 sample subset of the approximately 240 benthic macroinvertebrate sampling events occurring between 1983 and 1989. All geographic regions of the state were included as well as stream sizes from first to seventh order. The 145 samples were selected to ensure a uniform sampling method (rock-basket artificial substrates), typical free-flowing water habitat types, and freedom from other atypical influences (such as tidal or impoundment effects, disturbed samplers, atypical substrates, problems with sample retrieval, etc.). The objective was to assemble a uniform baseline dataset from one primary habitat type (free-flowing, stony bottom streams) free from the influence of determining physical or sample handling variables from which we could identify consistent and predictable biological characteristics associated with different catagories of water quality. To this end, much attention has been given to data quality assurance and quality control including 1) standardized, documented field collection procedures, performed under the direct supervision of a Department stream biologist; 2) supervised sample sorting with a proportion of each sorter's samples re-sorted by another person to determine sorting efficiency; 3) consistent taxonomic workup with about 80 percent of samples identified by the same taxonomist (100 percent since 1986). Data quality has been further assured by rigorous data entry and data editing protocols during transfer of raw data to the computerized database management system (dBASE III Plus, with upgrade to FOXPRO in 1993).

A final important decision concerning preparation of data for analysis was to adjust all richness-related (i.e. numbers of different types of organisms) data to the generic level. The identification of benthic macroinvertebrates to the species level is difficult or impossible for many groups due to the extremely subtle physical difference between closely related species within a genus, as well as the continuing discovery of new species every year. Yet there are other groups that are very easy to identify to species. Thus, it is quite common for data to be submitted at varying levels of taxonomic resolution, some organisms perhaps only identified to order or family (for example, Oligochaetes, or very immature individuals of other groups) while others might be identified to species. This inconsistency was felt to be incompatible with the need for clear-cut attainment guidelines for aquatic life. Any measures sensitive to richness would be vulnerable to varying levels of effort in the identification to the species level.

The generic level of identification, on the other hand, is much better established and much more easily accomplished. The Department continues to identify all well established taxa to the species level but these counts are then adjusted to the generic level prior to the computation of indices (Appendix A "Generic Richness"). Data submitted to the Department is also adjusted to genus prior to analysis. This decision was reviewed and approved by the Department's Technical Review Committee (discussed on page 9).

DATA ANALYSIS METHODS

Overview:

This set of quantitative baseline benthic macroinvertebrate community data provides the basis for establishing attainment criteria for each aquatic life standard in the classification system. The database of organism occurrence has been broken down into a set of approximately 30 quantitative variables that summarize the identity and abundance information that describes the makeup of the benthic macroinvertebrate community, for example, "Total Abundance of Individuals", "Generic Richness", "Taxonomic Diversity", etc. Additionally, variables describing the abundance (raw counts) and relative abundance (percent) of taxa collected were also included. (See Appendix A for a list and description of all the variables used in the Regulations). The decision-making approach in these regulations begins with statistical models (linear discriminant analysis) that use some of the variables to make an initial prediction of the water quality classification of an unknown sample by comparing it to characteristics of each classification identified in the baseline database. The output from analysis by the primary statistical model (First Stage LDM) is a list of probabilities of membership for each of four classes (A,B,C, and Non-Attainment of Class C). Class AA is not considered as a separate classification because the aquatic life standards are the same for Class AA and Class A. An explanation of the statistical procedures used and results of the analyses is presented in detail in Appendix F.

The four-way First Stage LDM provides an initial probability that a given site attains one of the four classes. This value, with a possible range from 0 to 1 is used, after transformation, as the first variable in each of the three other linear discriminant functions. These subsequent models are two-way models rather than four-way models. That is, they are designed to distinguish between a given class and any higher classes, as one group, and any lower classes as the second group (Fig. 1). This approach has been taken for two reasons. Firstly, the Department is primarily interested in a one-tailed analysis of attainment of classification. The pertinent question, in terms of identifying a need to initiate management action, is whether or not a site is attaining **at least** its assigned classification. A site that is shown to attain higher aquatic life standards than its legislated class will be evaluated for attainment of dissolved oxygen and bacteria standards. If all three water quality criteria attain the next higher classification the Department will recommend to the Legislature that the river reach be upgraded to the next class. If it fails to meet its assigned classification then corrective actions must be initiated; however, exceedence of assigned classification is acceptable. The second reason that two-way models are used is because they allow for greater statistical discrimination than models that attempt to isolate one class from all others, e.g. Class B versus Class A and Class C and Non-Attainment. The Department's proposed models are presented in Appendices B, C, D and E. These Appendices include all mathematical transformations of variables, constants and coefficients called for in the models. The linear function itself is provided in Appendix F.

The use of a system based on probabilities of attainment of standards allows for a determination to be made even in the "grey" area between classes, once the regulations establish the probability level required for attainment. The required probability may be set at different levels depending on the degree of certainty deemed necessary for a given decision. For example, a finding that is to serve as the basis for enforcement actions on a responsible discharger might require a higher level of certainty (i.e. higher probability of non-attainment of class) than would be required in a routine ambient monitoring assessment report. It also affords the Department and the public some insight into the relative strength of membership within that class, and shows whether the site is of higher or lower quality than the majority of sites in that class.

Construction of the Statistical Models:

Since nearly 70,000 individual organisms from about 300 distinct taxonomic groups are represented in the database it was necessary to distill the huge information content down to variables that would be most valuable for distinguishing water quality groups. Every sample in the dataset was assigned to an *a priori* classification to establish four water quality groups. Two different approaches were taken to establish the *a priori* groups: one approach evaluated the sample locations strictly from the standpoint of the degree of pollutional influence that was known or could be reasonably assumed to be present at the time the biological samples were collected (referred to as "Pollution Impact Rank"). This assignment was made by a group of five veteran DEP water quality professionals, having personal familiarity with the water bodies and pollutional influences in the database (municipal treatment plants, industries, non-point sources, etc.)(Appendix G).

In the other approach only the benthic macroinvertebrate community data for each sample was evaluated and was assigned an aquatic life attainment classification based on the degree to which the sampled community conformed to one of the aquatic life standards in the statute (referred to as "Biologist's Classification"). This assignment was made by the three benthic biologists at the Department (Appendix H). The two independent ranking systems were compared for correspondence and inconsistencies (Appendix I). It was concluded that the Biologist's Classifications were responsive to water quality differences among sites, and that they also provided new information, not available through the traditional water quality evaluation methods used in the assignment of Pollution Impact Ranks.

The outcome of this analysis resulted in the selection of the Biologist's Classification system as the *a priori* baseline against which to construct the predictive statistical model. Because of the importance of this baseline system, confirmation of the validity and reproducibility of the Biologist's Classification assignments was sought from two biologists not affiliated with the Department of Environmental Protection, but having considerable experience in the evaluation of stream macroinvertebrate data. They were each sent a subset of 35 percent of the data (40 sites) and asked to make a classification assignment for each sample, based on the narrative standards, but using their own evaluation methods. As in the case of the evaluation by Department biologists, they had no knowledge of the site locations or the type or degree of pollutional impact at the sites. Concurrence with the concensus assigment by DEP biologists was 83% for one biologist and 90% for the other biologist. It was concluded that the Biologist's Classifications were valid and reproducible and that they could be used as the *a priori* classification system. A summary of the results of the Biologist's Classification confirmation exercise is provided as Appendix J.

Underlying Rationale for the Statistical Approach:

The *a priori* "best professional judgement" classification assignments were required for several reasons. The most fundamental consideration is really a conceptual one, having it's basis in the difference in the realities and requirements that exist between the regulatory process and ecological systems, as explained below. The second consideration stems from principles of the exploratory multivariate statistical approach because the statistical procedure of linear discriminant analysis is dependent upon the existence of *a priori* groups of samples of "known" classification, against which to develop the predictive model for samples from unknown classifications.

Returning to the conceptual considerations, although the aquatic life standards for each classification were written by aquatic biologists, with great attention to trying to ensure that they be ecologically relevant, they are never-the-less, fundamentally, only a legal conceptualization of an extremely complex natural system that displays a continuum of responses to pollutional stress, as well as to stresses and subsidies from natural causes. The requirements of the regulatory process dictate that all water bodies be assigned a discrete water quality classification. Obviously, discrete classes of water quality do not exist in nature. However, by precisely defining a set of measurable criteria that describe observable differences between sets of samples, it is possible to establish empirically and statistically distinct groups of biological samples. The biological standards in the Water Quality Law were developed to legally define these discrete classes, in terms of measurable differences in aquatic communities, observable in nature, in areas of differing pollutional influence. They were developed by biologists after examining the extreme ends of the continuum of biological community response to increasing levels, and different types, of pollutional disturbance (i.e studying essentially pristine conditions and examining other conditions known to be severely influenced by pollution), and then making inferences about the relative degree of impact of intermediate conditions. From this experience a clear picture of biological community response to differing types and degrees of pollution emerged, and the basis was established for assigning an aquatic life sample to one of the aquatic life classes.

With this in mind, the goal of the statistical analysis of the baseline dataset has been to confirm that the professional, but essentially subjective observations of the biologists, represent an objective and measurable reality. In other words, the statistics were designed to determine, first, whether or not statistically distinct groups of macroinvertebrate communities could be distinguished using the community attributes which make up the aquatic life classification standards, and secondly, what are the most reliable measures for distinguishing the groups, and what are the statistically expected ranges (numeric criteria), of the groups.

FINAL EVALUATION OF STATISTICAL OUTCOME

Professional Judgement Methods

This process provides a mechanism for adjustment of the decision models. It is the responsibility of the Department to decide if an adjustment of a decision should occur, based on analytical, biological and habitat information and the Department may require additional monitoring of affected waters. The process relies on professional biological judgement, as well as documented evidence of conditions which can result in atypical findings. A description of the application of Professional Judgement is in Appendix M.

MODEL PERFORMANCE RESULTS

The results of the First and Second Stage Model's performance are presented in narrative form in Appendix F and in Appendix L and L-1, and O and O-1.

NON-AGENCY TECHNICAL PEER REVIEW

In the fall of 1989 a nine member Technical Review Committee was established to provide oversight of the development of the biological monitoring regulations by professional biologists not affiliated with the Department of Environmental Protection. Participants were selected on the basis of their technical expertise, their familiarity with the ecological setting and the regulatory climate of Maine, and their capacity to provide the perspective of a relevant interest group. Interest groups represented include: hydropower, the paper industry, a natural resource advocacy group, the academic community, private biological consultants, and non-DEP state biologists. A list of members and attenders is provided in Appendix K. The Committee has met, in full day sessions, in January, 1990, in September, 1990 and in December, 1990. The role of this group has been strictly to provide technical guidance and critique of the scientific process. The Department has found this to be an extremely valuable exchange that has significantly shaped the direction and the quality of the product.

PEER REVIEW BY THE SCIENTIFIC COMMUNITY

The Department has also submitted the technical details of the Instream Biological Monitoring Program to extensive review by the scientific community, nationwide, in the form of oral, technical presentations and peer-reviewed journal articles, throughout the developmental process. A list of presentations and publications is provided in Appendix N.

APPLICATION OF BIOCRITERIA

Various roles have been suggested for the use of biocriteria. These include general assessment of water quality and attainment of standards, monitoring of specific discharges (point and non-point), evaluation of treatment technologies and Best Management Practices, detection of toxics, detection and evaluation of spills, habitat evaluation, and enforcement of standards. To accommodate these uses, instructions must be included in the Department's regulations which will codify essential aspects of implementation. These may include 1) who is responsible for acquisition of biological information, 2) designation of sites, 3) schedules for sampling and 4) how the information is to be applied.

Data Aquisition Responsibilities:

Up to now, the DEP has conducted most of the data acquisition and has made all decisions regarding the application of biocriteria information. While this has been satisfactory during these first years of development and trial, it is the intent that at least some information can and should be provided by non-agency sources. By inclusion of standard sampling protocols in regulation, it is intended that reliable data may be acquired from a variety of sources.

Situations in which the Department may require a license applicant to provide aquatic life field sampling data would be dependent on the potential of an activity to cause a community to be in nonattainment of the classification standards including the nature of the activity, magnitude in relation to the affected water, variability of the activity and other pertinent information.

Site Selection:

Designation of sampling sites is a specific concern which may need to be addressed in regulation. The establishment of reference stations is important since these sites can establish a base of comparison for many decisions, although the decision-making protocol in the draft regulations is capable of providing a classification prediction with or without an upstream reference site. It is suggested that the Department be responsible for the designation of reference sites, but not necessarily their actual sampling. Reference sites should display similar habitat characteristics as that of sites to which they will be compared, particularly with respect to water velocity and substrate type. Ideally, reference sites located within the same reach are best, however reference sites within the same watershed are suitable provided there are no more than two stream orders of difference between a reference and test site, and other habitat conditions are comparable.

Test sites (e.g. downstream or pollution-influenced sites) may be selected anywhere within a waterbody where effluents and receiving waters are fully mixed. It is not appropriate to test sites within designated mixing zones (designated by the BEP according to Section 451). However, numerous other undesignated mixing areas occur, some extending substantial distances and covering a considerable area of habitat. Assessment may be made in these areas provided adequate initial mixing of the effluent and receiving water has occurred.

Schedules for Sampling:

The draft biocriteria for Maine have been developed from a set of data collected from a specific sampling period (July-September). Because of known temporal changes in the invertebrate community, it would not be appropriate to apply these criteria outside this season. This seasonal restriction may require considerable advanced planning, depending upon the ultimate use of the data and the regulatory deadlines involved.

There are three time sequences that may apply to biomonitoring data. The first would be a fixed schedule of monitoring (Classification Attainment Evaluation). In this sequence, monitoring is conducted on a routine basis at a fixed frequency. Presently, the agency does not have the resources to monitor all the existing stations. Therefore, some prioritization of monitoring must occur. This could be established based on dilution capacity of receiving waters, size of the discharge, recent performance, and/or unique values of the resource.

A second time sequence would involve response monitoring triggered by a complaint, or a report of a spill (Site Specific Impact Evaluation). While these events cannot be anticipated, a certain amount of agency resources must be reserved for this purpose.

The third sequence of monitoring would occur as a result of some management activity such as licensing, relicensing or issuance of water quality certification (Pre-Impact Baseline Evaluation and/or Classification Attainment Evaluation). Scheduling of these events is usually well known, but requires monitoring well in advance of the regulatory deadlines to allow for evaluation and modification of practices should a non-attainment condition be detected. Sampling 2-3 years in advance of such an activity may be appropriate and may be required in regulation as a condition for licensing or certification. Sampling in advance of a new license or certification may be required on a case by case basis, for the purpose of establishing the reference conditions, but would not be used as a means of setting specific license limits.

Application of Biological Findings:

Biomonitoring information may be applied for several purposes. These can be categorized as assessment (such as evaluation of attainment of standards for the biennial water quality reports required by federal agencies, e.g. 305b, or non-point source assessment report, 319); licensing or certification activities; and for enforcement of water quality standards. Biological information has been included in the first category, assessment reporting, for several years, though largely on the basis of best professional judgement. Application of the formal criteria, once they are adopted, will insure consistent review and assessment. As a condition of licensing or certification, it should be demonstrated that a receiving water is attaining its classification before issuance of a license, or as stated in Section 464, 4, F(3), that the activity does not contribute to the cause of non-attainment. A license should not be issued where the activity contributes to non-attainment.

It is recognized that the biocriteria provide a summation of the effects of various activities that affect a waterbody. Where it can be demonstrated that a discrete activity is responsible for non-attainment, that activity should not be relicensed until attainment is achieved or until a plan to achieve attainment, with a specific time schedule for implementation, is developed, as part of the license. Where the occurrence of multiple sources contributes to non-attainment, it should be the responsibility of the Department to develop a plan, with time schedules, that will use all appropriate programs collectively to achieve attainment. Issuance of a license may be contingent upon compliance with those aspects of the plan that are the licensee's responsibility.

Use of biocriteria for enforcement of water quality standards may be made where it can be demonstrated that a discrete activity is responsible for non-attainment. This may be achieved using paired (above/below) samples, where the above site shows attainment of standards, and the site below the pollutional influence shows clear evidence of detrimental change, and/or failure to meet the classification standards.

CITATIONS

Courtemanch, D.L. and S.P. Davies. 1987. Implementation of biological standards and criteria in Maine's Water Classification Law. In: Proc. Instream Biomonitoring and Biological Criteria Workshop. US EPA, Lincolnwood, IL.

Maine Department of Environmental Protection. 1987. Methods for Biological Sampling and Analysis of Maine's Waters. MDEP, Augusta Maine.

US EPA. 1988 a. Water Quality Program Highlights: Maine's biologically based water quality standards. Office of Water Regulation and Standards, Washington, D.C., April, 1988.

US EPA. 1988 b. National Symposium on Water Quality Assessment. Meeting Summary. Annapolis, MD. US EPA. Monitoring and Data Support Division (WH-553) Washington, D.C.

US EPA. 1990. Biological Criteria: National Program Guidance for Surface Waters. Office of Water Regulation and Standards, Washington, D.C. EPA-440/5-90-004.

Table 1. **Maine's water quality classification system for rivers and streams, with associated biological standards.**

Class	Management	Biological standard
AA	High quality water for recreation and ecological interests. No discharges or impoundments permitted.	Habitat natural and free flowing. Aquatic life as naturally occurs
A	High quality water with limited human interference. Discharges restricted to noncontact process water or highly treated wastewater equal to or better than the receivingwater. Impoundments allowed.	Habitat natural. Aquatic life as naturally occurs.
B	Good quality water. Discharge of well treated effluent with ample dilution permitted.	Habitat unimpaired Ambient water quality sufficient to support life stages of all indigenous aquatic species. Only non-detrimental changes in community composition allowed.
C	Lowest water quality. Maintains the interim goals of the Federal Water Quality Act (fishable/swimmable). Discharge of well treated effluent permitted.	Ambient water quality sufficient to support life stages of all indigenous fish species. Change in community composition may occur but structure and function of the community must be maintained.

METHODS FOR THE CALCULATION OF INDICES AND MEASURES OF COMMUNITY STRUCTURE USED IN THE LINEAR DISCRIMINANT MODELS

Variable
Number

1 **Total Abundance**

Count all individuals in all replicate samples from one site and divide by the number of replicates to yield mean number of individuals per sample.

2 **Generic Richness**

Count the number of different genera found in all replicates from one site.

Counting Rules for Generic Richness:

1) A family level identification with less than or equal to one taxon identified to a lower taxonomic level (i.e. one genus or species) will be counted as a separate taxon in Generic Richness counts.

2) A family with more than one taxon identified to a lower taxonomic level will not be counted towards Generic Richness. Counts will be split proportionately among the genera that are present.

3) Higher level taxonomic identifications (Phylum, Class, Order) are not counted toward Generic Richness <u>unless</u> they are the only representative.

4) Pupae are ignored in all calculations.

5) All population counts at the species level will be aggregated to the generic level.

3 **Plecoptera Abundance**

Count all individuals from the order Plecoptera in all replicate samples from one site and divide by the number of replicates to yield mean number of Plecopteran individuals per sample.

4 **Ephemeroptera Abundance**

Count all individuals from the order Ephemeroptera in all replicate samples from one site and divide by the number of replicates to yield mean number of Ephemeropteran individuals per sample.

5 **Shannon-Wiener Generic Diversity (Shannon, CE and W. Weaver, 1963. <u>The Mathematical Theory of Communication</u>. University of Ill. Press, Urbana, IL.)**

After adjusting all counts to genus as described under "Counting Rules for Generic Richness":

$$\bar{d} = \frac{c}{N}\left(N \log_{10} N - \sum n_i \log_{10} n_i\right)$$

where: $\bar{d}$ = Shannon-Wiener Diversity
c = 3.321928 (converts base 10 log to base 2)
N = Total Abundance of Individuals
n_i = Total Abundance of Individuals in the i^{th} taxon

6 **Hilsenhoff Biotic Index (Hilsenhoff, W. L. 1987. An improved biotic index of organic stream pollution. The Great Lakes Entomol. 20(1)31-39).**

$$BI = \sum \frac{n_i a_i}{N}$$

where: BI = Biotic Index
n_i = number of individuals in the i^{th} taxon
a_i = tolerance value assigned to that taxon
N = total number of individuals in sample

7 **Relative Abundance Chironomidae**

Find abundance of Chironomidae (as for abundance of Ephemeroptera) and divide by Total Abundance of individuals.

8 **Relative Richness Diptera**

Count the number of different genera from the order Diptera (follow counting rules for Generic Richness) and divide by Generic Richness.

9 ***Hydropsyche* Abundance**

Count all individuals from the genus *Hydropsyche* in all replicate samples from one site, and divide by the number of replicates to yield mean number of *Hydropsyche* individuals per sample.

10 **Probability (A+B+C) from First Stage Model**

Sum the probabilities for Classes A, B, and C derived from the 9 variables in the First Stage Linear Discriminant Model.

11 ***Cheumatopsyche* Abundance**

Count all individuals from the genus *Cheumatopsyche* in all replicate samples from one site and divide by the number of replicates to yield mean number of *Cheumatopsyche* individuals per sample.

12 **EPT Generic Richness Divided by Diptera Richness**

Find EPT Generic Richness and divide by Diptera Generic Richness.

13 **Relative Abundance Oligochaeta**

Find abundance of Oligochaetes (as for abundance of Ephemeroptera) and divide by Total Abundance of individuals.

14 **Probability Class (A+B) from the First Stage Model**

Sum the probabilities for Class A plus Class B derived from the 9 variables in the First Stage Linear Discriminant Model.

15 **Perlidae Abundance**

Count all individuals from the family Perlidae (Appendix 2) in all replicate samples from one site and divide by the number of replicates to yield mean number of Perlidae per sample.

16 **Tanypodinae Abundance**

Count all individuals from the subfamily Tanypodinae (Appendix 2) in all replicate samples from one site and divide by the number of replicates to yield mean number of Tanypodinae per sample.

17 **Chironomini Abundance**

Count all individuals from the tribe Chironomini (Appendix 2) in all replicate samples from one site and divide by the number of replicates to yield mean number of Chironomini per sample.

18 **Relative Abundance Ephemeroptera**

Find abundance of Ephemeroptera and divide by Total Abundance of individuals.

19 **EPT Generic Richness**

Count the number of different genera from the order Ephemeroptera (E), Plecoptera (P), and Trichoptera (T).(Follow counting rules for Generic Richness).

20 **Probability Class (A+B) from the A+B Sub-Model in the B or Better Model**

Sum the probabilities for Classes A plus B derived from Variables 15-19 in the A+B Sub-Model found in the B or Better Model.

21 **Summed Abundances of: *Dicrotendipes & Micropsectra & Parachironomus & Helobdella***

Find abundance of the 4 genera (as for abundance of Ephemeroptera) and sum them.

22 **Probability of Class A from the First Stage Model**

Insert the probability of Class A derived from the 9 variables in the First Stage Linear Discriminant Model.

23 **Relative Plecoptera Richness**

Find Plecoptera Richness and divide by Generic Richness.

24 **Relative Abundance *Brachycentrus***

Find abundance of *Brachycentrus* (as for Abundance of Ephemeroptera) and divide by Total Abundance of individuals.

25 **Summed Abundances of: *Cheumatopsyche & Cricotopus & Tanytarsus & Ablabesmyia***

Find abundance of the 4 genera (as for abundance of Ephemeroptera) and sum them.

26 **Summed Abundances of: *Acroneuria & Stenonema***

Find abundance of the 2 genera (as for the abundance of Ephemeroptera) and sum them.

27 **Probability of Class A from the A Sub-Model of the Class A Model**

Insert the probability for Class A derived from Variables 23-26 in the A Sub-Model found in the Class A Model.

28 **EP Generic Richness/14**

Sum Ephemeroptera Generic Richness plus Plecoptera Generic Richness and divide by 14 (maximum expected for Class A).

29 **Dominant A Indicator Taxa/5**

Find the 5 most abundant taxa in the community and calculate the proportion that are A indicator taxa from Appendix 1.

30 **Presence of A Indicator Taxa/7**

Count the number of A indicator taxa from Appendix 1 that are present in the community and divide by 7 (total possible number).

Indicator Taxa: Class A

Brachycentrus (Trichoptera: Brachycentridae)
Serratella (Ephemeroptera: Ephemerellidae)
Leucrocuta (Ephemeroptera: Heptageniidae)
Glossosoma (Trichoptera: Glossosomatidae)
Paragnetina (Plecoptera: Perlidae)
Eurylophella (Ephemeroptera: Ephemerellidae)
Psilotreta (Trichoptera: Odontoceridae)

FAMILY FUNCTIONAL GROUPS

PLECOPTERA

Perlidae

Acroneuria
Attaneuria
Beloneuria
Eccoptura
Perlesta
Perlinella
Neoperla
Paragnetina
Agnetina

CHIRONOMIDAE

Tanypodinae

Ablabesmyia
Clinotanypus
Coelotanypus
Conchapelopia
Djalmabatista
Guttipelopia
Hudsonimyia
Labrundinia
Larsia
Meropelopia
Natarsia
Nilotanypus
Paramerina
Pentaneura
Procladius
Psectrotanypus
Rheopelopia
Tanypus
Telopelopia
Thienemannimyia
Trissopelopia
Zavrelimyia

FAMILY FUNCTIONAL GROUP
(continued)

Chironomini

Pseudochironomus
Axarus
Chironomus
Cladopelma
Cryptochironomus
Cryptotendipes
Demicryptochironomus
Dicrotendipes
Einfeldia
Endochironomus
Glyptotendipes
Goeldichironomus
Harnischia
Kiefferulus
Lauterborniella
Microchironomus
Microtendipes
Nilothauma
Pagastiella
Parachironomus
Paracladopelma
Paralauterborniell
Paratendipes
Phaenopsectra
Polypedilum
Robackia
Stelechomyia
Stenochironomus
Stictochironomus
Tribelos
Xenochironomus

I. FIRST STAGE MODEL

VARIABLE NUMBER	VARIABLE NAME	TRANSFORMATION	COEFFICIENTS CLASS A	CLASS B	CLASS C	NON-ATTAINMENT
	CONSTANT		-81.05960	-90.45419	-94.53085	-87.69133
1	TOTAL ABUNDANCE	*nLOG*	8.10018	8.78244	9.77036	10.29463
2	GENERIC RICHNESS		-0.12886	-0.11147	-0.20637	-0.15975
3	PLECOPTERA ABUNDANCE	*nLOG*	0.14286	-0.28546	-1.23536	-2.08207
4	EPHEMEROPTERA ABUNDANCE	*nLOG*	-0.28635	-0.27814	-0.50926	-2.61034
5	SHANNON-WIENER GENERIC DIVERSITY		17.08893	16.85889	16.74591	14.07958
6	HILSENHOFF BIOTIC INDEX (0-10)		5.53397	6.85508	7.66419	7.14624
7	RELATIVE ABUNDANCE CHIRONOMIDAE	*nLOG*	-10.28287	-10.46995	-9.42318	-9.60312
8	RELATIVE RICHNESS DIPTERA		64.48643	65.00548	63.03596	58.45393
9	HYDROPSYCHE ABUNDANCE		-0.00372	-0.00079	-0.00332	0.00278

VARIABLE NUMBER	VARIABLE NAME	TRANSFORMATION	COEFFICIENT	
			CLASS ABC	NON-ATTAINMENT
	CONSTANT		-32.32477	-8.08631
10	PROBABILITY (A+B+C) FROM FIRST STAGE MODEL (9 VARIABLES)	***ARCSIN (radians)***	25.43321	3.79521
11	CHEUMATOPSYCHE ABUNDANCE	***nLOG***	-0.18901	-0.46639
12	EPT GENERIC RICHNESS DIVIDED BY DIPTERA RICHNESS	***SQ.ROOT***	3.37296	3.32932
13	RELATIVE ABUNDANCE OLIGOCHAETA	***nLOG***	-4.72098	-3.41434

VARIABLE NUMBER	VARIABLE NAME	TRANSFORMATION	COEFFICIENTS	
			CLASS AB	CLASS C-NA
	CONSTANT		-9.74762	-0.8158
14	PROBABILITY CLASS (A) + (B) FROM FIRST STAGE MODEL (9 VARIABLES)	*ARCSIN (radians)*	7.17828	0.91960
	FIVE VARIABLE A+B SUB-MODEL			
	CONSTANT		-9.81108	-4.53424
15	PERLIDAE ABUNDANCE	*nLOG*	-0.34810	-0.88270
16	TANYPODINAE ABUNDANCE	*nLOG*	-0.09987	0.22502
17	CHIRONOMINI ABUNDANCE	*nLOG*	-0.20354	0.05815
18	RELATIVE ABUNDANCE EPHEMEROPTERA		6.12273	-1.03415
19	EPT GENERIC RICHNESS		0.99431	0.54098
20	PROBABILITY (A + B) FROM A+B SUB-MODEL (variables # 15-19)	*ARCSIN (radians)*	6.97001	0.62398

VARIABLE NUMBER	VARIABLE NAME	TRANSFORMATION	COEFFICIENTS	
	INDICATOR TAXA		CLASS AB	CLASS C-NA
21	SUMMED ABUNDANCES OF: *DICROTENDIPES* *MICROPSECTRA* *PARACHIRONOMUS* *HELOBDELLA*	*nLOG (of sum)*	-0.05958	-0.04149

VARIABLE NUMBER	VARIABLE NAME	TRANSFORMATION	COEFFICIENTS CLASS A	COEFFICIENTS CLASS BC-NA
	CONSTANT		-9.26864	-2.53031
22	PROBABILITY CLASS A FROM FIRST STAGE MODEL (9 VARIABLES)	***ARCSIN (radians)***	4.71285	-0.79081
	FOUR VARIABLE 'A' SUB-MODEL			
	CONSTANT		-3.53463	-3.26997
23	RELATIVE PLECOPTERA RICHNESS		54.70984	27.34672
24	RELATIVE ABUNDANCE BRACHYCENTRUS		16.49089	1.12026
	INDICATOR TAXA			
25	SUMMED ABUNDANCES OF : *CHEUMATOPSYCHE*, *CRICOTOPUS*, *TANYTARSUS*, *ABLABESMYIA*	*nLOG (of sum)*	0.46118	1.19263
26	SUMMED ABUNDANCES OF: *ACRONEURIA*, *STENONEMA*	*nLOG (of sum)*	-0.05473	-0.32788

VARIABLE NUMBER	**VARIABLE NAME**	**TRANSFORMATION**	**COEFFICIENTS**	
			CLASS A	CLASS BC-NA
27	PROBABILITY CLASS A FROM 'A' SUB-MODEL (VARIABLES # 23-26)	*ARCSIN (radians)*	4.36590	1.88474
28	EP GENERIC RICHNESS /14		7.40183	7.61093
29	DOMINANT A INDICATOR TAXA / 5		9.22989	-0.26603
30	PRESENCE OF A INDICATOR TAXA / 7		5.94453	-1.82803

THE LINEAR DISCRIMINANT FUNCTION

INTRODUCTION

Univariate statistical methods are widely known for comparing two or more populations (t-test, analysis of variance and covariance, etc.). If more than one variable has been measured on each observation, however, an analysis restricted to single variables may not be sufficiently informative for classifying individual observations into groups. A method that uses all variables simultaneously is referred to as "multivariate". The advantage of the multivariate approach is that two or more classes or groups that overlap considerably when each variable is viewed separately may be more distinct when the variables are viewed together. A group of multivariate methods which are specifically suited for classifying samples (e.g. streams belonging to different pollution or water quality classes) is linear discriminant analysis. The literature on linear discriminant analysis can be confusing because there are several multivariate statistical methods which have been given the title of "linear discriminant analysis", but have different objectives and means of discriminating groups (e.g. canonical discriminant analysis, Fisher's linear model or Mahalanobis distance function, nearest neighbor discriminant analysis, log-linear categorical analysis, multiple analysis of variance, multiple regression analysis, etc.). As explained in the following section, we chose Fisher's linear discriminant model to analyze our data.

METHODS

The objective of this project is to classify streams in Maine according to four water quality ranks derived by the Maine Department of Environmental Protection (Biologists Classification: Appendix H). The approach taken involved the construction of two separate stages of statistical analysis, the first stage to determine the highest probability of membership of an unknown sample in one of the <u>four</u> water quality ranks, and the second stage to refine the prediction by use of a paired <u>two-group test</u> (Fig. 1). Thus, the initial four-group linear discriminant model, the FIRST STAGE MODEL, predicts the probability of sample membership in Class A versus Class B versus Class C versus Non-Attainment of Class C. The probabilities of membership in a given group reflect the strength of association of the sample to the water quality class. The model is based upon linear discriminant functions utilizing a subset of the more than 400 taxonomic and community structure measures computed for the sample data set. The first stage model acts as a screen, narrowing the initial prediction down to one class. The probabilities obtained from the FIRST STAGE MODEL are then used as the first variable in the subsequent two-group models. Application of the two-group tests is hierarchical in that it first determines the probability that an unknown sample belongs in the cluster of samples, A + B + C versus the probability that it belongs in the cluster of Non-Attainment of Class C samples. This is referred to as the C OR BETTER MODEL and it determines if the sample is *at least a Class C.* The B OR BETTER MODEL , similarly, determines if the unknown sample attains *at least Class B* standards. It discriminates between the cluster of A + B samples and the cluster of C + Non-Attainment of Class C samples. The CLASS A MODEL discriminates Class A samples from the cluster of samples in Classes B + C + Non-Attainment of Class C. All discriminant models (one four-way and three, two-way) utilize different variables, providing independent estimates of class membership. It is important to note that the derived probabilities for the three Second Stage Models are also independent and, combined, do not add up to 100%. The models are relying on different aspects of the multivariate representation of the benthic community to separate the groups.

The data set available for model construction consisted of 145 stream macroinvertebrate sampling events, each with a common set of quantitative measures (ca. 400 variables) representing the biotic (e.g., taxa abundances, biological indices, etc.) and physical attributes (stream width, temperature, land use of surrounding area, etc.) of the site. We chose Fisher's linear discriminant model to:

1. Identify relationships between the qualitative Biologist's Classification (4 water quality classes) and the quantitative predictor variables;

2. Identify the boundaries between the groups of streams, the boundaries being defined in terms of those variable characteristics which distinguish the objects in the respective criterion groups.

In this type of discriminant analysis a concept is employed which is similar to linear regression. A linear discriminant function is an equation that is a weighted linear combination of the predictor variables derived so as to discriminate best among the classification groups. This is achieved by the statistical decision-making rule of maximizing the between group variance relative to the within group variance. The linear combinations of the predictor variables are known as the discriminant function.

It has the form:

$$Z=C + W_1X_1 + W_2X_2 + \ldots W_nX_n$$

Where: **Z=Discriminant Score**

C=Constant

W_i=The Weights or Coefficients

X_i=the Predictor Variable Values

A linear combination such as above would be derived for each of the classes in the classification scheme i.e., if there are four classes to be predicted then there will be four different discriminant equations that result in the discriminant model. All four equations have the same predictor variables, but different coefficients or weights, and constants. The constants and coefficients for each model are provided in Appendix B,C,D and E. However, before a discriminant function is estimated, an analysis of variance is conducted in order to prove the existence of significantly distinct classes, but having similar variances or covariance matrices. It makes no sense to derive a predictive algorithm for separating classes of streams if, in reality, these streams are not significantly different from one another. The primary assumption made in the use of this method is that the water quality classes represent real populations of Maine streams and that streams not used to build the model are represented by one of the four classes.

Once the discriminant function is derived it should be tested to make sure that the estimates of the coefficients or weights are representative for the populations or classes that are being classified. There are two methods for testing the discriminant function. Both are based on assessing the percentage of sites which are correctly classified. The preferred method involves building the discriminant function with one data set and testing the predictions on a second, independent validation data set. This, of course, requires that a considerable data base exists, since just in building the discriminant model a minimum of ten samples is needed for each predictive variable that is included in the discriminant function (e.g., if four predictor variables are used a minimum of forty sampled streams is required).

Since our first stage linear discriminant model uses nine predictor variables and we had 145 samples, we chose to use a different method. It is used when the entire data set is just large enough for model construction and is referred to as the jackknife technique. In this method a series of discriminant functions are estimated utilizing the data set with a given percentage left out (e.g. 10-20 percent), then the entire data set is tested with the discriminant model derived from the selected 80-90 percent of the data set and the correctness of the classification on this smaller data set is evaluated. This procedure is repeated, each time leaving out a different portion of the data set. The average and variance of the correct percent classification and coefficient values are measures of the overall predictiveness of the discriminant function and sensitivity of the weights to small changes in the structure of the data (termed robustness of the model). The procedure serves to screen for the possibility that a few atypical samples are significantly affecting the predictive success of the model.

Other information that can be derived from the discriminant function is the significance of each of the predictors in the function. The weights and their standard errors allow one to estimate the partial contribution of each variable to the overall classification. If the weights are significantly different from zero (based upon the standard error) then an estimate of the importance of each variable can be derived from the size of the standardized coefficient weight. The magnitude of the standardized weight, in either a negative or positive direction from zero, may be correlated with the effect that the variable has on separating the classes. This is only a crude estimate, however, since these predictors are not operating entirely independently. Sometimes the action of one predictor is correlated with the action of another. For instance, if both species diversity and total abundance rise and fall in a correlated manner then they may both have high weights, but it is difficult to say which one is the most significant predictor. One method to alleviate this predicament is to use another technique, factor analysis, in conjunction with backward elimination discriminant analysis during the construction phase of the discriminant model, in order to screen the predictor variables and eliminate the redundant (highly correlated) and non-significant predictor variables.

The actual use of the discriminant function occurs when a stream with either an unknown classification (a new classification to be estimated) is sampled or a stream with a previous classification (legislated classification to be evaluated to see if it still represents the current status of the stream) is sampled. Each of the predictor variables represented in the linear discriminant function must be measured or calculated for the sampled stream. Then each value for the predictor variables in the sampled stream is substituted into each linear discriminant equation (one for each possible class). The class equation that yields the largest value when the predictor variable quantities are substituted is the class chosen for the predicted class. A probability of class membership can then be derived. This probability should be interpreted as the <u>strength</u>, on a 0 to 1 scale, that one can feel confident that the unknown test sample belongs to a particular water quality class. A validation of the discriminant model should be conducted every five to ten years with a set of standard stream sites so that factors such as climate change or other factors which are not related to pollution can be identified and the model can be re-calibrated if necessary.

<u>RESULTS</u>: First Stage Model

Physical variables appeared to have little direct correlation with the Biologist's Classification rankings (all r's < 0.6). Geographic locale of the sites was also not found to be correlated with classification. Year to year variations in the discriminating variables and the predictions, for locations with multiple year data, suggest that the variables which characterize stream classes change at a magnitude that does not affect correct classification for sites that are not borderline.

Functional group abundance (raw abundance, log and rank transformed) were not significant predictors of the Biologist's Classification. Only one generic level abundance was significant, *Hydropsyche* spp. For the most part, abundance of individual genera or species were not found to be good predictors in the discriminant model. This is not surprising since individual species abundances would be expected to fluctuate from year to year and stream to stream, within a particular water quality class. In the test data set of more than 300 genera, less than 30 genera occurred in at least 25 percent of the sites. We found that it is the higher level taxonomic groups (i.e. families, orders, etc.) and aggregated indices (i.e. richness, diversity, biotic index,etc.) that perform best for a discriminant model approach. Other approaches were used to develop an algorithm for separating water quality classes based on benthic macroinvertebrate data (cluster analysis, principle components analysis, two-way indicator species analysis) but did not yield satisfying results.

The best discriminant predictor model that we found for separating the four groups contains nine variables (Appendix B). The success rate for correctly predicting the assigned Biologist's Classification was between 67 percent and 84 percent for all classes (Appendix L). The correlation matrix suggests that the factor analysis was effective in providing a group of independent and non-redundant variables. Analysis of variance results suggest that the Biologist's Classification groups are significantly different and so a basis for discriminating between at least two populations exists.

RESULTS: Second Stage Model

The second stage discriminant functions for separating sites into their assigned Biologist Classification utilize 16 additional variables, as well as probabilities derived from the First Stage Model and sub-models within the Second Stage Models, (Appendix C,D, and E). The percent correct classification of the second stage models in the Jacknife procedure ranges from 92% to 99% (Appendix L).

A test of the First Stage and the Second Stage models was also performed using 46 sites not used in building the models. Results of the test sites are summarized in Appendix O. In no case did the models mis-predict a sample by more than one class. Of the 46 total sites only two mis-predicted sites were in complete disagreement with the three independent biologist rankings (Appendix O-1). In all other cases where mis-predictions occurred the biologists considered the sites borderline between two classes. The four mis-classified A sites were all considered by the biologists to be low A or high B sites. A summary of all mis-classified sites is included in Appendix O-1.

It was concluded that the results of the test data set run confirm that the model predictions reflect the biologist classification rankings and that the majority of mis-predictions are in borderline cases.

SUMMARY

In summary, a statistical procedure, in two stages, has been developed to predict the probability of membership of an unknown sample within one of four water quality classes, based on a total set of twenty five different biological community variables and five model probabilities derived from the different models. The first stage discriminant model uses nine variables to separate samples into one of four groups. It separates Class A samples from Class C or Non-Attainment of Class C samples with complete accuracy (no Class A sites mis-classified by the model into Class C or Non-Attainment). In no case did the model mis-classify a sample by more than one class. Further refinement of predictions is accomplished by a second stage analysis using sixteen additional variables in three additional discriminant models having a predictive success between 92 percent (for the B OR BETTER MODEL) and 99 percent (for the C OR BETTER MODEL) according to the Jacknife procedure. These statistical models provide the foundation for the assignment of aquatic life classification attainment in the Department's Aquatic Life Regulations, serving as the first step in the decision-making sequence. Results of the models, as well as all pertinent facts concerning the sampling and analysis process (irregularities in habitat criteria, loss or disturbance of replicates, sub-sampling protocol, etc.) are reviewed by the professional biological staff in the final stage of decision-making (Appendix M). The final result is a step-wise decision making protocol that is based on strength of membership within classes.

PROCESS AND CRITERIA

FOR THE ASSIGNMENT OF POLLUTION IMPACT RANKS

Raters:

Don Albert

Degree in Civil Engineering; employed in the Water Bureau for 10 years, writing industrial and municipal wastewater discharge licenses for the first 3 1/2 years, then promoted to his current position as head of the Technical Assistance Section for municipal and industrial facilities in the Division of Operations and Maintenance.

Norm Marcotte

Degree in Soil Science; Certified Soil Scientist; employed in the Water Bureau 10 years, writing and reviewing municipal and industrial wastewater discharge licenses; currently Maine's Non-Point Source Program Coordinator

Dennis Merrill

Degree in Biology; employed by the Water Bureau for 17 years, working in the Division of Operations and Maintenance for the first 6 years and in the Enforcement Section of the Division of Licensing and Enforcement for the last 10 years, addressing municipal and industrial wastewater discharge license violations.

Paul Mitnik

Degrees in mathematics and Forest Engineering; presently employed as a professional Engineer in the Division of Environmental Evaluation and lake Studies. Has done water quality modeling for the Water Bureau for 11 years.

Barry Mower

Master's Degree in Fisheries with a minor in Water Quality; Certified Fishery Scientist. Employed by the Water Bureau for 16 years, currently in charge of the effluent toxicity evaluation program.

Ranking Process:

Sampling event information (date, location in relation to known sources, etc.) was provided to the ranking team. Each member independently assigned a relative pollution impact rank to the site for the time period during which biological sampling occurred. Criteria used to assign ranks and a description of characteristics defining each rank are listed below. Following the independent rankings, the group reconvened to arrive at a consensus rank. This process involved an airing of all known facts about the site at the time of sampling, and an open exchange of the rationales behind the individual rankings. In 95% of the cases the group was able to unanimously agree on the consensus rank based upon new information provided by other raters. The ranks that are listed in the Pollution Impact Rank Justification Report are the independently assigned ranks. These were revised during the group discussion to produce the consensus rank. For 8 sites a unanimous or definitive rank was not given by the group. These sites are indicated in the Report with parentheses () around the final assigned rank. In several cases pertinent information was not available to the group at the time it met and it was agreed by the group that a rank should be assigned by other Water Bureau staff people having a more direct, personal knowledge of the site. These persons are named in the Report for the sites they rated. There was also a small number of sites for which agreement could not be reached. In these cases, the prevailing rank was the rank number agreed upon by the greatest number of raters.

Impact Rank Criteria:

The evaluation team agreed upon a set of rating criteria and rank characteristics prior to their independent rank assignments. These criteria were:

Dilution:

the relative size of any known point sources and estimated non-point source impacts in relation to the available flows (at low flow periods) in the waterbody. In some cases actual 7Q10 flow values and discharge volumes were known so minimum flow dilution ratios were available. In many cases loading was estimated from a direct knowledge of point source volumes and an estimation of the relative size of the receiving watershed. Non-point source loadings from untreated domestic waste was evaluated from available knowledge about the number of residences contributing sewage to the waterbody. Agricultural impacts were evaluated from first hand knowledge or mapped inference of land use patterns in the watershed.

Nature of the Pollutants:

Higher ranks were assigned to sites receiving more toxic and more persistent materials. This outcome could be moderated by very high dilution ratios. In general, sites heavily loaded by metals and organic chemicals received the highest rank (4). Organic solids and nutrient enrichment into streams with inadequate dilution tended to yield a rank 3. Rank 2 was frequently assigned to sites with agriculture or urbanization in the watershed, or with suspected loading with inorganic solids from erosion. Rank 1 was primarily reserved for locations that had largely undeveloped and/or forested watersheds.

Dissolved Oxygen:

Adequacy of levels of dissolved oxygen to support indigenous fish species and to attain water quality classification standards. Dissolved oxygen was considered to be a significant limiting factor if levels were known to fall below 5 ppm (minimum Class C standards), or if water quality modeling indicated that the site was in the vicinity of a major D.O. sag from a point source.

Episodic Events:

Evaluation of the frequency and severity of license violations and spills from licensed point sources, as well as any other intermittent events known to cause water quality impairment.

Cumulative impacts:

Evaluation of the occurrence of pollution sources in series that contribute to the loading of a given reach. For example, it was decided that in most cases, unless there was a very significant input of high quality water from a tributary, or there was an impoundment that would serve to remove solids loadings, a downstream reach of a waterbody should not be assigned a higher quality rank that an upstream reach, due to cumulative effects. Also, a reach downstream of a relatively benign discharge had to be assigned a rank reflective of any more detrimental conditions that might exist upstream.

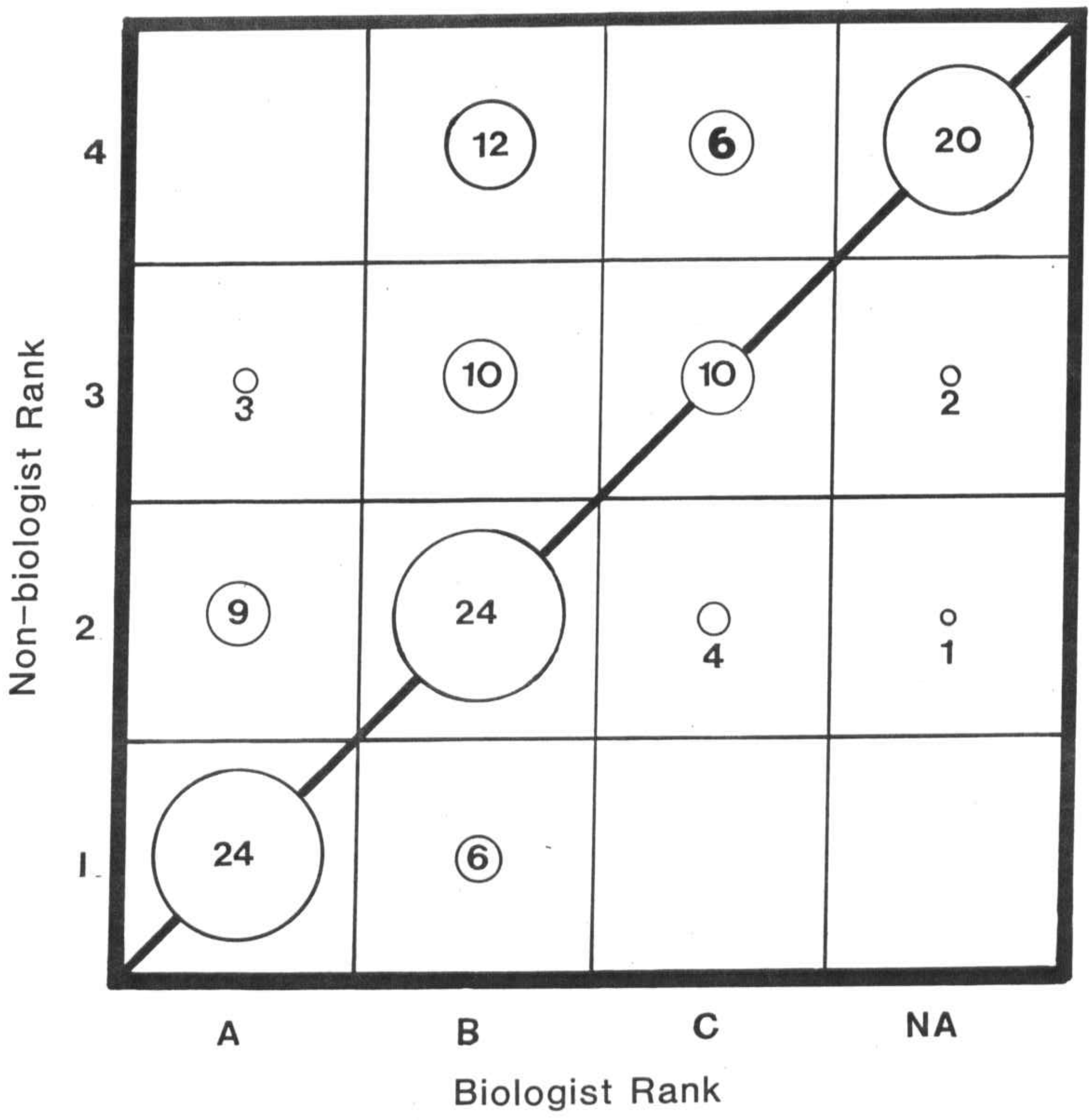
Descriptions of Ranks:

RANK 1: Very low impact conditions; no known significant sources of water quality impairment, or, flow volume sufficient to ameliorate any sources that do exist.

RANK 2: Waterbody has plenty of assimilative capacity remaining; there is adequate dilution to accommodate all existing sources without a significant, measurable lowering of ambient water quality.

RANK 3: Some assimilative capacity remaining; dilution in general is sufficient to maintain water quality, however, there may at times be measurable lowering of ambient water quality due to episodic changes in dilution ratios (extreme low flow conditions in the waterbody, license violations, or spills, etc.); or the pollutants are known to be toxic and/or persistent.

RANK 4: There is no assimilative capacity remaining; the inputs are known to have had a significant, measurable, detrimental impact on water quality; discharges contain one or more constituents or characteristics in an amount that cannot be sufficiently diluted by receiving water flows to prevent significant water quality impacts.


4
3
2
1
Non-biologist Rank
12
6
20
3
10
10
2
9
24
4
1
24
6
A
B
C
NA
Biologist Rank

PROCESS AND CRITERIA

FOR THE ASSIGNMENT OF BIOLOGIST'S CLASSIFICATION

Raters:

David Courtemanch
MS in aquatic entomology; PhD in Environmental Science; employed as a Biologist with the Department of Environmental Protection for 19 years; currently Director of Division of Environmental Assessment (DEA).

Susan Davies
MS aquatic entomology; employed as a Biologist in the River and Stream section of DEA for 11 years, coordinating the Instream Biological Monitoring Program.

Leon Tsomides
MS aquatic entomology; employed as a Biologist in the River and Stream Section of DEA for 6 years, working with the Instream Biological Monitoring Program.

Ranking Process:

Each biologist independently reviewed biological information for each sampling event, as listed below, including identities and abundances of taxa occurring in the biological sample and computed index values for the biological data (e.g. diversity, richness, EPT, etc). Physical habitat information was also reviewed including water depth, velocity, substrate composition, canopy cover, etc., in order to evaluate the effects of various habitat conditions on the structure of the macroinvertebrate community. Sample information was reviewed for the values of the given measures, relative to values for other samples in the data set. The actual classification assignment was determined by how closely the biological information conformed to the aquatic life classification standards, correcting for habitat effects, and according to assessment guidelines provided by the list of Ecological Attributes associated with each Class (see Table 3 Ecological Attributes and Aquatic Life Standards). Numerical ranges, per se, were not established, a priori, for each measure. Instead, the information was reviewed for it's compatibility with the mosaic of findings expected for each Class, listed in Table 4, Relative Findings Chart. The biologists did not have any knowledge of the actual location of the sampled sites, nor did they have knowledge of any pollutional influences. Following the independent assignment of classes the biologists established a concensus classification, following an open exchange of justifications for each biologist's assignment.

Biologist's Classification Criteria:

Each biologist reviewed the sample data for the values of a list of measures of community structure and function. Criteria used by biologists to evaluate each measure are listed in Table 4.

TOTAL ABUNDANCE OF INDIVIDUALS
TOTAL ABUNDANCE OF EPHEMEROPTERA
TOTAL ABUNDANCE OF PLECOPTERA
ABUNDANCE OF EPHEMEROPTERA/TOTAL ABUNDANCE
ABUNDANCE OF PLECOPTERA/TOTAL ABUNDANCE
ABUNDANCE OF HYDROPSYCHIDAE/TOTAL ABUNDANCE
ABUNDANCE OF EPHEMEROPTERA+PLECOPTERA/TOTAL ABUNDANCE
ABUNDANCE OF GLOSSOSOMA/TOTAL ABUNDANCE
ABUNDANCE OF BRACHYCENTRUS/TOTAL ABUNDANCE
ABUNDANCE OF OLIGOCHAETES/TOTAL ABUNDANCE
ABUNDANCE OF HIRUDINEA/TOTAL ABUNDANCE
ABUNDANCE OF GASTROPODA/TOTAL ABUNDANCE
ABUNDANCE OF CHIRONOMIDAE/TOTAL ABUNDANCE
ABUNDANCE CONCHAPELOPIA+THIENNEMANNYMIA/TOTAL ABUNDANCE
ABUNDANCE OF TRIBELOS/TOTAL ABUNDANCE
ABUNDANCE OF CHIRONOMUS/TOTAL ABUNDANCE
GENERIC RICHNESS
EPHEMEROPTERA RICHNESS
PLECOPTERA RICHNESS
EPT RICHNESS
EPHEMEROPTERA RICHNESS/GENERIC RICHNESS
PLECOPTERA RICHNESS/GENERIC RICHNESS
DIPTERA RICHNESS/GENERIC RICHNESS
EPHEMEROPTERA+PLECOPTERA RICHNESS/GENERIC RICHNESS
EPT RICHNESS/DIPTERA RICHNESS
NON-EPT OR CHIRONOMIDAE RICHNESS/GENERIC RICHNESS
PERCENT PREDATORS
% COLLECTOR FILTERERS+GATHERERS/%PREDATORS+SHREDDERS
NUMBER OF FUNCTIONAL FEEDING GROUPS REPRESENTED
SHANNON-WEINER GENERIC DIVERSITY
HILSENHOFF BIOTIC INDEX

In addition, in cases where a valid, clean-water, upstream reference station existed, the following comparative index data was also reviewed:

JACCARD TAXONOMIC SIMILARITY
TAXONOMIC SIMILARITY OF DOMINANT TAXA
COEFFICIENT OF COMMUNITY LOSS
PERCENT SIMILARITY

RESULTS

In 64% of the cases there was unanimous agreement among the independent raters, and in an additional 34% of the samples two of the raters were in agreement and one had assigned a different classification. In 3 of the rated samples there was disagreement among all three raters.

RELATIVE FINDINGS CHART

MEASURE OF COMMUNITY STRUCTURE	RELATIVE FINDINGS			
	A	B	C	NA
Total Abundance of Individuals	often low	often high	variable	variable - often very low or high
Abundance of Ephemeroptera	high	high	low	low to absent
Abundance of Plecoptera	highest	some present	low to absent	absent
Proportion of Ephemeroptera	highest	variable depending on dominance by other groups	low	zero
Proportion of Plecoptera	highest	variable depending on dominance by other groups	low	zero
Proportion of Hydropsychidae	intermediate	highest	variable	low to high
Proportion of Ephemeroptera & Plecoptera	highest	variable	low	absent
Proportion of Glossosoma	highest	low to intermediate	very low to absent	absent
Proportion of Brachycentrus	highest	low to intermediate	very low or absent	absent
Proportion of Oligochaetes	low	low	low to moderate	highest
Proportion of Hirudinea	low	variable	variable	variable to highest

	A	B	C	NA
Proportion of Gastropoda	low	low	variable	variable to highest
Proportion of Chironomidae	lowest	variable depending on dominance of other groups	highest	variable
Proportion of Conchapelopia & Thienemannimyia	lowest	low to variable	variable	variable to highest
Proportion of Tribelos	low to absent	low to absent	low to variable	variable to highest
Proportion of Chironomus	low to absent	low to absent	low to variable	variable to highest
Generic Richness	variable	highest	variable	lowest
Ephemeroptera Richness	highest	high	low	very low to absent
Plecoptera Richness	highest	variable	low to absent	absent
EPT Richness	high	highest	variable	low
Proportion Ephemeroptera Richness	highest	high	low	low to zero
Proportion Plecoptera Richness	highest	variable	low	zero
Proportion Diptera Richness	low to variable	variable	highest	high to

	A	B	C	NA
				variable
Proportion Ephemeroptera & Plecoptera Richness	highest	high	low to variable	low to absent
EPT Richness divided by Diptera Richness	high	highest	low to variable	lowest to zero
Proportion Non-EPT or Chironomic Richness	high	high	low	lowest
Percent Predators	low	low	high to variable	highest
Percent Collector Filterers & Gatherers divided by Percent Predators & Shredders	high	highest	low	lowest
Number of Functional Feeding Groups Represented	variable	highest	variable	lowest
Shannon-Weiner Generic Diversity	low to intermediate	highest	variable to intermediate	lowest
Hilsenhoff Biotic Index	lowest	low	intermediate	highest

COMPARISON OF BIOLOGIST'S CLASSIFICATION AND POLLUTION IMPACT RANK ASSIGNMENT

BioClass = A — Total of 36 Stations

*Pollution Rank = 1 — 24 Stations (67%)

Pollution Rank = 2 — 9 Stations

- Expected Non Point Source (NPS) problems — 7
- Enrichment or BOD — 2

Pollution Rank = 3 — 3 Stations

- Expected enrichment from inadequate treatment 3

Pollution Rank = 4 — 0 Stations

BioClass = B — Total of 52 Stations

Pollution Rank = 1 — 6 Stations

- Expected NPS — 4
- Atypical Habitat — 2

*Pollution Rank = 2 — 24 Stations (46%)

Pollution Rank = 3 — 10 Stations

- Enrichment or BOD — 6
- Chlorine — 2
- Low dissolved oxygen — 1
- Siltation — 1

Pollution Rank = 4 — 12 Stations

- Combined Toxic & Conventional — 10
- Low dissolved oxygen — 2

BioClass = C — Total of 12 Stations

Pollution Rank = 1 — 0 Stations

Pollution Rank = 2 — 4 Stations

- Probable industrial NPS — 2
- Atypical Habitat — 2

*Pollution Rank = 3	10 Stations (50%)
Pollution Rank = 4	6 Stations
Low dissolved oxygen	4
Toxic or combined	3

BioClass = Non-Attainment	Total of 23 Stations
Pollution Rank = 1	0 Stations
Pollution Rank = 2	1 Station
Atypical Habitat	1
Pollution Rank = 3	2 Stations
Cumulative impacts	2
*Pollution Rank = 4	20 Stations (87%)

* Assignment of stations in agreement

CMPRSN

Summary of Non-DEP Biologists

Percent Concurrence

with

DEP Rankings of Sites

	Biologist 1 (n=40)		Biologist 2(n=40)
	A(10)	80%	90%
	B(10)	60%	80%
	C(10)	90%	90%
	NA(10)	100%	100%
Total:		83%(33/40)	90%(36/40)

MAINE DEPARTMENT OF ENVIRONMENTAL PROTECTION
BIOLOGICAL MONITORING PROGRAM TECHNICAL REVIEW COMMITTEE

Committee Members

DAVID DOMINIE
Central Maine Power Company
Corporate Offices
Edison Drive
Augusta. Maine 04330
(207)623-3521

BRUCE GRANTHAM
LOTIC, Inc.
PO Box 279
Connor Mill Office Park
Unity, Maine 04988
(207)948-3062

PAUL LEEPER
Eco-Analysts
PO Box 224
Bath, Maine 04530
(207)443-2629

ROBERT NUZZO
Massachusetts DEP/DWPC
Technical Services
P.O. Box 116
Grafton, Massachusetts 01536
(508)792-7470

JOAN TRIAL, PhD
Maine Department of Inland Fisheries and Wildlife
PO Box 1298
Bangor, Maine 04401
(207)941-4457

LESLIE WATLING, PhD
The Darling Center for Marine Studies
25 Clarks Cove Road
Walpole, Maine 04573
(207)563-3146

DAVID WEFRING
(formerly of:
International Paper Company
6400 Poplar Ave.
Memphis, Tennessee 38119)

Attenders

DENNIS BORTEN, PhD
National Council of the Paper Industry
for Air and Stream Improvement
PO Box 2868
New Bern, North Carolina 28561
(919)637-4326

PETER WASHBURN
Natural Resources Council of Maine
271 State Street
Augusta, Maine 04330
(207)622-3101

Staff

Susan Davies, Leon Tsomides, David Courtemanch
Maine Department of Environmental Protection
State House Sta. No. 17
Augusta, Maine 04333
(207)289-3901

Francis Drummond, PhD
Department of Entomology
Deering Hall
University of Maine
Orono, Maine 04469
(207)581-2989

Percent concurrence between biologist assigned and model predicted classification in the Jacknife Procedure.

FIRST STAGE LINEAR DISCRIMINANT MODEL

Model Predicted Class

Assigned	A	B	C	NA
A	79%	21%	0%	0%
B	11%	77%	11%	0%
C	0%	29%	67%	4%
NA	0%	0%	16%	84%

SECOND STAGE LINEAR DISCRIMINANT MODELS

C OR BETTER MODEL

Model Predicted

Assigned	A,B,C	NA
A,B,C	99%	1%
NA	12%	88%

B OR BETTER MODEL

Model Predicted

Assigned	A,B	C,NA
A,B	92%	8%
C,NA	4%	96%

CLASS A MODEL

Model Predicted

Assigned	A	B,C,NA
A	93%	7%
B,C,NA	7%	93%

Professional Judgement

(a) For Evaluation of Test Samples of Organisms which Conform to criteria provided in Ch.530 Sec. 2 D 2 Sampling Procedures, and Sec. 12 B 1 Minimum Provisions and are thus suitable to be run through the linear discriminant models.

Where there is documented evidence of conditions which could result in uncharacteristic findings, allowances may be made to account for those situations. The Department can make adjustments based on analytical, biological, and habitat information and may require additional monitoring of affected waters.

Professional Judgement may be utilized when conditions atypical to the derivation of the decision criteria as provided in Ch. 530 Sec. 2 D 2 are found. Examples of specific conditions which may allow adjustments are:

Habitat Factors
1. Lake outlets
2. Substrate character
3. Tidal movement

Sampling Factors
1. Disturbed samples
2. Unusual taxa assemblages
3. Human error in sampling

Analytical Factors
1. Subsample Vs whole sample analysis
2. Human error in processing

It shall be the responsibility of the Department to decide if adjustments of a decision should occur. The following adjustments may be made to correct for these conditions:

1. Resample
The Department may require that additional monitoring of the Test Community of the affected waters be done following a decision that specific sampling factors may have influenced the results.

2. Raise The Finding
A. The Department may raise the decision of the model from nonattainment to indeterminate or attainment based on documented evidence of specific conditions, as defined above.
B. The Department may raise the decision of the model from one class of attainment to a higher class of attainment based on documented evidence of specific conditions, as defined above.

3. Lower the Finding
The Department may lower the decision of the model to indeterminate or to the next lower class of attainment based on documented, substantive evidence that the narrative aquatic life criteria for the assigned class are not met.

4. Determination of Non-Attainment: Minimum Provisions not met
Samples having any of the Ecological Attributes described in subsection B 1 of this rule, Minimum Provisions, for which there is no evidence of conditions which could result in uncharacteristic findings, as defined above, shall be determined to be in nonattainment of the minimum provisions of Class C aquatic life criteria.

(b). For the evaluation of samples which do not fit the provisions of Ch. 530 Sec 2 D 2, and are therefore not suitable to be run through the linear discriminant models.

For samples collected for the purpose of Classification Attainment Evaluation, which do not conform to the provisions of Ch. 530 Section 2 D; or for samples collected for purposes of Aquatic Life Impact Evaluation, or Pre-Impact Baseline Evaluation which do not conform to Ch. 530 Section 2 D the Department shall make an assessment of classification attainment or aquatic life impact in accordance with the following procedures:

1. A quantitative sampling and data analysis plan shall be developed in accordance with methods established in the literature on water pollution biology;
2. Sampling methods shall be determined on a site specific basis, based on habitat conditions of the sampling site, and the season sampled;
   a) soft-bottomed substrates shall, whenever ecologically appropriate and practical, be sampled by core or dredge of known dimension or volume;
   b) the preferred method for sampling hard-bottomed substrates shall be the rock basket/cone as described in <u>Methods for Biological Sampling and Analysis of Maine's Waters.</u>
   c) Other methods may be used where ecologically appropriate and practical.
3. Classification Attainment decisions shall be based on a determination of the degree to which the sampled site conforms to the narrative aquatic life classification criteria provided in MRSA Title 38 Article 4-A Sec. 465 . The decision shall be based on established principles of water pollution biology and shall be fully documented.
4. Site Specific Impact decisions may rely on established methods of analysis of comparative data between a Test Sample of Organisms and an approved Reference Sample of Organisms.
5. A determination of detrimental impact to aquatic life of a Test Community without an approved, matched Reference Community can be made if it can be documented, based on established methods of the interpretation of macroinvertebrate data, and based on established principles of water pollution biology, that the community fails to demonstrate the Ecological Attributes of its designated class as defined by the narrative standards in MRSA Title 38 Article 4-A Sec. 465.

PEER REVIEW BY THE SCIENTIFIC COMMUNITY

Courtemanch, D.L. 1984. A closing artificial substrate device for sampling benthic macroinvertebrates in deep rivers. Freshwater Invert. Biol. 3:143-146.

Courtemanch, D.L. 1987. Can benthic organisms direct our water quality programs? Presentation at North American Benthological Society annual meeting, Orono, Maine, June 2-5, 1987.

Courtemanch, D.L. 1987. Maine's biological water quality standards. Presentation at Northeast Regional meeting, National Council of the Paper Industry for Air and Stream Improvement. November 8, 1987, Boston, MA.

Courtemanch, D.L. 1989. Implementation of biocriteria in the water quality standards program. In: Water Quality Standards for the 21st Century, U.S. Environmental Protection Agency, Washington D.C. p.135-138.

Courtemanch, D.L. 1989. The development of biocriteria in a water quality program. Executive Enterprises, Inc. Boston, MA, June 16, 1989.

Courtemanch, D.L. 1991. Final biocriteria for Maine. Presentation to New England Association of Environmental Biologists annual meeting, Fairlee, Vermont, March 20-22, 1991.

Courtemanch, D.L. 1993. Maine's proposed biological criteria. Presentation at Northeast Regional meeting, National Council of the Paper Industry for Air and Stream Improvement. November 9, 1993, South Portland, Maine.

Courtemanch, D.L. and S.P. Davies. 1987. A coefficient of community loss to assess detrimental change in aquatic communities. Water Research 21:217-222.

Courtemanch, D.L. and S.P. Davies. 1988. Implementation of biological standards and criteria in Maine's water classification law. In: Proceedings of the First National Workshop on Biological Criteria, Lincolnwood, IL, December 2-4, 1987 p4-9.

Courtemanch and S.P. Davies. 1988. Biological standards in Maine. National Symposium on Water Quality Assessment, Annapolis, MD, June 1-3, 1988.

Courtemanch, D.L., S.P. Davies and E.B. Laverty 1989. Incorporation of biological information in water quality planning. Environmental Management 13: 35-41.

Courtemanch, D.L., S.P. Davies, L. Tsomides and F.A. Drummond. 1991. Establishing biological criteria for assessment of water quality integrity. Presentation at North American Benthological Society annual meeting, Santa Fe, New Mexico, May 21-24, 1991.

Courtemanch, D.L., S.P.Davies, L. Tsomides, F.A. Drummond. 1994. Use of multivariate analysis for establishing biological criteria for water quality classification. Presented by S.P. Davies at North American Benthological Society 42nd Annual Meeting, Orlando, FL. May 24-27, 1994.

Davies, S.P. 1989. Update of Maine's biomonitoring policy development, and discussion of national biocriteria development. Presentation to New England Association of Environmental Biologists annual meeting, Falmouth, Massachusetts, march 1-3, 1989.

Davies, S.P. 1992. Identification and use of indicator taxa in Maine's biological monitoring program. Presentation to New England Association of Environmental Biologists annual meeting, Laconia, New Hampshire, March 4-6, 1992.

Davies, S.P. 1994. Case study: Development and implementation of biological criteria in the State of Maine. Presentation to USEPA Multi-Regional Meeting on Water Quality Standards/Criteria. Nov. 29-Dec. 2, 1994, Seattle, WA.

Davies, S.P. and D.L. Courtemanch. 1988. Use of standards of biological integrity to determine attainment of water quality classification. Presentation at North American Benthological Society annual meeting, Tuscaloosa, Alabama, May 17-20, 1988

Davies, S.P., D.L. Courtemanch and L. Tsomides. 1990. Water quality classification attainment criteria for the State of Maine. Presentation at North American Benthological Society annual meeting, Blacksburg, Virginia, May 22-25, 1990.

Davies, S.P., L. Tsomides and A. Friedman. 1990. Invertebrate data management system. Presentation to New England Association of Environmental Biologists annual meeting, Ogunquit, Maine, April 4-6, 1990

Davies, S.P., L. Tsomides, D.L. Courtemanch and F. Drummond. 1991. Development of biological criteria for use in Maine's water quality classification program. In: Biological Criteria: Research and Regulation EPA-440/5-91-005 Washington D.C. p147-150.

Davies, S.P., L. Tsomides, D.L. Courtemanch and F. Drummond. 1992. Five year analysis of changes in the benthos of three streams of differing water quality in Maine. Presentation at North American Benthological Society annual meeting, Louisville, Kentucky, May 26-29, 1992.

Drummond, F.A., E. Groden, S.P. Davies, L. Tsomides and D.L. Courtemanch. 1990. A statistical model based upon benthic invertebrate rock basket sampling for the discrimination among classes of pollution impacted streams in Maine. Presentation at North American Benthological Society annual meeting, Blacksburg, Virginia, May 22-25, 1990.

Tsomides, L., S.P. Davies, D.L. Courtemanch and F. Drummond. 1993. Biological criteria in Maine's water quality classification program. Poster presentation at New England Association of Environmental Biologists annual meeting, meriden, Connecticut, March 9-12, 1993

Tsomides, L., S.P. Davies and F. Drummond. 1993. Biological criteria in Maine's water quality classification program. Poster presentation at North American Benthological Society annual meeting, Calgary, Alberta, Canada, May 25-28, 1993.

Tsomides, L. 1994. Case studies using stream biocriteria. Presentation to New England Association of Environmental Biologists, March 2-4, 1994, Newport, R.I.

Maine Department of Environmental Protection. 1987. Methods for the biological sampling and analysis of Maine's waters. MDEP, Augusta, Maine. 19p.

USEPA. 1988. Maine's biologically based water quality standards. Water Quality Program Highlights, U.S. Environmental Protection Agency, Washington, D.C.

APPENDIX O

Percent concurrence between biologist assigned and model predicted classification for the test data.

	Model Predicted Class			
Assigned	A	B	C	NA
A(17)	**76%**	24%	0%	0%
B(12)	25%	**50%**	25%	0%
C(12)	0%	0%	**67%**	33%
NA(5)	0%	0%	0%	**100%**

APPENDIX O-1

Misclassification of Test Site Samples by Linear Discriminant Models: Class A sites predicted as Class B.

	Biologist Independent Rankings	Consensus	Model Prediction
Site			
1	B A- A	A	B(.451 A)
2	B B+ A	A	B
3	B B+ A	A	B
4	A- A A-	A	B(.326 A)

Class B sites predicted as Class A.

	Biologist Independent Rankings	Consensus	Model Prediction
Site			
1	B B A-	B	A
2	B++ A B+	B	A
3	A B B	B	A

Class B sites predicted as Class C.

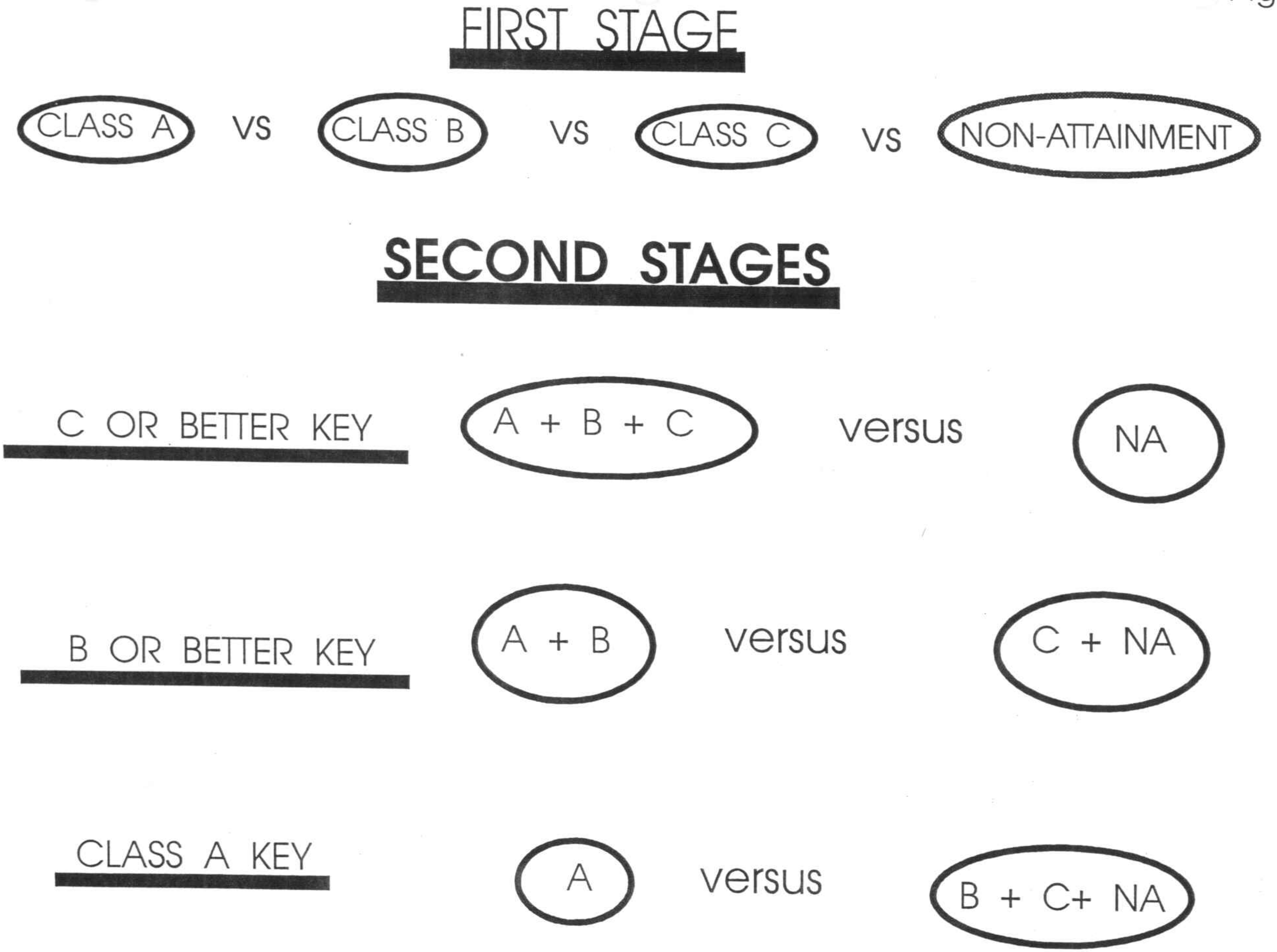
	Biologist Independent Rankings	Consensus	Model Prediction
Site			
1	B- B A	B	C(.25 B)
2	C B-/C+ B-	B	C
3	B C B	B	C

Class C sites predicted as Class NA.

	Biologist Independent Rankings	Consensus	Model Prediction
Site			
*1	C C C	C	NA
2	C NA C-	C	NA
3	C+ C NA	C	NA
*4	C C C	C	NA

*Site 1 and 4 are the same site sampled in different years.

Fig.1


FIRST STAGE
CLASS A
VS
CLASS B
VS
CLASS C
VS
NON-ATTAINMENT
SECOND STAGES
C OR BETTER KEY
A + B + C
versus
NA
B OR BETTER KEY
A + B
versus
C + NA
CLASS A KEY
A
versus
B + C+ NA