Concept Biological Criteria for Fresh Surface Waters

Concept criteria to quantify aquatic life standards for Classes AA, A, B, C and GPA fresh surface waters. Benthic macroinvertebrate and algal communities are used as surrogates to determine conformance with aquatic life standards, related definitions, and provisions for the implementation of biological water quality criteria provided in Maine's Water Classification Program statute (38 M.R.S. §§464-470).

1. **Definitions.** The following terms are defined for use in this chapter:

Association value. The probability (0.0-1.0), computed in the final decision models (sections 4 through 7 below), that a sample from a test community is comparable to statistically defined classification groups representing the ecological attributes described in Maine's Water Classification Program statute.

Classification attainment evaluation. An assessment to determine whether the aquatic life standards of a specified class are achieved. Classification attainment evaluations are performed by the Department and reported in the Department's biennial assessment report to the Legislature and the U.S. Environmental Protection Agency or may be required of an applicant for a waste discharge license or water quality certificate, as defined in this chapter.

Ecological attribute. A process, function, or characteristic of a biological community that is used to evaluate the attainment of the aquatic life standards of a classification and that can be quantified or documented using metrics and indices of the aquatic community structure and function.

Epilithic algae. Algae found growing on hard substrates, including rocks and logs.

Epiphytic algae. Algae found growing on emergent, submerged or floating aquatic vegetation.

Eurytopic taxa. Taxa that are adapted to a wide variety of habitats and tolerate a wide range of environmental conditions.

Indeterminate. A term that describes a class attainment outcome that is inconclusive due to 1) Failure of the sample to meet minimum provisions for total mean abundance, total generic richness or other provisions in section 3.A; or 2) A model outcome with an association value (probability) for a class of equal to or greater than 0.4 and less than 0.6 as described in section 8.F.1; or 3) Confounding factors exist such as described in section 8. F.1 and additional monitoring is required before a determination of class attainment can be made.

Linear discriminant function. An equation that is a weighted linear combination of predictor variables, derived to best distinguish among a set of classification groups having known statistical properties.

Linear discriminant model (LDM). A set of one or more linear discriminant functions, used in combination, to derive strength of membership of an unknown sample within a water quality classification. Membership strength can be converted into an association value (probability) for class membership on a 0.0-1.0 scale.

Maine tolerance index (MTI). A community level weighted-average index calculated from individual Maine tolerance values. Separate MTIs were developed for different biological assemblages and habitats including 1) macroinvertebrates in soft-bottom streams, marshes and shallow lakes and ponds, 2) algae occurring on hard substrates in eroded, mineral-bottom streams and rivers, and 3) epiphytic algae occurring in soft-bottom streams, marshes and shallow lakes and ponds.

Maine tolerance values. Stressor tolerance values for individual macroinvertebrate and algal taxa developed using Maine data and scaled from 1 (least tolerant) to 100 (most tolerant). Separate sets of taxa tolerance values were developed for specific biological assemblages and habitats, including 1) macroinvertebrates in soft-bottom streams, marshes and shallow lakes and ponds, 2) algae occurring on hard substrates in eroded, mineral-bottom streams and rivers, and 3) epiphytic algae occurring in soft-bottom streams, marshes and shallow lakes and ponds. Taxa tolerance values are included in applicable Department SOPs available upon request.

Reference community. Populations of organisms inhabiting a sampling site which is free from known pollution sources or other anthropogenic stressors that could adversely affect the natural biological community. A reference community sampling site is chosen for its similarity to a test community sampling site and must be approved by the Department.

Samplers. Devices used to collect organisms for biological monitoring and assessment, including but not limited to devices providing artificial substrates for organisms to colonize (rock-filled mesh bags, wire baskets or cones), and D-frame nets.

Sampling plan. A plan submitted to, and approved by, the Department that addresses study design, sample collection methods, sample processing methods, provisions for a taxonomic reference collection, data management and analysis methods, report preparation, professional oversight of technical staff, and management and security accountability.

Standard Operating Procedures (SOPs). A set of written protocols that document routine activities related to sampling, analysis and quality assurance to ensure consistency and data integrity.

Taxonomic reference collection. An archived collection of one or more representative macroinvertebrate specimens or digital algal images of individual taxa identified by a professional taxonomist preparing data for submission to the Department. Reference specimens must be preserved in a separate vial for each taxon, labeled with collection site, collection date, taxonomic name, taxonomist name, and unique sample tracking number (i.e., stream macroinvertebrate 'log number', wetland macroinvertebrate 'Sample ID' or algal sample 'bottle number').

Test community. A population of organisms inhabiting a sampling site where classification attainment evaluation is being conducted.

The Department. The Maine Department of Environmental Protection.

2. Responsibility for Sampling.

A. Assessment. 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 (classification attainment evaluation).

B. Licensing. Under certain conditions, as listed below, sampling may be required of an applicant for a waste discharge license, water quality certification, or other Department issued permit.

Sampling must be performed by persons who can demonstrate their qualifications and ability to carry out the Department's sampling protocols set forth in the applicable Department SOPs.

Prior to issuance or reissuance of a waste discharge license or other activities pursuant to 38 M.R.S. Chapter 3, Protection and Improvement of Waters and the State's responsibilities under the federal Clean Water Act, or issuance of a water quality certification pursuant to section 401 of the Federal Clean Water Act, an applicant may be required to conduct sampling and provide all data to the Department for evaluation to determine water quality class attainment. The Department may also require monitoring as a condition of any license, permit or certification that it issues. Such monitoring must be conducted according to a sampling plan provided to and approved by the Department.

The decision by the Department to require biological monitoring is based on the classification of the water, existing information about the condition of the biological community and other water quality information, past performance of existing controls for point and nonpoint sources of pollution, and the nature, magnitude, and variability of the activity relative to the affected water.

3. Aquatic Life Classification Criteria for Fresh Surface Water. Methods described in this section are used to make decisions about classification attainment. Standards for classification of fresh surface waters are described in 38 M.R.S. §§465 and 465-A. There are 4 water quality classes (AA, A, B and C) for fresh surface waters that are not classified as great ponds. Class GPA is the sole classification for great ponds and natural lakes and ponds less than 10 acres in size. The models are constructed to sequentially amass evidence concerning the highest level of classification criteria that a test community attains, using quantitative predictor variables defined in sections 4-7 below. The pertinent question, in terms of the classification attainment, is whether a test community is attaining at least its statutory classification. The methods described in this rule may also be used to determine if a given waterbody attains a higher class and therefore may be subject to statutory antidegradation provisions found in 38 M.R.S. §464(4)(F)(4) and considered for water quality reclassification. The methods may also be used, where appropriate, for other purposes including assessment of pre-impact baseline conditions or site-specific impact evaluations.

A. General provisions for aquatic life standards. Except as otherwise provided in section 8.F, all samples of benthic macroinvertebrates or algae that are collected for the purpose of classification attainment evaluation using the LDMs described in the following sections, whether collected by the Department or by any person submitting data to the Department, must be collected, processed and identified in conformance with applicable Department SOPs appropriate for the habitat and biological assemblage to be sampled (summarized below), as determined and approved by the Department.

Selection of an appropriate sampling site and quantitative analysis of the sample must also conform to criteria set forth in applicable Department SOPs. Quantitative analysis of the sample must conform to the requirements set forth in sections 4-7 of this chapter and must include a sampling plan approved by the Department. Macroinvertebrate samples must be identified to the genus level of taxonomy, where practicable. Computation of indices and measures of community structure required for the macroinvertebrate LDMs must be adjusted to the genus level. Algal samples must be identified to the lowest practicable taxonomic level (genus or species where possible).

Waterbody Type	Biological Assemblage	Target Habitat	Sampling Method	LDM description
Eroded, mineral-bottom streams and rivers	Aquatic macroinvertebrates	Riffles, runs, pools	Artificial substrate samplers (rock bags, baskets or cones)	See section 4
Soft-bottom streams, marshes and shallow lakes and ponds	Aquatic macroinvertebrates	Shallow areas having aquatic vegetation beds	Standardized D-net sweeps	See section 5
Eroded, mineral-bottom streams and rivers	Epilithic algae	Stream reaches having a rocky bottom or containing submerged large woody debris	Rock or log scrapings	See section 6
Soft-bottom streams, marshes and shallow lakes and ponds	Epiphytic algae	Shallow areas having aquatic vegetation beds	Plant stem rubbings	See section 7

(1) *Macroinvertebrate Sample Minimum Provisions.* Samples that have been properly collected and analyzed but fail to meet either of the following criteria are unsuitable for further analysis through the LDMs:

(a) Total mean abundance must be at least 50 individuals (average for all replicate samples at a site); and

(b) Total generic richness for all replicate samples at a site must be at least 15.

(2) *Algal Sample Minimum Provisions.* Samples that have been properly collected and analyzed must have a total richness of at least 15 to be suitable for further analysis through the LDM. The total richness is calculated by adding the number of distinct families, genera, species, and subspecies.

Samples not attaining these provisions are considered to be indeterminate and must be evaluated by the Department using best professional judgement in accordance with section 8.F.

B. Aquatic life statistical decision models. The following statistical decision models consist of linear discriminant functions developed to use quantitative ecological attributes to determine the strength of the association of a test community to any of the water quality classes.

The coefficients or weights (see sections 4-7) 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.

The linear 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

(1) *Statistical decision model for macroinvertebrate samples collected from eroded, mineral-bottom streams and rivers using the rock bag/basket/cone method.* Association values are computed, using variable values from a test sample, for each classification by employing 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 (AA/A, B, or C) or is in nonattainment (NA) of the minimum criteria for any class. Class AA and Class A have the same aquatic life standards and therefore are treated as the same aquatic life class in the model. The probabilities from the four-way model 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 AA/A+B+C vs. NA; Classes AA/A+B vs. Class C+NA; Class AA/A vs. Classes B+C+NA).

(2) *Statistical decision model for macroinvertebrate samples collected from soft-bottom streams, marshes and shallow lakes and ponds using the standardized D-net sweep method.* Association values are computed, using variable values from a test sample, for each classification by employing a single four-way model. The four-way model uses 8 variables pertinent to the evaluation of all classes and provides four probabilities that a given site attains one of three classes (AA/A, B, or C) or is in nonattainment (NA) of the minimum criteria for any class. Class AA and Class A have the same aquatic life standards and therefore are treated as the same aquatic life class in the model. Lakes and ponds having a statutory class of GPA also have the same aquatic life standard as Class AA/A and are considered to be in attainment based on the macroinvertebrate community if the model result is Class A. These probabilities have a possible range from 0.0 to 1.0.

(3) *Statistical decision model for algal samples collected from eroded, mineral-bottom streams and rivers using the rock or log scraping method.* Association values are computed, using variable values from a test sample, for each classification by employing a single four-way model. The four-way model uses 6 variables pertinent to the evaluation of all classes and provides four probabilities that a given site attains one of three classes (AA/A, B, or C) or is in nonattainment (NA) of the minimum criteria for any class. Class AA and Class A have the same aquatic life standards and therefore are treated as the same aquatic life class in the model. These probabilities have a possible range from 0.0 to 1.0.

(4) *Statistical decision model for epiphytic algal samples collected from soft-bottom streams, marshes and shallow lakes and ponds using the plant stem rubbings method.* Association values are computed, using variable values from a test sample, for each classification by employing a single four-way model. The four-way model uses 6 variables pertinent to the evaluation of all classes and provides four probabilities that a given site attains one of three classes (AA/A, B, or C) or is in nonattainment (NA) of the minimum criteria for any class.

Class AA and Class A have the same aquatic life standards and therefore are treated as the same aquatic life class in the model. Lakes and ponds having a statutory class of GPA also have the same aquatic life standard as Class AA/A and are considered to be in attainment based on the epiphytic algal community if the model result is Class A. These probabilities have a possible range from 0.0 to 1.0.

The equations for the final decision models use the predictor variables relevant to the class being tested. The resultant discriminant scores are known as the Mahalonobis Distance where:

Mahalonobis Distance = Z_t (sample x) = g_1 (x, t) + g_2 (t)

Where: Z_t = discriminant score for sample x, class t
$g_1 (x,t) = (x-m_t)' S^{-1} (x-m_t)$
$g_2 (t) = -2 \log_e (q_t) = 0$ (if prior probabilities are equal)

Where: x = a vector containing all the values of all the variables for a given linear discriminant function, for a given sample, of class t
m_t = a vector, as for x, but containing the means of all predictor variables in the given linear discriminant function, for the given sample, of class t
S = pooled covariance matrix (the variance of the multivariate observation)
q_t = value of the prior probability that a given sample is Class A, B, C, or NA.

The probability (association value) of a sample x, belonging to a particular class t, is:

$$P_t(x) = \frac{e^{[Z_t(x)]}}{\sum_A^{\mathrm{NA}}\left[e^{[Z_{\mathrm{A,B,C,NA}}(x)]}\right]}$$

Where: $P_t(x)$ = the probability that sample x belongs to class t (for Classes A, B, C, NA)
e = the exponential function
Z_t = the discriminant score or Mahalonobis Distance for class t (Classes A, B, C, NA)

4. Metrics, indices, and coefficients used for macroinvertebrate samples collected from eroded, mineral-bottom streams and rivers using the rock bag/basket/cone method.

A. Methods for the calculation of indices and measures used in the LDMs Variables (1) to (30) are as follows. Lists for macroinvertebrate Class A indicator taxa and Family Functional Groups are included in applicable Department SOPs. A comprehensive macroinvertebrate taxa list including functional feeding group categories and Hilsenhoff Biotic Index values is available upon request.

(1) *Total mean abundance.* Count all individuals in all replicate samplers from a site and divide by the number of replicates to yield the mean number of individuals per sampler.

(2) *Total generic richness.* Count the number of different genera found in all replicate samplers from one site.

Counting rules for generic richness:

(a) Species-level counts. All population counts at the species level are aggregated to the generic level.

(b) Family-level counts, no more than one genus. A family level identification that includes no more than one taxon identified to the generic level is counted as a separate taxon in generic richness counts.

(c) Family-level counts, more than one genus. A family level identification with more than one taxon identified to generic level is not counted toward generic richness. Counts are divided proportionately among the genera that are present.

(d) Phylum, Class, or Order counts. A higher-level taxonomic identification (Phylum, Class, Order) is not counted toward generic richness unless it is the only representative.

(e) Pupae. 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 the mean number of Ephemeropteran individuals per sampler.

(5) *Shannon-Wiener Generic Diversity.* Shannon-Wiener generic diversity is computed after adjusting all counts to genus, as described under paragraph (2) above.

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

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.* HBI is calculated using all taxa in the sample that have assigned tolerance values. Tolerance values are provided in Hilsenhoff, William. 1987. An Improved Biotic Index of Organic Stream Pollution, *The Great Lakes Entomologist* 20:31-39.

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

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 the counting rules in Variable 4, and divide by total abundance (Variable 1).

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

(9) *Hydropsyche mean abundance.* Count all the individuals from the genus *Hydropsyche* in all replicate samplers from a 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.* The 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.* Divide EPT generic richness (Variable 19) 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 of the class Oligochaeta, following counting rules in Variable 4, and divide by total abundance (Variable 1).

(14) *Probability (A+B) from first stage model.* The sum of probabilities for Classes A and B from first stage model.

(15) *Perlidae mean abundance.* Count all individuals from the family Perlidae 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.* Count all individuals from the subfamily Tanypodinae 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.* Count all individuals from the tribe Chironomini 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 abundance of Dicrotendipes* & *Micropsectra* & *Parachironomus* & *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.*

(23) *Relative generic 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 abundance of Cheumatopsyche* & *Cricotopus* & *Tanytarsus* & *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 abundance of Acroneuria* & *Stenonema.* Sum the number of individuals in each genus in all replicate samplers and divide by the number of replicates (as 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 that are present in the community and divide by 7 (total possible number).

B. Model coefficients for macroinvertebrate samples collected from eroded, mineral-bottom streams and rivers using the rock bag/basket/cone method.

First Stage Model

Coefficients

Variable number	Transformation	Class A	Class B	Class C	Nonattainment
Constant	--	-99.95508	-105.70948	-112.67581	-107.74283
1	nLog (value +0.001)	10.77061	11.46981	11.80888	11.26793
2	--	-0.38619	-0.43340	-0.50051	-0.48822
3	nLog (value +0.001)	0.23940	0.03946	-0.60923	-0.95480
4	nLog (value +0.001)	-0.59970	-0.55500	-0.67722	-1.79032
5	--	21.22732	20.91256	21.07602	19.46547
6	--	8.01620	9.12163	10.31492	10.72746
7	nLog (value +0.001)	-11.70298	-11.52650	-11.49414	-11.66371
8	--	70.77937	71.09637	72.46514	70.22517
9	--	-0.00535	-0.00398	-0.00152	0.00007

Final Classification Models

Class C or better model

Coefficients

Variable number	Transformation	Class A-B-C	Nonattainment
Constant	--	-25.70020	-8.55844
10	Arcsin	19.98470	3.36032
11	nLog (value +0.001)	-0.26001	-0.43781
12	Sq. root	5.57672	5.92732
13	nLog (value +0.001)	-2.33229	-1.89945

Class B or better model

		Coefficients	
Variable number	Transformation	Class A-B	Class C-nonattainment
Constant	--	-17.81016	-6.93836
14	Arcsin	12.04826	3.63707
15	nLog (value +0.001)	-1.11091	-1.03934
16	nLog (value +0.001)	-0.10582	0.01978
17	nLog (value +0.001)	0.17813	0.10825
18	--	4.03202	-1.14508
19	--	0.87400	0.63310
21	nLog (value +0.001)	-0.69360	-0.53194

Class A model

		Coefficients	
Variable number	Transformation	Class A	Class B-C-nonattainment
Constant	--	-9.59254	-4.08552
22	Arcsin	8.34341	1.52080
23	--	3.78999	4.27447
25	nLog (value +0.001)	0.53110	0.77851
26	nLog (value +0.001)	-0.55838	-0.51448
28	--	12.32529	9.81592
30	--	6.94828	-0.67475

5. **Metrics, indices, and coefficients for aquatic macroinvertebrate samples collected from soft-bottom streams, marshes and shallow lakes and ponds using the standardized D-net method.** The statistical model consists of a single LDM with 8 variables. It is applicable to samples collected from shallow, vegetated aquatic habitats including soft-bottom streams, marshes, lakes and ponds. Maine tolerance values and sensitivity categories used in calculations for this LDM were developed specifically for these habitats and are available in the applicable Department SOP. A comprehensive macroinvertebrate taxa list including functional feeding group categories and Hilsenhoff Biotic Index values is available upon request.

A. Methods for calculating metrics used in the LDM (Variables 1-8) are as follows:

(1) *Shannon-Wiener Generic Diversity Index* (Section 4.A.5).

(2) *Relative abundance of collector-gatherers.* Calculate the mean number of individuals in the collector-gatherer functional feeding group for all replicate samplers from a site and divide by the total mean abundance.

(3) *Relative Generic Richness of Ephemeroptera, Odonata, and Trichoptera.* Count the number of different genera that are mayflies, caddisflies, dragonflies, and damselflies in the sample, and divide by the total generic richness.

(4) *Ephemeroptera mean abundance* (Section 4.A.4).

(5) *Maine Tolerance Index.* The MTI is calculated using all taxa in the sample that have assigned Maine tolerance values.

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

Where: MTI = Maine Tolerance 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 Maine tolerance values

(6) *Generic Richness of Sensitive Taxa.* Count the number of different genera having Maine tolerance values in the sensitive range for all replicates for the sample.

(7) *Mean Abundance of Sensitive Taxa.* Calculate the mean number of individuals having Maine tolerance values in the sensitive range for all replicate samplers from a site.

(8) *Ratio of Sensitive to Eurytopic Taxa Abundance.* Divide the mean abundance of sensitive taxa by the sum of 1 and the mean abundance of individuals having Maine tolerance values in the eurytopic range.

B. Model coefficients for macroinvertebrate samples collected from soft-bottom streams, marshes and shallow lakes and ponds using the standardized D-net method.

Coefficients

Variable number	Transformation	Class A	Class B	Class C	Nonattainment
Constant	--	-161.543	-136.581	-129.993	-140.016
1	--	24.5791	23.06538	23.67237	18.93293
2	Sq. root	56.1496	54.63462	55.63192	43.26608
3	Sq. root	189.5138	176.7903	167.3398	146.2942
4	4^{th} root	-4.21759	-4.43895	-4.82144	-5.67683
5	--	1.898626	1.822415	1.906884	2.712681
6	Sq. root	8.412304	6.008252	4.501556	6.084531
7	Log_{10}+1	-6.63102	-4.34576	-5.60927	-5.50135
8	4^{th} root	24.88097	19.74993	17.32717	17.53744

6. **Metrics, indices, and coefficients for algal samples collected from eroded, mineral-bottom streams and rivers using the rock or log scraping method.** The statistical model consists of a single LDM with 6 variables. It is applicable to samples collected from submerged rocks and other hard substrates, such as logs. Maine tolerance values and sensitivity categories used in calculations for this LDM were developed specifically for these habitats and are available in the applicable Department SOP. Additional diatom indicator values used in LDM calculations are described in van Dam, H., A. Mertens, and J. Sinkeldam. 1994. A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. *Netherlands Journal of Aquatic Ecology* 28:117–133 (available on request).

A. Methods for calculating metrics used in the LDM (Variables 1-6) are as follows.

(1) *Relative richness of sensitive algae.* Count the number of algal taxa having Maine tolerance values in the sensitive range and divide by the total number of sensitive, intermediate, and tolerant taxa. Exclude taxa without assigned Maine tolerance values. This variable includes diatoms, cyanobacteria, and other algae (e.g., red, green). Algal taxa richness counts include the number of distinct families, genera, species, and subspecies. Maine tolerance values are described in Danielson, T. J., C. S. Loftin, L. Tsomides, J. L. DiFranco, and B. Connors. 2011. Algal bioassessment metrics for wadeable streams and rivers of Maine, USA. Journal of the North American Benthological Society 30:1033–1048.

(2) *Richness of algae having intermediate tolerance.* Count of the number of algal taxa having Maine tolerance values in the intermediate range (Danielson et al. 2011). This variable includes diatoms, cyanobacteria, and other algae (e.g., red, green). Algal taxa richness counts include the number of distinct families, genera, species, and subspecies.

(3) *Relative abundance of tolerant algae.* Sum the abundance (densities) of algal taxa having Maine tolerance values in the tolerant range (Danielson et al. 2011) and divide by the total abundance of sensitive, intermediate, and tolerant taxa. Exclude taxa without assigned Maine tolerance values. This variable includes diatoms, cyanobacteria, and other algae (e.g., red, green).

(4) *Relative abundance of diatoms that are tolerant of salt and brackish water*. Sum the abundance (densities) of diatoms having salinity tolerance values of 4 and divide by the total abundance of diatoms with salinity tolerance values. Exclude non-diatom algae and diatoms that do not have a salinity tolerance value from computations. Salinity tolerance values are described in van Dam et al. 1994.

(5) *Richness of eutrophentic diatoms.* Count the number of diatom taxa having trophic state tolerance values of 5 (eutraphentic) and 6 (hypereutraphentic) (van Dam et al. 1994). Algal taxa richness counts include the number of distinct families, genera, species, and subspecies.

(6) *Relative abundance of motile diatoms.* Sum the abundance (densities) of motile and highly motile diatom taxa and divide by the summed abundance of all diatoms having motility ratings. Diatom motility ratings are described in Fore, L. S., and C. Grafe. 2002. Using diatoms to assess the biological condition of large rivers in Idaho (USA). Freshwater Biology 47:2015–2037, and Wang, Y. K., R. J. Stevenson, and L. Metzmeier. 2005. Development and evaluation of a diatom-based Index of Biotic Integrity for the Interior Plateau Ecoregion, USA. Journal of the North American Benthological Society 24:990–1008.

B. Model coefficients for algal samples collected from eroded, mineral-bottom streams and rivers using the rock or log scraping method.

Coefficients

Variable number	Transformation	Class A	Class B	Class C	Nonattainment
Constant		-47.7498	-37.6661	-30.7909	-34.3411
1	Arcsine Sq. root	12.74281	8.890575	4.554797	2.92692

Variable number	Transformation	Class A	Class B	Class C	Nonattainment
2	Sq. root	12.2867	10.33789	6.01114	1.817007
3	4th root	7.899358	13.11909	21.87007	33.4012
4	4th root	-6.50943	-4.47783	1.486956	-1.22376
5	Sq. root	-1.94064	-0.35065	1.370636	3.591617
6	4th root	-10.6338	-1.27934	13.62663	25.10755

7. Metrics, indices, and coefficients for epiphytic algal samples collected from soft-bottom streams, marshes and shallow lakes and ponds using the plant stem rubbings method. The statistical model consists of a single LDM with 6 variables. It is applicable to samples collected from shallow, vegetated aquatic habitats including soft-bottom streams, marshes, lakes and ponds. Maine tolerance values and sensitivity categories used in calculations for this LDM were developed specifically for these habitats and are available in the applicable Department SOP. Additional diatom indicator values used in LDM calculations are described in van Dam et al. 1994 (available on request).

A. Methods for calculating metrics used in the LDM (Variables 1-6) are as follows.

(1) *Relative Richness of diatoms in the Eunotiaceae Family.* Count the number of Eunotiaceae diatom taxa and divide by the total generic richness. Algal taxa richness counts include the number of distinct families, genera, species, and subspecies.

(2) *Relative Abundance of Eutrophentic Diatoms.* Sum the abundance (densities) of diatoms having trophic state tolerance values of 5 (eutraphentic) and 6 (hypereutraphentic) (van Dam et al. 1994) and divide by the abundance of diatoms having trophic state values of 1 through 6. Exclude diatoms with trophic state values of 7 or diatoms without assigned trophic state values.

(3) *Relative Richness of Oligosaprobic Diatoms.* Count the number of diatom taxa having saprobic tolerance values of 1 (oligosaprobic, van Dam et al. 1994) divided by the count of diatoms with saprobic tolerance values. Exclude diatoms without assigned saprobic tolerance values from computations. Algal taxa richness counts include the number of distinct families, genera, species, and subspecies.

(4) *Relative Richness of Intermediate Taxa.* Count the number of algal taxa having Maine tolerance values in the intermediate range and divide by the total number of taxa having Maine tolerance values. Exclude taxa without assigned Maine tolerance values for epiphytic algae. Algal taxa richness counts include the number of distinct families, genera, species, and subspecies.

(5) *Relative Richness of Sensitive Taxa.* The number of sensitive algal taxa based on Maine tolerance values divided by the number of taxa with Maine tolerance values. Exclude taxa without assigned Maine tolerance values for epiphytic algae. Algal taxa richness counts include the number of distinct families, genera, species, and subspecies.

(6) *Maine Tolerance Index.* See section 5.A.5 above.

B. Model coefficients for epiphytic algal samples collected from soft-bottom streams, marshes and shallow lakes and ponds using plant stem rubbings method.

Coefficients

Variable number	Transformation	Class A	Class B	Class C	Nonattainment
Constant	--	-258.1647495	-257.1190828	-224.7542754	-222.7828704
1	Sq. root	-13.63093995	-6.791484023	-11.4493779	-16.27325923
2	4th root	85.84867886	89.64715806	89.40347772	89.38860194
3	Sq. root	176.3177542	171.8750904	161.1565337	184.8001292
4	--	259.5701353	253.2858536	223.3879692	162.8025981
5	Sq. root	199.273381	178.2932481	152.6551021	111.117886
6	--	2.657234576	2.907996234	2.974285651	3.625543226

8. Determination of decision results. A waterbody's attainment class is determined by Department biologists using the process described below, and as shown in Appendices 1 through 3. If the provisions in section 3.A are not met, or if the sample is Indeterminate for other reasons defined in section 1, professional judgment (section 8.F) must be used to decide the appropriate course of action.

A. Assess data appropriateness and minimum requirements. For all sample types, the first step is to determine if the sample meets minimum requirements (section 3.A) and is appropriate for use with the applicable LDMs.

B. Macroinvertebrate samples collected from eroded, mineral-bottom streams and rivers using the rock bag/basket/cone method. Determination of attainment results is based on one four-way model whose probabilities are used as inputs into three additional two-way models described in section 3.B above.

(1) *Determine if sample attains at least Class C.* The second step is to use the association value from the "C or better" LDM (pABC) to determine if the sample meets at least Class C or is in nonattainment of minimum aquatic life criteria. If the association value is equal to or greater than 0.6, the sample attains Class C. If the association value is less than 0.4, the sample does not attain Class C and is determined to be in nonattainment of any classification. If the association value (pABC) is equal to or greater than 0.4 and less than 0.6, then the sample is indeterminate for Class C and professional judgment is used to decide if the sample is (1) Class C, or (2) in nonattainment.

(2) *Determine if the sample attains at least Class B.* For those samples that attain at least Class C, the next step is to use the association value from the "B or better" LDM (pAB) to determine if the sample is (1) at least Class B with an association value equal to or greater than 0.6, (2) Class C with an association value less than 0.4, or (3) indeterminate for Class B with an association value equal or greater than 0.4 and less than 0.6.

(3) *Determine if the sample attains Class A.* For those samples that are at least Class B, the final step is to use the association value from the "A" LDM (pA) to determine if the sample is (1) Class A with an association value equal to or greater than 0.6, (2) Class B with an association value less than 0.4, or (3) indeterminate for Class A with an association value equal to or greater than 0.4 and less than 0.6.

C. **Macroinvertebrate samples collected from soft-bottom streams, marshes and shallow lakes and ponds using the standardized D-net sweep method.** Determination of attainment results is based on a single four-way model described in section 3.B above.

(1) *Determine model association value equal to or greater than 0.6.* If the model association value for any class (pA, pB, pC,) is equal to or greater than 0.6, the sample attains the corresponding class (Class A/AA, Class B, or Class C). A model result of Class A is also considered to attain aquatic life criteria for Class GPA waters. If the association value for non-attainment of any class (pNA) is equal to or greater than 0.6, the sample determination is Non-Attainment (NA).

(2) *Model association values between 0.4 and 0.6.* If no model association value is equal to or greater than 0.6 and one or more values are equal to or greater than 0.4 and less than 0.6, the sample is Indeterminate and professional judgment (section 8.F) is used to decide whether it meets the indeterminate class, an adjacent class, or is in nonattainment of any class.

D. **Algal samples collected from eroded, mineral-bottom streams and rivers using the rock or log scraping method.** Determination of attainment results is based on a single four-way model described in section 3.B above.

(1) *Determine model association value equal to or greater than 0.6.* If the model association value for any class (pA, pB, pC,) is equal to or greater than 0.6, the sample attains the corresponding class (Class A/AA, Class B, or Class C). If the association value for non-attainment of any class (pNA) is equal to or greater than 0.6, the sample determination is Non-Attainment (NA).

(2) *Model association values between 0.4 and 0.6.* If no model association value is equal to or greater than 0.6 and one or more values are equal to or greater than 0.4 and less than 0.6, the sample is Indeterminate and professional judgement (section 8.F) is used to decide whether it meets the indeterminate class, an adjacent class, or is in nonattainment of any class.

E. **Algal samples collected from soft-bottom streams, marshes and shallow lakes and ponds using the plant stem rubbings method.** Determination of attainment results is based on a single four-way model described in section 3.B above.

(1) *Determine model association value equal to or greater than 0.6.* If the model association value for any class (pA, pB, pC,) is equal to or greater than 0.6, the sample attains the corresponding class (Class A/AA, Class B, or Class C). A model result of Class A is also considered to attain aquatic life criteria for Class GPA waters. If the association value for non-attainment of any class (pNA) is equal to or greater than 0.6, the sample determination is Non-Attainment (NA).

(2) *Model association values between 0.4 and 0.6.* If no model association value is equal to or greater than 0.6 and one or more values are equal to or greater than 0.4 and less than 0.6, the sample is Indeterminate and professional judgement (8.F) is used to decide whether it meets the indeterminate class, an adjacent class, or is in nonattainment of any class.

F. **Professional judgment.** Where there is documented evidence of conditions that could result in uncharacteristic findings, or when a class attainment result is Indeterminate as defined in Section 1, Department biologists may make allowances to account for those situations by adjusting the classification attainment decision through use of professional judgement as provided in this section. Adjustments to the classification attainment decision may be based on analytical, biological, and habitat information, or the Department may require that additional monitoring of affected waters be conducted prior to issuing a classification attainment decision.

(1) *Sampling procedures and minimum provisions conform but other confounding factors exist.* Samples of test communities must be collected in accordance with approved Department SOPs to be suitable for analysis using the applicable LDMs for classification attainment evaluation. The LDMs are not suitable for use with samples collected in areas of lakes or impoundments that thermally stratify. The Department may use professional judgement when conditions are found that are atypical to the derivation of the applicable LDM, as provided in section 8.F. Factors that may allow adjustments to the model outcome include but are not limited to: habitat factors, including lake outlets from waters classified GPA, unusual naturally-caused substrate character, tidal effects, cataclysmic events, oligotrophic conditions; sampling factors including disturbed samples, unusual taxa assemblages, and documented human error in sampling; and sample processing factors, including subsample vs. whole sample analysis and documented human error in processing. The following adjustments may be made to correct for these conditions:

(a) Raise the finding. On the basis of documented evidence of specific conditions such as those defined above, the Department may decide:

(i) To raise the classification attainment outcome predicted by the model from nonattainment of any class to attainment of Class C; or

(ii) To raise the classification attainment outcome predicted by the model from attainment in one class to attainment in the next higher class; or

(iii) To determine that a sample with an indeterminate outcome for a given class attains that class.

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

(c) Indeterminate result. Where the Department cannot make a finding as described in 8.F.1(a-b), additional monitoring of the test community will be required before a determination of class attainment can be made.

(d) *Model association values between 0.4 and 0.6.* If one or more model association value is equal to or greater than 0.4 and less than 0.6, the sample is Indeterminate and professional judgement is used to decide whether the sample meets the indeterminate class, an adjacent class, or is in nonattainment of criteria for any class. In most cases, this occurs in samples having borderline communities that exhibit ecological attributes of two adjacent classes. Department biologists evaluate the sample community with respect to statutory narrative aquatic life criteria to decide which class description most closely matches the ecological characteristics of the sample and also consider any documented confounding factors such as those described in this section.

(2) *Minimum provisions do not conform.* For classification evaluation of test communities that do not conform to minimum provisions provided in section 3.A, the LDMs are not used to

determine classification attainment and the result is Indeterminate. The Department may use professional judgement as follows:

(a) Determination of nonattainment. Those samples having ecological attributes that do not conform with minimum narrative aquatic life criteria for Class C described in 38 M.R.S. §465, and where there is no evidence of confounding factors that could result in uncharacteristic findings, must be determined to be in nonattainment of the aquatic life criteria for any class.

(b) Determination of class attainment. The Department may use professional judgment to make a finding of attainment of the narrative aquatic life criteria for any class. The decision is based on established principles of aquatic ecology and must be fully documented.

(c) Indeterminate finding. The Department may find that there is insufficient evidence to make a determination of class attainment or nonattainment of aquatic life criteria for any class, therefore the result remains Indeterminate and additional monitoring of the test community will be required before a determination of class attainment can be made.

(3) *Standard sampling procedures are not feasible or appropriate.* For classification attainment evaluation of test communities that do not conform with criteria provided in applicable Department SOPs, the Department may make an assessment of classification attainment or aquatic life impact in accordance with the following procedures:

(a) Approved sampling plan. A quantitative sampling and data analysis plan must be developed in accordance with methods established in the scientific literature of aquatic ecology, and the Department must approve the plan.

(b) Determination of sampling methods. Sampling methods are determined on a site-specific basis, depending on habitat conditions of the sampling site and must be approved by the Department.

(i) The preferred method for sampling macroinvertebrates in rivers and streams having eroded, mineral-bottom substrates is the rock bag/basket/cone method described in applicable Department SOPs, available upon request.

(ii) The preferred method for sampling macroinvertebrates in soft-bottom streams, marshes and shallow lakes and ponds is the standardized D-net sweep method described in applicable Department SOPs, available upon request.

(iii) The preferred method for sampling algae in rivers and streams having eroded, mineral-bottom substrates is the rock or log scraping method described in applicable Department SOPs, available upon request.

(iv) The preferred methods for sampling epiphytic algae in soft-bottom streams, marshes and shallow lakes and ponds is the plant stem rubbings method described in applicable Department SOPs, available upon request.

(v) Other methods. Other methods may be used where ecologically appropriate and practical and must be approved by the Department prior to sampling. Samples collected using non-preferred methods are not suitable for use with the LDMs.

(c) Classification attainment decisions. Department biologists may make classification attainment decisions based on a determination of the degree to which the test community conforms to the narrative aquatic life criteria described in 38 M.R.S. §465.The decision is based on established principles of aquatic ecology and must be fully documented.

(d) Site specific impact decisions. Department biologists may make site specific impact decisions based on established methods of analysis of comparative data between a test community and an approved reference community.

(e) Determination of detrimental impact. Department biologists may make a determination of detrimental impact to aquatic life of a test community without an approved reference community if it can be documented that the community fails to demonstrate the ecological attributes of its assigned statutory class as defined by the narrative aquatic life criteria for that class. Such determinations are based on established principles of aquatic ecology and must be fully documented.

9. Application of decision results

A. Attainment of statutory classification. Applicable narrative and numeric standards and criteria contained in 38 M.R.S. §464 et al. and Chapter 579 rules are applied independently, and all must be met for a waterbody to attain its legislatively assigned water quality class. A waterbody is considered not to attain its assigned class if it fails to meet aquatic life criteria for either macroinvertebrates or algae, or other criteria described in section 9.A.2, for any given sampling event. A waterbody shall be determined to be in attainment of the designated aquatic life uses and characteristics of its assigned water quality classification if:

(1) The association value determined according to sections 4-7 above is shown to be equal to or greater than 0.6 for that class or any higher class, or where Department biologists determine based on provisions for professional judgement in section 8.F that the waterbody attains its statutory class or any higher class; and

(2) Where all other narrative and numeric standards and criteria pertinent to protecting the aquatic life uses of the statutory classification are determined to be attained, including but not limited to aquatic habitat characteristics, quality and quantity, support of indigenous fish species, and protection of other aquatic life defined in 38 M.R.S §466.1 including freshwater mussels, as required in Maine's Water Classification Program statute.

B. Federal Reporting. Results of classification attainment evaluations are reported in the Department's biennial water quality assessment report as required under section 305(b) of Federal Clean Water Act using protocols described in Maine's Consolidated Assessment and Listing Methodology. Waters that are confirmed not to attain their statutory classification are added to the state's list of impaired waters as required under section 303(d) of the Federal Clean Water Act.

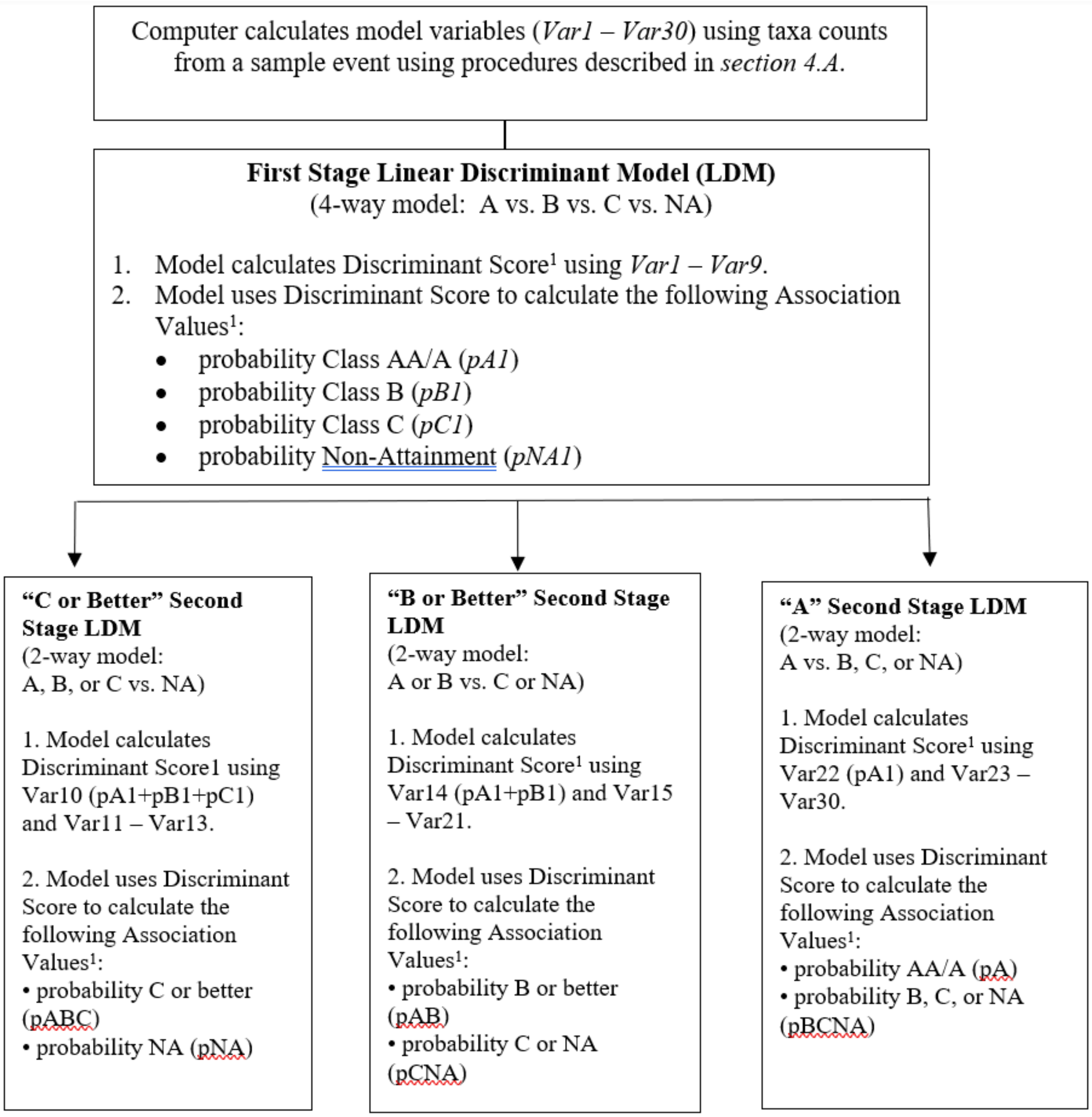
C. Classification upgrades. Waters that consistently attain the aquatic life use criteria of a higher classification must be protected in accordance with the applicable statutory provisions for antidegradation (see 38 M.R.S. §464.4 F(4)).

D. Licensing, permitting and water quality certification activities. An activity regulated by the Department may be approved when a classification attainment evaluation finds that all standards of classification of the water body are met and will continue to be met, notwithstanding the approved activity. An activity regulated by the Department may be approved when a classification attainment evaluation finds that the standards of classification of the water body are not met if it can be shown that the activity does not, or a new activity will not, cause or contribute to nonattainment of designated aquatic life uses and other standards of the assigned water classification.

If the classification attainment evaluation shows that any standards of classification of the water body are not met, and the applicant is unable to show that the activity does not, or a new activity will not cause or contribute to nonattainment, then a license, permit or water quality certification may only be issued when it can be demonstrated that modifications to the proposed activity, as required by the new license, permit or certification, will provide attainment conditions. If two or more activities can be shown to cause or contribute to nonattainment of the assigned water classification, then a license, permit or water quality certification may only be issued when it can be demonstrated that modifications to the activity, as required in the license, permit or certification, in conjunction with modifications to other sources in the watershed that contribute to the nonattainment, including modifications that are a part of a Department approved total maximum daily load, will provide attainment. The Department may require additional monitoring of the affected waters following issuance of any license, permit or water quality certificate.

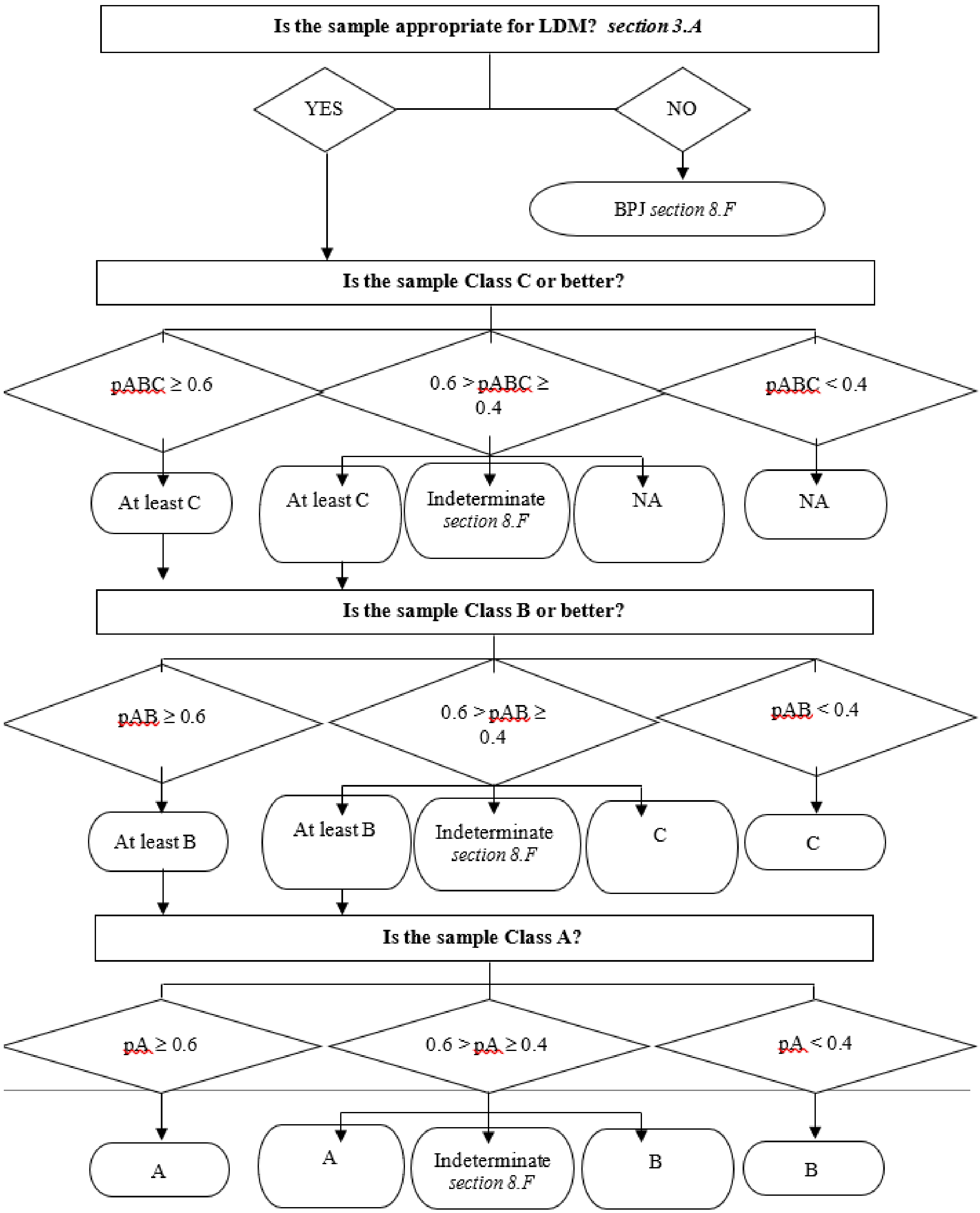
APPENDICES

APPENDIX 1: Process for calculating LDM model variables and association values described in section 4 for macroinvertebrate samples collected from eroded, mineral-bottom streams and rivers.



[1] – Discriminant Scores and Association Values are defined in *section 3.B*.

APPENDIX 2: Process for determining attainment class using association values for multi-stage models described in section 4 (macroinvertebrate samples collected from mineral-bottom streams and rivers).



APPENDIX 3: Process for determining attainment class using association values for 4-way models described in sections 5-7 (macroinvertebrate and epiphytic algae samples collected from soft-bottom habitats and epilithic algae samples collected from mineral-bottom streams and rivers).