Methods for Biological Sampling and Analysis of Maine's Rivers and Streams

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MAINE DEPARTMENT OF ENVIRONMENTAL PROTECTION

METHODS

FOR

BIOLOGICAL SAMPLING AND ANALYSIS OF

MAINE'S RIVERS AND STREAMS

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FOREWORD

This manual describes the field, laboratory and data preparation methods required by the Maine Department of Environmental Protection to collect and analyze benthic macroinvertebrate samples for the River and Stream Biological Monitoring Program. The biological classification of Maine's inland waters was authorized by the Maine State Legislature with the passage of Public Law 1985 Chapter 698 - The Classification System for Maine Waters. This law states that it is the State's objective "to restore and maintain the chemical, physical and biological integrity" of its waters, and establishes a water quality classification system to enable the State to manage its waters so as to protect their quality. The classification system further establishes minimum standards for each class, which are based on designated uses, and related characteristics of those uses, for each class of water.

Each water quality class contains standards that, among other things, describe the minimum condition of the aquatic life necessary to attain that class. The Maine Department of Environmental Protection (the Department) has developed numeric criteria in support of the narrative aquatic life standards in the Water Quality Classification Law. The Department has collected a large, standardized database consisting of benthic macroinvertebrate samples from above and below all significant licensed discharges in the State, from areas impacted by non-point sources, as well as from relatively unperturbed areas. These sampling locations were chosen to represent the range of water quality conditions in the State. This information has been used to develop numeric criteria which are specific to the natural biotic community potential of the State of Maine (see Davies et al., 1995 and 1999 for a description of the development and application of numeric criteria) and is established in DEP regulation Chapter 579 : Classification Attainment Evaluation Using Biological Criteria for Rivers and Streams.

Standardization of data collection and analytical methods is fundamental to the consistent, unbiased and scientifically sound evaluation of aquatic life impacts. This manual sets forth the standardized practices and procedures used by the Department to acquire or accept benthic macroinvertebrate data for use in regulation, assessment or program development.

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I GENERAL METHODS FOR RIVER AND STREAM AQUATIC LIFE CLASSIFICATION ATTAINMENT EVALUATION

Each water quality class is defined by standards that describe the minimum condition of the aquatic community necessary to attain that class. The benthic macroinvertebrate community is used as an indicator community of the general state of the aquatic life in flowing waters for the purpose of assessment of classification attainment. Standardized sampling techniques and sample analysis are required for assessment of biological attainment of stream water quality classification. This manual presents the standard practices and procedures that have been adopted by the Department to acquire benthic macroinvertebrate data for purposes of aquatic life classification attainment evaluation.

Purpose:

To determine the water quality class attained by a particular river or stream reach in terms of the aquatic life standards set forth in 38 MRSA Sec. 465 (The Classification System for Maine Waters).

Requirements:

All samples of aquatic life that are collected for purposes of classification attainment evaluation, whether collected by the Department or by any party required to make collections by the Department, must be collected, processed and identified in conformance with the standardized methods outlined in this manual. Selection of appropriate sampling sites and micro-habitat to sample, as well as procedures for quantitative analysis of the sample must conform to methods set forth in this manual. Data submitted by any party required to make collections by the Department must be accompanied by a Quality Assurance Plan, approved by the Commissioner.

1. Qualifications of Sampling Personnel

Biological sampling must be performed by a professional aquatic biologist or by qualified personnel under the supervision of a professional aquatic biologist. The professional aquatic biologist must have, as a minimum, a Bachelor of Science degree in biological sciences with aquatic entomology, invertebrate zoology, fisheries or closely related specialization, and greater than 6 months experience working with macroinvertebrate sampling methods and taxonomy. (See also Qualifications of Laboratory Personnel, Sec. II-1.)

2. Apparatus, Equipment, Supplies, Instruments

(1) Sampling devices

a) Rock-filled wire basket introduced substrate

Use: flowing wadeable, eroded, mineral-based bottom rivers and streams.

Description: cylindrical plastic coated or chrome wire, baskets with at least 1.5 cm spaces between wires, a hinged opening, and secure closure (Klemm, D.J. et al, 1990).

Substrate material: clean, washed, bank-run cobble, graded to uniform diameter range of 3.8 to 7.6 cm (1.5 to 3 inches) in size (#2 roofing stone).

Baskets must be filled to 7.25 +/- 0.5 kg (16 lbs +/-1 lb) of substrate material.

b) Rock-filled mesh bag introduced substrate

Use: small flowing streams, too shallow for rock baskets to be fully submerged.

Description: mesh bags of sufficient size to hold 7.25 +/- 0.5 kg of cobble substrate as described above, with at least 2.54 cm aperture mesh, and secure closures.

c) Closing introduced substrate cone

Use: deep, non-wadeable rivers having sufficient flow to have an eroded, mineral based bottom.

Description: cone shaped wire, or plastic coated wire basket filled with substrate material and closed by means of an inverted, weighted funnel (Courtemanch, 1984).

Substrate material: (see above Rock-filled wire basket substrate material).

(2) Sieves, sieve buckets, nets

Samples are concentrated on sieves having a mesh size between 500 - 600 microns (USA Standard Testing Sieve ASTM-E-11 Specification size No. 30 or No. 35).

(3) Optical equipment

a) Binocular microscope: Magnification range from 10x or less to 30x or greater.

b) Compound microscope: Magnification range from 10x to at least 400x; 100x with oil immersion lens is advisable.

3. Sampling Season, Sampler Exposure Period, Placement and Retrieval

(1) Sampling season

The standard sampling season upon which all macroinvertebrate classification criteria are based is the late summer, low flow period (July 1 to September 30). All baseline data for the biological classification program has been collected during this time period. This period often presents conditions of maximal stress to the biological community due to decreased dilution of pollutional material and increased stream water temperatures. Furthermore, because the composition of the benthic macroinvertebrate community changes with season, due to natural life history features, this period defines a standardized seasonal community.

As noted, the Department's linear discriminant models define biological classification criteria derived from a macroinvertebrate community defined by the specific sampling methods and index season under which they were collected. Samples collected at other times of year may yield valuable water quality related information, however classification attainment may not be assigned solely on the basis of results of the linear discriminant models for these non-standard samples.

(2) Exposure period

Standard methods require that substrate samplers be exposed in the water body for a period of 28 days +/- four days within the above-specified sampling season. However, extended exposure periods may be necessary to allow for adequate colonization in the case of assessments of low velocity or impounded habitats. If such conditions exist a 56 days +/- four days exposure period may be used.

(3) Sampler placement

Rock Baskets/Bags

The actual sampler location should be approached so as to avoid any disturbance in, or upstream of, the sampled site. Position baskets in locations of similar habitat characteristics. Orient baskets with the long axis parallel to stream flow. Provide for relocation of baskets by flagging trees in the vicinity and/or by drawing a diagram with appropriate landmarks indicated.

Cones

Cone samplers should be marked with individual marker buoys (milk jugs or other suitable float) leaving about 5 extra feet of line to allow for water level changes and to provide for easy retrieval. They should be placed on the substrate with a minimum of disturbance, in an apex-up position, and located in the approximate middle fifty percent of the channel. (Note however, care should be taken not to create an obstruction to boat traffic.) In areas subject to vandalism, or in rivers having extensive macrophyte beds, it may be necessary to attach the sampler lines to a common anchor and thence to one unobtrusive surface float. Retrieval funnels will not properly close when lines are fouled with drifting macrophytes.

(4) Sampler retrieval

Rock Baskets/ Bags

Baskets are approached from downstream. Excessive accumulations of macrophytes, algae or debris clinging to the outside of the basket should be carefully removed, taking care to avoid jarring the basket itself. An aquatic net or drift net (mesh size 500 - 600 microns) is positioned against the substrate immediately downstream of the basket which is then quickly lifted into the net. The contents of the basket and all net washings are emptied into a sieve bucket (500 - 600 microns); the basket wires are carefully cleaned first, then rocks are hand washed and inspected and returned to the basket. All sieve bucket contents are placed in sample jars. A small amount of stream water and 95% ethyl alcohol is added to yield an approximately 70% solution of alcohol. Especially dense samples should be re-preserved in the laboratory, with fresh 70% ethyl alcohol. Rock baskets should be thoroughly cleaned and allowed to desiccate prior to re-use.

Cones

Cone samplers should be retrieved with the boat anchored directly upstream of the samplers. Once the float is retrieved and removed, the line should be held as vertically as possible while the weighted funnel is released down the line to enclose the cone. Cone and funnel should be retrieved quickly and smoothly from the bottom, and released directly into a sieve bucket or tub. Field processing should then proceed as described above for rock baskets.

4. Site Selection Criteria

Classification criteria apply to a strictly defined sample of the benthic macroinvertebrate community. Habitat type from which the community is obtained is a significant determinant of the make-up of the target community. Benthic macroinvertebrate communities of flowing streams and rivers having a hard, eroded substrate comprise the majority of samples in the baseline data set. This habitat is characteristic of the majority of the river and stream waters of the State. Exceptions to these conditions may require special consideration and the exercise of professional judgment. (Note: See Section III-2. (3) "Classification attainment evaluation of waters subjected to flow regulation" page 13, for procedures relating to the assessment of regulated flow sites.) While it is useful to obtain both an upstream and downstream sample to evaluate the effect of a pollution source, classification attainment evaluation does not require data from a matched reference site in order to arrive at a determination of aquatic life class. Analytical methods for classification attainment evaluation are described in Section III.

(1) Site attributes

a) The area selected should be generally representative of the habitat of the stream reach as a whole;
b) Where there is alternating riffle/pool habitat, the riffle/run is the habitat of choice;
c) A location should be selected where there is a high degree of certainty that the rock basket samples will remain fully submerged even if the water level drops significantly.

(2) Precautions

a) Avoid atypical influences such as bridges, entering culverts, channelized areas such as road crossings, culverts, or obstructions to flow;
b) Avoid bank effects: samplers should be located in the middle 50% of the bank to bank width, or in an area with a flow regime typical of the overall character of the stream segment;
c) Avoid slackwater areas and eddies immediately upstream or downstream of large rocks or debris.

(3) Matching reference and effluent impacted sites

If possible both stream reaches should be viewed prior to selection of sampling sites. Efforts should be made to sample habitats which are comparable in the following characteristics:

a) Water velocity;
b) Substrate composition (i.e., size ranges and proportions of particles making up the substrate);
c) Canopy coverage;
d) Depth;
e) Other upstream influences except the pollution source in question (for example, use caution when one site is just below a lake outfall and the other is not).

(4) Factors to be considered in site selection below point sources

The area of initial dilution of an effluent should be determined by visual observation of the plume pattern; by observations of biotic effects attributable to the plume, if evident (periphyton growth, die-off patterns); and by transects of specific conductance measurements from the outfall, in a downstream direction. The site selected should be in an area where reasonable opportunity for mixing of the effluent has occurred. If a mixing zone has been defined in a license, sampling should occur immediately downstream of it. In cases where the effluent plume channels down one bank for great distances (>1 km), or where localized effluent impact is expected to be severe for a distance beyond the zone of initial dilution, it is advisable to have a sampling site upstream of the source, one or more in the plume, and at least two farther downstream. One downstream site should be located at the point of presumed bank to bank mixing and subsequent sites should be located to assess the extent of impact downstream.

5. Sample Size

The biological community is evaluated on the basis of benthic macroinvertebrates obtained from at least three samplers which yield an average of at least 50 organisms per sampler. Matched upstream and downstream sites must be sampled using identical methods and level of effort, preferably by the same personnel.

Subsampling may be performed on samples if the mean number of organisms in a sampler exceeds 500 and subsampling will yield at least 100 organisms per rock/cone sampler. All samplers in a site should be treated consistently. Subsampling methods are described in Section II-5. Note: Subsampling will

reduce sample richness by an indeterminate amount. This may affect the outcome of linear discriminant analysis. See Section III-2. (2).

6. Physical Habitat Evaluation

A field data sheet (Appendix A) is to be completed at the time of sampler placement. This form records site specific information concerning natural variables that may affect community structure. Items addressed include exact site location (latitude and longitude, narrative description of the mapped location and/or a topographic map with site indicated); substrate composition; canopy coverage; land use and terrain characteristics; water velocity, temperature, dates of exposure and investigator name. The form is to be completed by observation as well as instrument measurement of water velocity, specific conductance, dissolved oxygen, global positioning device, temperature, etc.

II LABORATORY METHODS

1. Qualifications of Laboratory Personnel

Sample processing and taxonomy in the laboratory must be performed or supervised by a professional freshwater macroinvertebrate taxonomist who is certified by the Society of Freshwater Science in the identification of eastern US taxa. Certification must include Genus level categories, such as Ephemeroptera, Plecoptera and Trichoptera (EPT), General Arthropods and Chironomidae taxa. Taxonomic data will not be accepted without verification that the supervising laboratory taxonomist has been certified in relevant categories.

2. Sample Preservation, Sorting

All sample material collected in the field, as described in Section I, is preserved in 70% ethyl alcohol. Samples are stored in airtight containers until sorted. Sorting of macroinvertebrates from detritus and debris should follow methods described in Appendix B. One out of every ten samples is evaluated by a biologist for sorting completeness.

After sorting, recommended storage for macroinvertebrates is in 70% ethyl alcohol with 5% glycerin, in vials sealed with tightly fitting rubber stoppers.

3. Sample Labeling

All samples are labeled in the field immediately upon collection. The label must include the following information:

Date of sample retrieval
Waterbody
Town or target discharge
Whether above or below the discharge (if applicable)
Replicate number

4. Sample Log Book

In the laboratory, the samples from each sampled site are to be assigned a sample log number, written on all items generated by the sample (e.g., sample vials, slides, records, count sheets, etc.). Log numbers are sequentially recorded in a master log book. The log book shall also contain site identification, date of placement and retrieval, investigator name, sampler type and any comments regarding sampler retrieval or data quality.

5. Subsampling

(1) Methods

If it is determined that a sample should be subsampled (see criteria in Section I-5 Sample Size) methods of Wrona et al, (1982) are followed. These are summarized below:

a) Fit a plastic or glass Imhoff-type settling cone with an aquarium air stone sealed in the bottom and connected to a compressed air supply.

b) Place the sorted macroinvertebrate sample in the cone and fill the apparatus with water to a total volume of one liter.

c) Agitate gently for 2 to 5 minutes with the air stone.

d) Remove 25% of the sample in 5 aliquots with a wide-mouth 50 ml dipper and combine into one sample vial. The dipper should be submerged and withdrawn over a five second interval.

e) Ascertain whether or not the required 100 organisms have been obtained in the subsample.

f) Indicate clearly on the sample label and on the data sheet the fraction of the sample that the subsample represents.

(2) Precautions

a) Especially large or dense organisms such as crayfish, molluscs or caddisflies with stone cases, which do not suspend randomly in the sample, should not be included in the subsample. They should be counted separately.

b) When removing aliquots, the subsampler should be careful to avoid biased capture of organisms in the cone. Avoid watching the cone as the dipper is withdrawn.

This method has been tested by the Department and has been found to randomly distribute the sample. The five separate counts conform to a Poisson series and thus can be combined into one sample (Elliott, 1979).

(3) Chironomidae subsampling

A subsampling plan for Chironomidae shall be approved by the Department. A Department recommended subsampling plan follows the following criteria:

a) For samples having less than 100 midges, all midges will be identified to genus/species level.

b) For samples having 100 to 199 midges, a subsample of one half (0.5) will be removed by randomly selecting the specimens to be identified and identified to genus/species level. Remaining unsampled midges will be examined for unusual or rare specimens, which will be removed and identified to genus/species level separate from the subsample of the sample.

c) For samples having 200 to 499 midges, a subsample of one quarter (0.25) will be removed by randomly selecting the specimens to be identified and identified to genus/species level. Remaining unsampled midges will be examined for unusual or rare specimens, which will be removed and identified to genus/species level separate from the subsample of the sample.

d) For samples having 500 or more midges, midges will be grouped by genus for those for which it is possible to confidently identify them to genus level without mounting. For remaining midges not grouped by genus, a subsample of 100 specimens will be randomly selected and identified to genus/species level. Remaining unsampled midges will be examined for unusual or rare specimens, which will be removed and identified to genus/species level separate from the subsample of the sample.

e) Reporting of the subsample of the sample will be as follows. Numbers reported on the Excel spreadsheet will be converted to reflect the sample total. Any round-off errors between the subsample total and the sample total will be equalized by adding or deducting the difference from the most numerous taxon. If unusual or rare specimens are removed from the sample following the subsample removal, the conversion of the subsample total to a "partial" sample total will be based on the sample total minus the number of unusual or rare specimens. Following this procedure, the number of unusual or rare specimens will be added to the "partial" sample total to bring it back to the sample total.

6. Sample Taxonomy

All taxonomic data submitted to the Department must be accompanied by the name(s) of the individual(s) actually performing the identifications. A list of taxonomic references used, and a reference collection of organisms must also be submitted (see below).

(1) Taxonomic resolution

Macroinvertebrate organisms are identified to genus in all cases where possible. If generic keys are not available or taxonomic expertise is lacking for a taxon it should be identified to the lowest level possible. Identification of organisms to species is highly recommended whenever possible. Although quantitative analysis of benthic macroinvertebrate samples by the Department is based on counts adjusted to the generic level of resolution, species designations are recorded in the Department database and can contribute to the final stage of data analysis, Professional Judgment Evaluation of the model outcome. This is especially important for Class Insecta. Taxonomists submitting data for use by the Department must use current taxonomic references.

(2) Identification of Chironomidae

Specimens of chironomid midges are identified from slide mounts of the cleared head capsule and body parts. Euparol or Berlese mounting medium is recommended for preparation of slides. CMCP-9 is recommended for the preparation of permanent slide mounts of reference material, for voucher specimens or for permanent collections. These slides should be prepared under a fume hood. Instructions for preparation and slide mounting may be found in Wiederholm, (1983). In samples in which a given taxon is represented by a large number of individuals, the identification to genus may be made from slide mounts of a sufficient proportion of the individuals to give a high degree of certainty that they are all the same (10-50% depending on

the distinctiveness of the taxon visible under binocular microscope). A subsampling plan for Chironomidae is described in Section II-5. Each permanent slide mount is to be fully labeled or coded in a manner which positively associates the slide with the sample from which it originated.

(3) Quality control

All organisms and records from any sampling event intended to serve regulatory purposes must be preserved for a period of at least ten years. In the course of identifying taxa collected as part of the Department's biological monitoring program, or in other collection activities, a special reference collection of separate taxa is established. This collection allows subsequent identifications of the same taxon to be confirmed and thus serves to standardize taxonomy for the program.

Each contracted taxonomist, working for the Department or working for anyone submitting data to the Department, will be required to submit a reference collection of taxa identified, as well as a list of the taxonomic references used in the identifications. Organism identifications will be checked against the Department's collection by a Department taxonomist.

III ANALYTICAL METHODS

In general, it is the responsibility of the Department, or its agents, to conduct sampling for the purpose of making decisions on the attainment of water quality classification. Under certain conditions, sampling may be required of applicants for waste discharge licenses, or applicants requiring Section 401 Water Quality Certification. Sampling may be performed by corporations, businesses, organizations or individuals who can demonstrate their qualifications and ability to carry out the Department's sampling and analytical protocol, described in this manual. Such monitoring will be conducted according to a quality assurance plan provided to the Department and approved by the Commissioner.

Classification attainment evaluation is established in DEP regulation Chapter 579: Classification Attainment Evaluation Using Biological Criteria for Rivers and Streams. Davies et al, 1995 details the conceptual and technical basis for the State’s application of linear discriminant analysis to assess attainment of aquatic life standards. A synopsis of Chapter 579 follows in this section.

1. Minimum Provisions

Properly collected and analyzed samples that fail to achieve the following criteria are unsuitable for further analysis through the numeric criteria statistical models:

- Total Mean Abundance must be at least *50* individuals (average per basket/bag/cone);
- Generic Richness for three replicate basket/bag/cone samplers must be at least *15*.

Samples not attaining these criteria shall be evaluated by Professional Judgment. A determination will be made whether the affected community requires re-sampling or whether the community demonstrates non-attainment of minimum provisions of the aquatic life standards.

2. Aquatic Life Statistical Decision Models

The four statistical decision models consist of linear discriminant functions developed to use quantitative ecological attributes of the macroinvertebrate community (Appendix C-1) to determine the strength of the association of a test community to any of the water quality classes (Appendix D). The coefficients or weights are calculated using a linear optimization algorithm to minimize the distance, in multivariate space, between sites within a class, and to maximize the distance between sites between classes.

(1) Linear discriminant models

The discriminant function has the form:

$$Z = C + W_1X_1 + W_2X_2 + ...W_nX_n$$

Where: Z = discriminant score
C = constant
W_i = the coefficients or weights
X_i = the predictor variable values

Association values are computed, using variable values from a test sample, for each classification using one four-way model and three two-way models. The four-way model uses nine variables pertinent to the evaluation of all classes and provides four initial probabilities that a given site attains one of three classes (A, B, or C), or is in non-attainment (NA) of the minimum criteria for any class. These probabilities have a possible range from 0.0 to 1.0, and are used, after transformation, as variables in each of the three subsequent final decision models. The final decision models (the three, two-way models)

are designed to distinguish between a given class and any higher classes as one group and any lower classes as the other group (i.e., Classes A+B+C vs. NA; Classes A+B vs. Class C+NA; Class A vs. Classes B+C+NA). The equations for the final decision models use the predictor variables relevant to the class being tested (Appendix E). The process of determining attainment class using association values is outlined in Appendix F.

(2) Application of professional judgment

Where there is documented evidence of conditions which could result in uncharacteristic findings, allowances may be made to account for those situations by adjusting the classification attainment decision through use of professional judgment as provided in DEP regulation Chapter 579: Classification Attainment Evaluation Using Biological Criteria for Rivers and Streams. The Department may make adjustments to the classification attainment decision based on analytical, biological, and habitat information or may require that additional monitoring of affected waters be conducted prior to issuing a classification attainment decision.

Professional Judgment may be utilized when conditions are found that are atypical to the derivation of the linear discriminant model. Factors that may allow adjustments to the model outcome include but are not limited to:

a) Habitat factors
- Lake outlets
- Impounded waters
- Substrate characteristics
- Tidal waters

b) Sampling factors
- Disturbed samples
- Unusual taxa assemblages
- Human error in sampling

c) Analytical factors
- Subsample vs. whole sample analysis
- Human error in processing

(3) Classification attainment evaluation of waters subjected to flow regulation

The Maine State Legislature, in 38 MRSA Article 4-A Sec. 464 (9)-(10), *The Water Classification Program*, acknowledges that changes to aquatic life and habitat occur as the result of the impoundment of riverine waters and has modified the standards of waters so affected. The habitat and aquatic life criteria of riverine impounded waters of Class A, Class B or Class C are

deemed to be met if the impoundment attains the standards of Class C (e.g., maintenance of structure and function of the resident biological community). Impoundments managed as Great Ponds must also attain Class C aquatic life standards. If the actual water quality attains any more stringent characteristic or criterion than the Class C standards dictate, then the waterbody must be managed so as to protect those higher characteristics. Class C standards also apply to the *downstream* waters below certain specified riverine impoundments on the Kennebec River and the Saco River (Wyman Dam, Moosehead East Outlet Dam, West Buxton Dam and Skelton Dam) that are classified as A or B. All other waters subjected to flow regulation are managed according to standards of the water quality classification assigned by the Legislature.

(4) Adjustments of a decision

It is 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:

a) Resample
The Department may require that additional monitoring of the test community be done before a determination of class attainment can be made, based on documented evidence of specific sampling factors that may have influenced the results.

b) Raise the finding
   i. The Department may raise the classification attainment outcome predicted by the model from non-attainment of any class to indeterminate or to attainment of Class C, based on documented evidence of specific conditions, as defined above.

   ii. The Department may raise the classification attainment outcome predicted by the model from attainment in one class to attainment in the next higher class, based on documented evidence of specific conditions, as defined above.

c) Lower the finding
The Department may decide to lower the classification attainment finding, on the basis of documented, substantive evidence that the narrative aquatic life criteria for the assigned class are not met.

d) Determination of non-attainment: minimum provisions not met
Samples having any of the ecological attributes not attaining the minimum provisions, and where there is no evidence of conditions which could result in uncharacteristic findings, as defined above, must be determined to be in non-attainment of the minimum provisions of the aquatic life criteria for any class.

e) Determination of attainment: minimum provisions not met
Where there is evidence of factors that could result in minimum provisions not being met, professional judgment may be used to make a professional finding of attainment of the aquatic life criteria for any class. Such decisions will be provisional until appropriate resampling is carried out.

(5) Sampling procedures do not conform

For classification attainment evaluation of test communities that do not conform to criteria provided in Section I General Methods, or Section III-1, Minimum Provisions, of this manual, and are therefore not suitable to be run through the linear discriminant models, the Department may make an assessment of classification attainment or aquatic life impact in accordance with the following procedures:

a) Approved assessment plan
A quantitative sampling and data analysis plan must be developed in accordance with methods established in the scientific literature on water pollution biology, and shall be approved by the department.

b) Determination of sampling methods
Sampling methods are determined on a site-specific basis, based on habitat conditions of the sampling site, and the season sampled:

i. Soft-bottomed substrates shall, whenever ecologically appropriate and practical, be sampled by core or dredge of known dimension or volume.

ii. The preferred method for sampling hard-bottomed substrates shall be the rock basket/cone/bag as described in Section I-2.

iii. Other methods may be used where ecologically appropriate and practical.

c) Classification attainment decisions
Classification attainment decisions may be based on a determination of the degree to which the sampled site conforms to the narrative aquatic life classification criteria provided in 38 MRSA Section 465 and found in Appendix D. The decision is based on established principles of water pollution biology and must be fully documented.

d) Site-specific impact decisions
Site-specific impact decisions may rely on established methods of analysis of comparative data between a test community and an approved reference community.

e) Determination of detrimental impact
A determination of detrimental impact to aquatic life of a test community without an approved reference community may 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 aquatic life standards in the water quality classification law.

Appendix A

Maine DEP Biological Monitoring Unit
Stream Macroinvertebrate Field Data Sheet

Log Number _____ Directions_____ Type of Sample_____
Station Number_____ _____ Date Deployed_____
Waterbody_____ _____ Number Deployed_____
River Basin_____ Lat-Long Coordinates (WGS84, meters) Date Retrieved_____
Municipality_____ Latitude_____ Number Retrieved_____
Stream Order_____ Longitude_____ Agency/Collector(s)_____

1. Land Use (500 m radius upstream)
☐ Urban ☐ Upland conifer
☐ Cultivated ☐ Swamp hardwood
☐ Pasture ☐ Swamp conifer
☐ Upland hardwood ☐ Marsh

2. Terrain (500 m radius upstream)
☐ Flat
☐ Rolling
☐ Hilly
☐ Mountains

3. Canopy Cover (upstream view)
☐ Dense (75-100% shaded)
☐ Partly open (25-75% shaded)
☐ Open (0-25% shaded)
(% daily direct sun) _____

4. Physical Characteristics of Bottom (estimate % of each component over 12 m stretch of site; total = 100%)
[ ] Bedrock [ ] Rubble (3" – 10") [ ] Sand (<1/8") [ ] Detritus
[ ] Boulders (<10") [ ] Gravel (1/8" – 3") [ ] Silt-clay-muck

5. Habitat Characteristics (immediate area)

Time _____AM PM	Time _____AM PM
Width (m) _____	Width (m) _____
Depth (cm) _____	Depth (cm) _____
Flow (cm/s) _____	Flow (cm/s) _____
Diss. O_2 (ppm)_____	Diss. O_2 (ppm)_____
Temp (°C) _____	Temp (°C) _____
pH _____	pH _____
SPC (µS/cm) _____	SPC (µS/cm) _____
TDS (ppm) _____	TDS (ppm) _____

Temperature Probe # _____
☐ deployed ☐ retrieved

6. Observations (describe)
Fish_____
Algae_____
Macrophytes_____
Habitat quality_____
Dams/impoundments_____
Discharges_____
Nonpoint stressors _____

7. Water Samples
☐ Standard
☐ Metals
☐ Pesticides

Lab Number

8. Photographs

9. Landmarks of Sampler Placement (illustrate or describe landmarks to be used for relocation)

Appendix B

Instructions for Macroinvertebrate Sorters

1. Pick the sample **in small portions** (1-2 TBS of material) at a time.

2. Pick all organisms you can see. If in doubt it's usually best to include it.

3. Some types of samples can be easily floated by adding a saturated solution of Epsom salt or sugar to the water. Maintain the saturated solution for the lab by adding enough salt or sugar to water to maintain a thick layer of crystals on the bottom of the storage jar. Use the supernatant solution for picking. Large numbers of organisms can be removed with a sieve spoon from the water surface. After the floaters have been removed, proceed to pick the rest of the sample as usual. A significant portion of the sample will not float and must be picked out with forceps.

4. The sample can be considered done when a careful 45 second search, after swirling the sample, yields no further organisms.

5. The samples are picked in water but should not remain unpreserved for more than 8 hours. Be certain that the final sample vial is preserved with 70% alcohol and 5% glycerin solution when done.

6. Return the detrital material to the original sample jar and preserve with 70% alcohol.

7. Write on the sample jar label "Picked X1 (your initials)".

8. Include in the vial of organisms a slip of index card label in hard pencil (No. 2) including **all information appearing on the original jar label**:

   Log Number — River
   Date - month/day/year — Location (Town or industry name)
   whether above or below
   Basket or Cone number
   Vial number if more than 1 vial is needed per basket

   ex. Log 621 Sandy R. 9/5/97
   Below Farmington (disturbed)
   Basket 2 vial #1 of 2

9. Complete all samples from one log number before beginning a new log number.

10. Keep a record of samples picked including log number

    Basket number — Time spent per basket
    Your name — Date

Appendix C-1

Methods for the Calculation of Indices and Measures of Community Structure Used in the Linear Discriminant Models

Variable Number

1 **Total Mean 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:

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

b) A family level identification which includes no more than one taxon identified to the generic level is counted as a separate taxon in generic richness counts.

c) A family level identification with more than one taxon identified to generic level is not counted towards generic richness. Counts are to be divided proportionately among the genera that are present.

d) Higher level taxonomic identifications (Phylum, Class, Order) are not counted toward generic richness unless they are the only representative.

e) Pupae are ignored in all calculations.

3 **Plecoptera Mean Abundance**

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

4 **Ephemeroptera Mean Abundance**

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

5 **Shannon-Wiener Generic Diversity (Shannon and Weaver, 1963)**

After adjusting all counts to genus following counting rules in Variable 2:

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

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, 1987)**

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

where: HBI = Hilsenhoff 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 with tolerance values.

7 **Relative Chironomidae Abundance**

Calculate the mean number of individuals of the family Chironomidae, following counting rules in Variable 4, and divide by total mean abundance (Variable 1).

8 **Relative Diptera Richness**

Count the number of different genera from the Order Diptera, following counting rules in Variable 2, and divide by generic richness (Variable 2).

9 ***Hydropsyche* Mean Abundance**

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

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

Sum of probabilities for Classes A, B, and C from First Stage Model.

11 ***Cheumatopsyche* Mean Abundance**

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

12 **EPT - Diptera Richness Ratio**

EPT Generic Richness (Variable 19) divided by the number of genera from the order Diptera, following counting rules in Variable 2. If the number of genera of Diptera in the sample is 0, a value of 1 is assigned to the denominator.

13 **Relative Oligochaeta Abundance**

Calculate the mean number of individuals from the Order Oligochaeta, following counting rules in Variable 4, and divide by total mean abundance (Variable 1).

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

Sum of probabilities for Classes A and B from First Stage Model.

15 **Perlidae Mean Abundance (Family Functional Group)**

Count all individuals from the family Perlidae (Appendix C-3) in all replicate samplers from one site and divide by the number of replicates to yield mean number of Perlidae per sampler.

16 **Tanypodinae Mean Abundance (Family Functional Group)**

Count all individuals from the subfamily Tanypodinae (Appendix C-3) in all replicate samplers from one site and divide by the number of replicates to yield mean number of Tanypodinae per sampler.

17 **Chironomini Mean Abundance (Family Functional Group)**

Count all individuals from the tribe Chironomini (Appendix C-3) in all replicate samplers from one site and divide by the number of replicates to yield mean number of Chironomini per sampler.

18 **Relative Ephemeroptera Abundance**

Variable 4 divided by Variable 1.

19 **EPT Generic Richness**

Count the number of different genera from the Order Ephemeroptera (E), Plecoptera (P), and Trichoptera (T) in all replicate samplers, according to counting rules in Variable 2, generic richness.

20 **Variable Reserved**

21 **Sum of Mean Abundances of**: ***Dicrotendipes, Micropsectra, Parachironomus* and *Helobdella***

Sum the abundance of the 4 genera and divide by the number of replicates (as performed in Variable 4).

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

Probability of Class A from First Stage Model.

23 **Relative Plecoptera Richness**

Count number of genera of Order Plecoptera, following counting rules in Variable 2, and divide by generic richness (Variable 2).

24 **Variable Reserved**

25 **Sum of Mean Abundances of *Cheumatopsyche, Cricotopus, Tanytarsus* and *Ablabesmyia***

Sum the number of individuals in each genus in all replicate samplers and divide by the number of replicates (as performed in Variable 4).

26 **Sum of Mean Abundances of *Acroneuria* and *Stenonema***

Sum the number of individuals in each genus in all replicate samplers and divide by the number of replicates (as performed in Variable 4).

27 **Variable Reserved**

28 **Ratio of EP Generic Richness**

Count the number of different genera from the order Ephemeroptera (E), and Plecoptera (P) in all replicate samplers, following counting rules in Variable 2, and divide by 14 (maximum expected for Class A).

29 **Variable Reserved**

30 **Ratio of Class A Indicator Taxa**

Count the number of Class A indicator taxa as listed in Appendix C-2 that are present in the community and divide by 7 (total possible number).

Appendix C-2

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)

Appendix C-3

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

Appendix C-3

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
Paralauterborniella
Paratendipes
Phaenopsectra
Polypedilum
Robackia
Stelechomyia
Stenochironomus
Stictochironomus
Tribelos
Xenochironomus

Appendix D

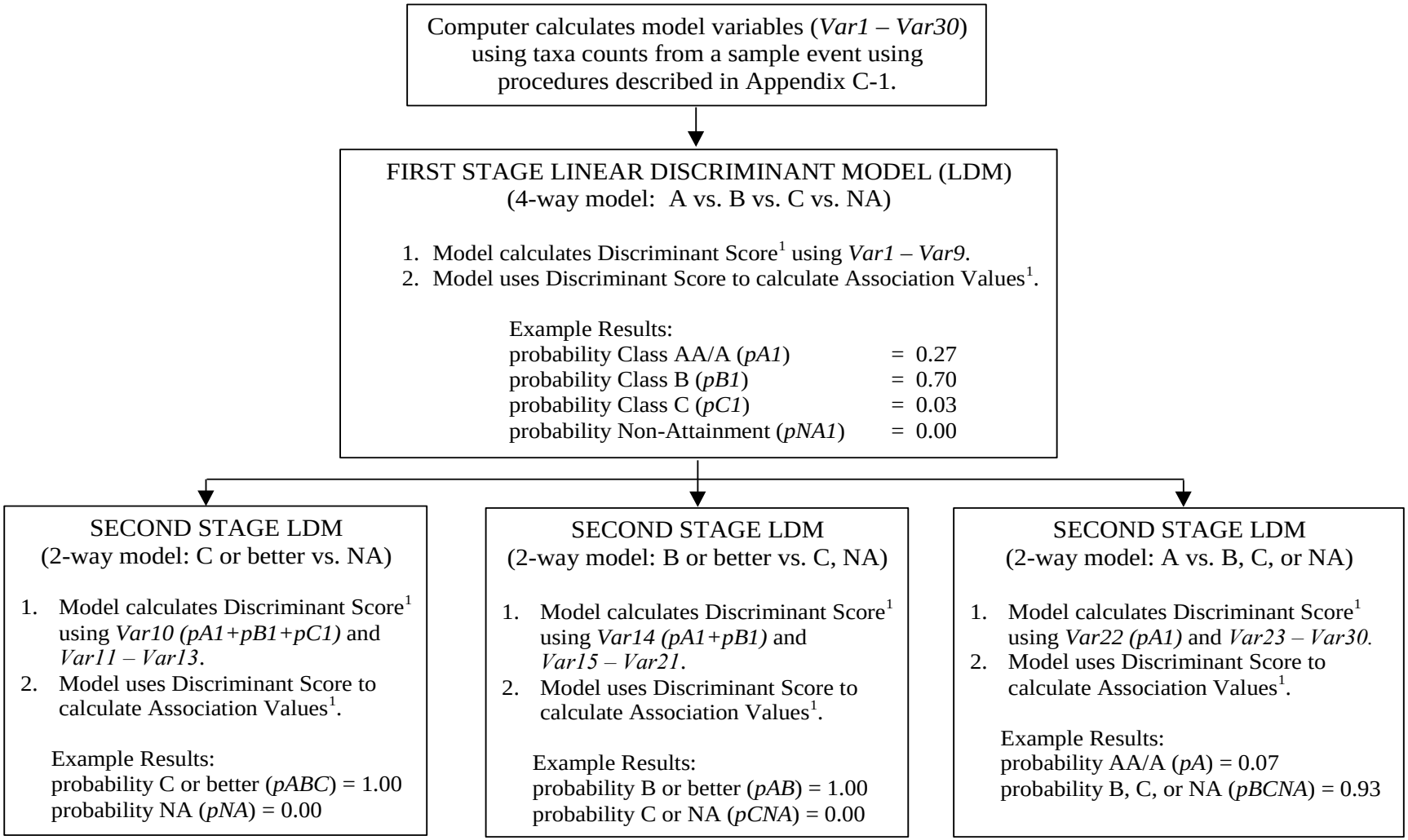
MRSA 38, 4-A Sec 464-465

Aquatic Life Standards for the State of Maine

Classification	Biological Standards
AA	No direct discharge of pollutants; aquatic life shall be as naturally occurs.
A	Natural habitat for aquatic life; aquatic life shall be as naturally occurs.
B	Unimpaired habitat for aquatic life; discharges shall not cause adverse impact to aquatic life in that the receiving waters shall be of sufficient quality to support all aquatic species indigenous to the receiving water without detrimental changes in the resident biological community.
C	Habitat for aquatic life; discharges may cause some changes to aquatic life, provided that the receiving waters shall be of sufficient quality to support all species of fish indigenous to the receiving waters and maintain the structure and function of the resident biological community.

Appendix E

Process of Calculating Model Variables and Association Values Using Linear Discriminant Models

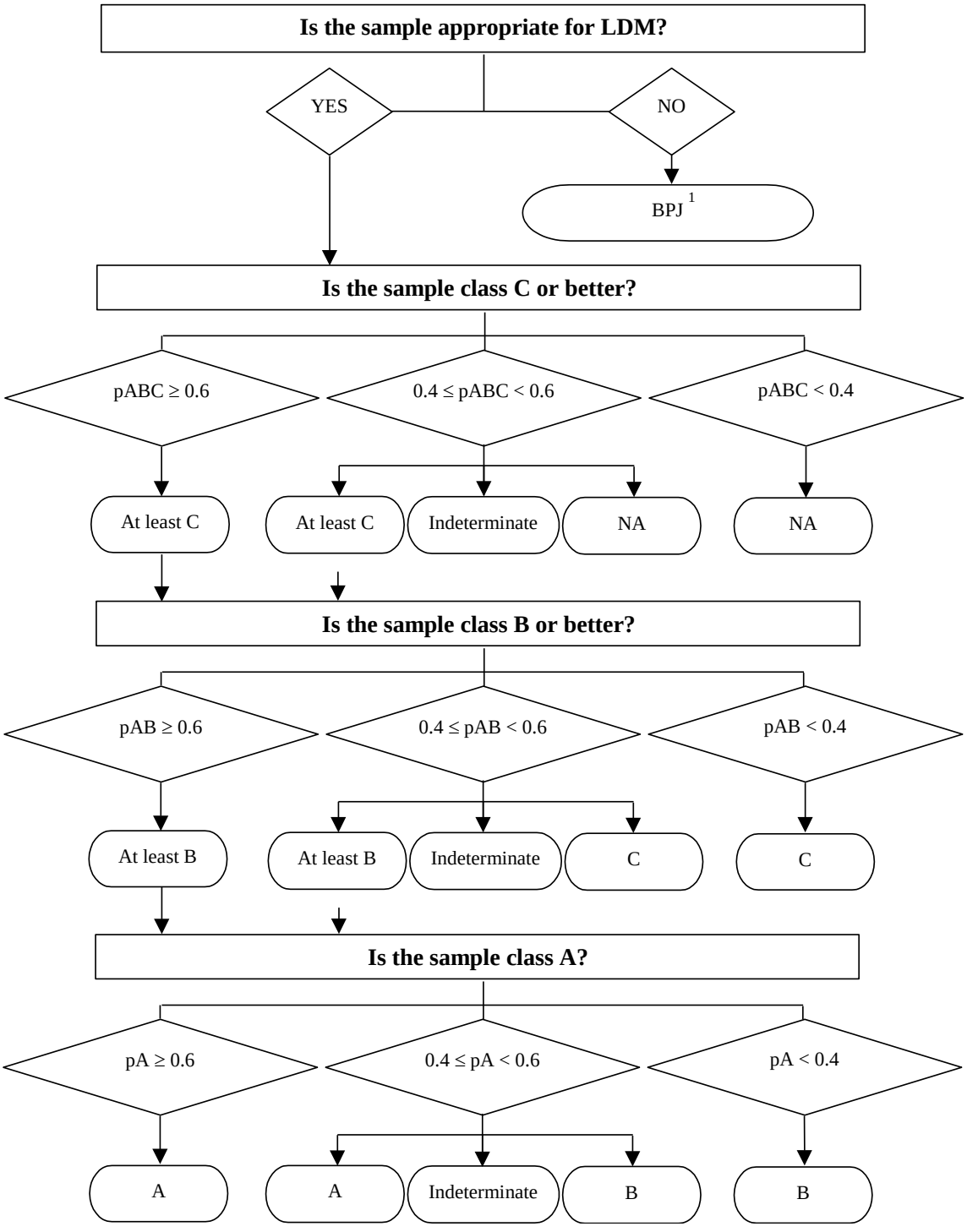


[1] Discriminant Score and Association Values are defined in Section III-2.(1).

Chart by Thomas J. Danielson

Appendix F

Process for Determining Attainment Class Using Association Values



[1] Best Professional Judgment (BPJ) is defined in Section III-2. (2), (4), and (5)

Chart by Thomas J. Danielson

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