Site classification for the New Hampshire benthic index of biotic integrity (B-IBI) using a non-linear predictive model

NEW HAMPSHIRE DEPARTMENT OF Environmental Services

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ABSTRACT

The New Hampshire Department of Environmental Services (NHDES) intends to distinguish naturally varying biological stream conditions from those resulting from human pressures. In an assessment framework, this allows identification of impaired biological conditions that might be improved through management of environmental stressors. Variations in the natural baseline conditions are distinguished by association of benthic macroinvertebrate sample characteristics with environmental conditions in minimally disturbed (reference) sites throughout the state. The development of a revised classification system proceeded by grouping similar biological samples (ordination and cluster analysis), identifying environmental factors that were associated with the distinct groups, and then establishing rules for assigning sites into classes based on natural factors. The classification rules allowed uncertain cases to be partially assigned to multiple site classes (as in fuzzy set theory). The resulting classification therefore recognizes those cases that are not definitively part of a crisp set, but that resemble the core characteristics of more than one set. Application of proportional classification in the assessment framework is accomplished by adjusting biological index thresholds in proportion to site class membership probabilities. Five core biological groupings were identified across New Hampshire and four were identifiable using environmental predictors. The site classes included mountains, hills, plains, and naturally acidic.

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1. INTRODUCTION

Since 1997 the New Hampshire Department of Environmental Services (NHDES) has collected biological information from wadable streams. The NHDES biomonitoring unit uses benthic macroinvertebrate and fish samples to assess the biological integrity of these waterbodies. In 2003, NHDES developed a multi-metric index to characterize the structure and function of macroinvertebrate communities (benthic index of biotic integrity, B-IBI). As part of this development process, a binomial classification system based on two primary ecological regions (North, South) was identified based on the composition of macroinvertebrate communities from reference sites. Index thresholds were subsequently defined for each ecological region.

The B-IBI used for assessment of New Hampshire streams is based on reference biological conditions and comparisons to those conditions. The reference condition is defined as minimally impacted (Stoddard et al. 2006) and can vary due to natural differences in the biological composition among reference sites. If the biological differences are consistently associated with natural environmental characteristics, then identification of multiple reference classes, or strata, will allow for the definition of specific, yet separate, expectations of the reference condition for each biological group. Known as classification, the advantage of identifying multiple distinct reference strata is gained though a reduction in natural variability associated with each unique biological group. In turn, by narrowing the variability in index scores for individual reference groups, there is a higher likelihood of correctly identifying degraded and unimpacted sites and decreased chance of erroneously assessing a site's biological condition (type I or II error).

While NHDES is confident that the ecological regions as identified through previous analyses provide a basic level of classification, a refinement of the system is needed to fully comprehend the natural distribution of distinct macroinvertebrate communities. The current binomial ecoregional classification employed by NHDES is thought to represent a coarse division of multiple macroinvertebrate assemblages. Through a re-analyis and incorporation of additional data, a refinement of the current system will allow index expectations to be more specific to the respective macroinvertbrate assemblages. In completing the re-analysis, environmental variables that describe these assemblages can be identified and ranked in order of importance. In evaluating environmental variables, ecological regions may again emerge as a top-ranked determinant of biological classes, though a suite of individual environmental variables may be discovered that provide a more accurate classification of distinct macroinvertebrate assemblages. The goal of the project is to improve upon the existing classification system using the best predictors of macroinvertebrate assemblages.

As part of the refinement of the current classification system, the primary questions to be answered are:

1) *Are there distinct, reference-quality macroinvertebrate communities that can be identified across the state?*

2) *If distinct communities exist, do they correspond to an ecoregional classification scheme?*

3) *Or, are specific local or watershed scale environmental variables more important in structuring the macroinvertebrate community?*

Multivariate statistical analyses can be used to answer the questions above and to produce a predictive model of site classes. The analyses will include:

1) *Identification of macroinvertebrate community groupings based on similarity of taxonomic representation and community structure.*
2) *Identification of indicator taxa associated with each macroinvertebrate community.*
3) *Evaluation of current ecoregional classification systems as explanatory variables associated with biological groups.*
4) *Simultaneous analysis of macroinvertebrate data and up to 20 environmental variables to determine the importance of these variables in predicting macroinvertebrate community composition.*

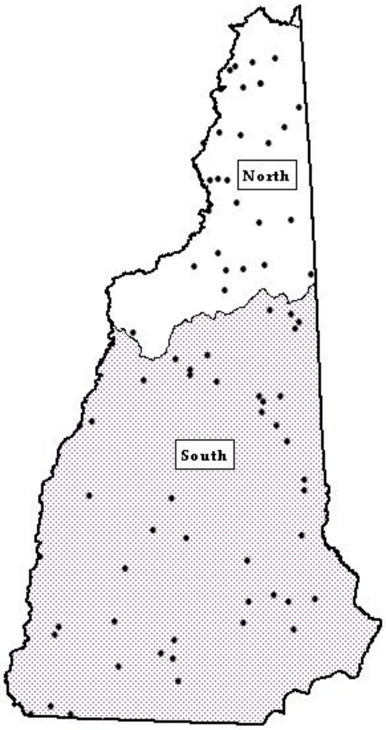
2. METHODS and RATIONALE

2.1 Dataset

The data used for analysis were provided by NHDES and consisted of biological sample data, biological metrics, and environmental information for 74 reference sites visited between 1997 and 2006. The sites were identified by NHDES through an objective process using geographic information system (GIS) to quantify the relative intensity of human influence within the upstream watershed of a given sample point. In cases where this information was not available topographic map examination and professional judgment based on site visits was used to qualify sites as reference quality. Originally, 61 sites were identified as reference quality. After examining the spatial distribution of these primary reference sites 12 secondary reference sites were added to fill the spatial gaps in site coverage throughout the state (Map 1).

A total of 29 environmental characteristics were compiled for each site (Table 1, Appendices A and B). These data were expected to explain or predict, in part, biological group variability. A site by environmental variable matrix included location information, physical characteristics, water quality data, habitat information, and coarse landuse categories. Expected nutrient concentrations were predicted based on prevailing land uses in the watershed, modeled through the SPARROW data set (Moore et al. 2004).

Map 1. Current ecoregional-based classification boundaries, reference sites, and NHDES benthic index of biotic integrity (B-IBI) metrics and respective B-IBI thresholds.



Metrics	Bioregional Criteria
Number of all taxa	
Number of Order: Plecoptera taxa	
Number of tolerant taxa	
Percent of Family: Chironomidae individuals	North = 65
Percent of clinger individuals	
Percent of intolerant individuals	South = 54
Percent of non-insect individuals	

Table 1. Environmental variables used in analysis of NHDES biomonitoring sites. See Appendix A for variable abbreviation and description. See Appendix B for raw data.

Variable Category	Variable Name
Ecologically defined regional schemes	Benthic IBI bioregions Level 4 ecounits
Site coordinates	Longitude Latitude
Site physical characteristics	Elevation Stream order Drainage Area Percent slope (SPARROW) Dominant particle size category Percent dominant size particle category Percent dominance top 3 particle size categories Wetted stream width Average water depth Standard deviation of depth Average water velocity Standard deviation of velocity Streamflow
Water quality	Alkalinity pH Specific conductance
Geology	Percent dominant geologic division Diversity of geologic divisions Dominant geologic division (watershed) Dominant geologic division (sample point)
Land cover	Percent surface water (SPARROW) Percent coniferous forest (SPARROW) Percent deciduous forest (SPARROW)
Nutrients	Predicted total phosphorus concentration (SPARROW) Predicted total nitrogen concentration (SPARROW)

2.2 Macroinvertebrates

Macroinvertebrates were collected by NHDES biomonitoring staff. Protocols for collection included the placement of three replicate artificial substrates (rock baskets) in the stream for a period of six to eight weeks. Deployment of artificial substrates generally occurred in late June through late July. Collection of samples began in August and continued into the beginning of October. Upon collection, macroinvertebrate samples are prepared by cleaning individual substrates contained within wire mesh cylinders into a 500µm bottomed sieve bucket and then transferring the debris and organisms into 1-liter containers. Each replicate is then preserved with 70% ethanol.

Macroinvertebrate samples are processed using a Caton grid whereby a minimum of 25 percent of the sample is sorted and identified to genus for most taxa. Midges (Family: Chironomidae) are identified to family, non-insects have varying levels of identification specificity, and damaged or early instar insect individuals are commonly identified to family. If 100 individuals are contained in the original 25 percent effort, no further sorting is required. If fewer than 100 individuals are present, then complete 25 percent sub-sample increments are processed until the 100-individual threshold is reached.

After sorting and identification is complete, data is input into EDAS, a customized relational database, as individual replicates. We used EDAS to compute the raw metrics and the B-IBI score for each site.

2.3 Environmental Setting

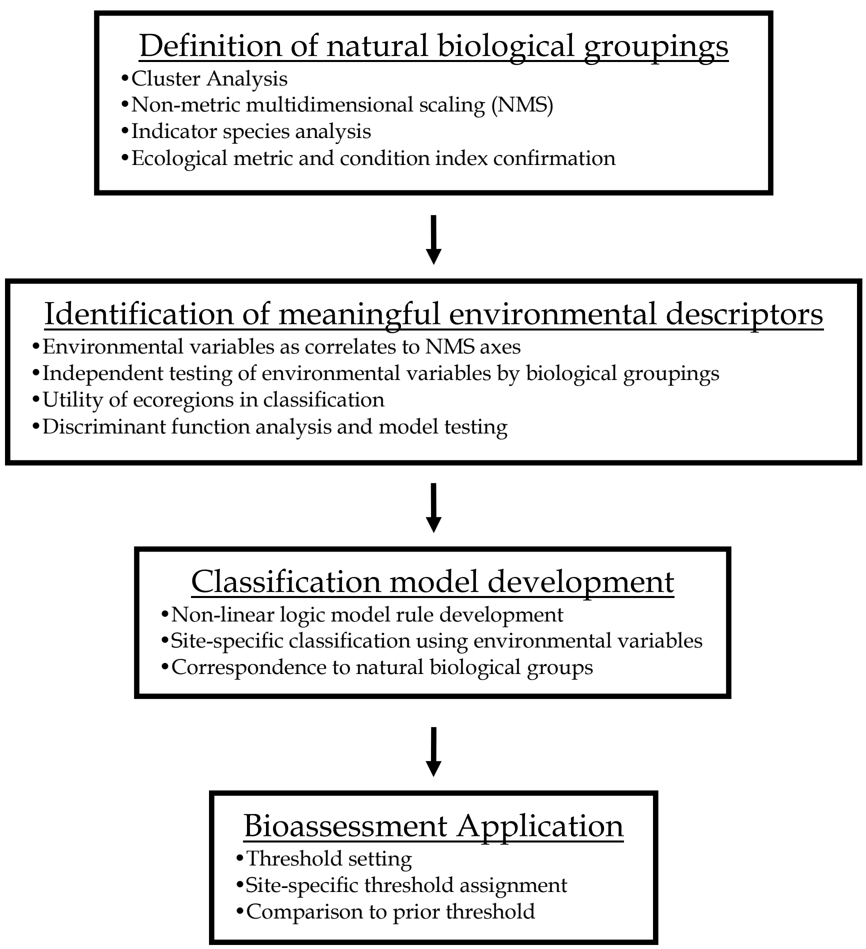
Streams included in the reference set had elevations that ranged from the lowest in southeast (~100 ft) to the highest in the northern Appalachians (~1800 ft). The gradient from mountains to hills to coastal plains generally followed a north to south gradient. The westernmost portions of the state included hills of moderate elevation (~500-750 ft.) and the Connecticut River Valley, a distinctly unique geologic area in New Hampshire. These conditions have resulted in potentially unique ecological settings across the state as evidenced by the ecoregional classification boundaries established separately by the Nature Conservancy (Olivero 2003) and most recently by the EPA (USEPA 2010).

In New Hampshire human development is densest in lower elevation areas in the southern portion of the state, towards the coast, along the highway system, and in the Connecticut River Valley. As noted above, efforts were focused on minimally developed watersheds for classification purposes. Development affected reference site selection opportunities most in southern areas of New Hampshire, resulting in the potential for fewer sites, reference sites with more developed watersheds, or sites biased towards smaller watersheds in pockets of undeveloped areas. Efforts were made to avoid these problems by carefully selecting reference streams and making sure to include a sufficient spatial distribution of sites for adequate detection of unique macroinvertebrate assemblages.

2.4 Data Analysis

The analysis of macroinvertebrate and environmental data towards a site classification system followed a series of procedural steps (Figure 1). First, we identified biological groupings based on similarities observed in the taxonomic composition for each sample and identified unique indicator taxa. Second, we sought environmental reasons for the biological groupings. Third, we devised a system for predicting site classes based on the environmental factors. We also tested ability of the existing ecoregional classification system to successful identify the unique biological groupings. And finally, we recommended applications for a classification scheme in the existing bioassessment program.

Figure 1. General process followed for development of a revised New Hampshire classification system for the B-IBI.



2.5 Defining Biological Groupings

2.5.1 Cluster analysis

Biological groups were first explored using cluster analysis. A site-by-taxon matrix was compiled to reflect the presence or absence of each taxon at each site. Sites were then arranged in a branched hierarchy of similar groups by first calculating all pair-wise Bray-Curtis (B-C) similarities between samples and then clustering sites with the flexible-beta algorithm (McCune and Grace 2002). Using cluster analysis, biologically distinct groups can be defined by breaking the dendrogram into several branches. Each group can then be associated with environmental factors on ordination diagrams or using discriminant analysis (see below). Here the terms "groups" and "clusters" are used interchangeably. They are different from "classes" in that classes are the result of predictive models, whereas groups and clusters are the condition to be predicted.

Rare and ambiguous taxa can be problematic in cluster analysis and were eliminated. Rare taxa were defined as those that occurred in less than 4 of the 74 reference samples. Ambiguous taxa were those that were identified at a higher, less specific taxonomic level because of damaged or undeveloped specimens. The site-by-taxa matrix was therefore reduced to retain as much information as possible while excluding rare and ambiguous taxa. When several rare genera occurred within one Family or when several identifications were at the Family level, then all individuals were counted at the Family level. When most identifications within a Family were made at Genus level, then the identifications made at Family level were excluded from the analysis.

2.5.2 Ordination

Biological groups discerned through cluster analysis were confirmed using non-metric multidimensional scaling (NMS). The same site by taxon matrix was used to complete NMS ordinations as in cluster analysis. In NMS, samples that have a similar taxonomic composition plot closer together than dissimilar samples. Natural environmental variables can be associated with the biological groups through visual inspection of the ordination diagrams and correlations with the biologically defined axes of the NMS diagram. NMS is a robust method for detecting similarity and differences among ecological community samples and works well with presence/absence data (McCune and Mefford 1999, Reynoldson et al. 1995).

As in cluster analysis, similarity among reference biological samples was determined using the B-C similarity measure. The ordination software (PC-Ord, MJM software, McCune and Mefford 1999) calculates a site-by-site matrix of B-C similarity from which the arrangement of samples in the ordination diagram is derived. Multiple dimensions are compressed into two or three dimensions.

2.5.3 Indicator taxa

Indicator taxa analysis was conducted using PC-Ord to identify those taxa that were unique to each of the biological groups. Indicator taxa were the product of a taxon's relative abundance (across group occurrences) and frequency (within group occurrences) in each of the biological groups. Indicator values represented a taxon's relative affinity to a biological group. Indicator taxa were of interest in order to provide a biological identity to a group and as a subjective check on whether distinct groups are logical relative to the environmental variables that are used to describe them.

2.5.4 Metric distributions

Metrics are the ecological measurements of a benthic assemblage's structure and function. When combined in unison, as with a multimetric index, metrics are used in determination of biological condition. Comparative distributions of metric values were used to determine whether the distinct groupings based on individual taxa also have distinct metric signatures. Metrics can be insensitive to the clustered groups depending on the constancy of taxonomic characteristics among taxa that are substituted across groups. In other words, the metrics measure an assemblage's structure or function independent of the specific taxa contained in the sample. Therefore, we used metric distributions to confirm or refute the proposed groupings and assure that the groupings are meaningful in the assessment context. Alternatively, metric distributions can also indicate when groupings are inadequate. Metric distributions that show considerable variability within a single group suggest that the natural ecological variability of the site grouping has not been accounted for. In these cases, we would look into the possibility of multiple refined groupings within the variable group. Comparisons of individual metrics and B-IBI scores across biological groups were completed using the Kruskal-Wallis (K-W) test to detect overall significance and the Mann-Whitney U test for individual group comparisons.

2.6 Discerning Environmental Factors

The most important environmental determinants of biological groups were identified using two primary techniques: 1) ordination overlays and correlations of the environmental variables to the ordination axes (see Ordination above), and 2) environmental variable distributions with respect to the biological groups. Following these steps, discriminant function analysis (DFA) was used as a confirmatory step in identifying the most important environmental factors and a testing of a model that could potentially be used for overall site classification.

2.6.1 Variable distributions

Just as metric distributions were used to inform appropriate biological groupings, comparisons of environmental variable distributions by biological groupings were used to identify those variables that differed among groups. Box and whisker plots were utilized in determining breakpoints of the variable values among groups. Kruskal-Wallis tests were used across biological groups to determine the environmental variables that were significantly different.

2.6.2 Discriminant function analysis (DFA)

DFA is typically used to identify the variables most useful in predicting class membership and assigning probabilities of class membership to sites. DFA calculations result in probabilities ranging from 0 to 1. The predicted site class is the one with the highest probability of membership. The probabilities are based on a discriminant function which is created as a linear combination of independent variables. This is analogous to multiple regression and is subject to the assumptions of linear and homoscedastic relationships. If DFA is effective, the model predicts correct groups for a high percentage of sites. The environmental variables that repeatedly appear as significant determinants in the models are the ones that affect the natural biological community.

We used DFA as method for confirming environmental variable importance as determined through correlations with ordination axes and comparisons of variable distributions among the biological groups. DFA model predictions were also completed in order to assess overall model success and consideration as a possible classification option.

2.7 Predicting Site Classes

2.7.1 Discrete classes

Site classes can be defined as discrete bins (one class or another) or as continuous gradients (partial membership to multiple classes). For bioassessments using stream macroinvertebrates, discrete classification has been used because it simplifies index development. In addition, the ecoregion concept was developed as an inclusive method for identifying geographic areas with similar environmental characteristics that would lead to comparable biological assemblages. In turn, ecoregions could be used *a prioi*, in the absence of impacts, to classify sites for bioassessment (Omernik and Bailey 1997). However, discrete classification can result in misclassification errors at sites that exhibit characteristics of multiple classes or are near the thresholds used for class definition (e.g., a site located close to an ecoregional boundary).

2.7.2 Non-linear predictions

Fuzzy set theory is based on non-linear functions that allows for degrees of group membership compared to an all-or-nothing approach in classical (discrete) set theory. The advantage of this in site classification is that it allows sites that do not resemble “pure” members of stereotypical classes to be recognized and treated as “hybrids” in an assessment context. In contrast, distinct classification forces assignment of each site to a single class. For example, if site classes were defined by ecoregions, a site that is 100 meters to the south of an ecoregional boundary would be placed in the southern site class. A site that is 100 meters to the north of the same boundary, yet only 200m from the first site, would be placed in the northern site class. Each site would then have distinct assessment expectations based on their assessment class, despite their relative close proximity. In fuzzy set theory, both sites could have nearly equal membership to both classes and could be assessed accordingly.

Site classification along a continuous gradient is common in predictive models of observed and expected (O/E) taxa, such as the River Invertebrate Prediction and Classification System (RIVPACS, Clarke et al. 2003). Such models use DFA to assign class membership probabilities to individual sites. However, DFA-based models assume linear relationships between the probabilities of site membership to a specific class and the predictive variables used to generate these probabilities. In this sense, DFA ignores potential logical breakpoints along the environmental gradient. With fuzzy sets, natural, non-linear breakpoints can be recognized and the membership rules can be communicated in terms that are common to human reasoning (Zadeh 1997).

Fuzzy set theory has been used to assess biological conditions based on codifying expert opinions of taxonomic characteristics in logic-based membership rules (Snook et al. 2007, Gerritsen et al. 2006). Fuzzy sets and fuzzy logic are common in engineering applications worldwide (e.g., Demicco and Klir 2004), though examples of environmental applications are less common (e.g., Bosserman and Ragade 1982; Castella and Speight 1996; Ibelings et al. 2003).

2.8 Applications in Assessment

2.8.1 Existing classification

The classification scheme currently used for bioassessment in New Hampshire by NHDES is defined by two ecological regions (union of ecological drainage units - EDUs) corresponding to delineations used by the Nature Conservancy (Map 1). These regions are generally described by latitude and river basin. The northern region (above latitude 44.2) includes the Androscoggin and the Upper Connecticut EDUs and the southern region includes the Coastal-Merrimack, and Lower Connecticut EDUs. The multimetric index is calculated based on a consistent metric suite and scoring across the entire state. Thresholds of biological impairment vary among the two ecological regions.

2.8.2 Thresholds and expectations

It was expected that a new classification scheme would enhance the correct detection of biological impairment by refining the biological expectations for individual sites. Using a continuous, rather than discrete, classification system would allow sites that were obviously part of one class to be compared to a threshold derived from the distribution of reference values in that respective class. A site that was near the theoretical boundary of a class criterion would be recognized as having partial characteristics of more than one class (“hybrid” site). In that case, a site specific threshold could be derived by multiplying the threshold of each pure class by the proportion the individual site resembles each class.

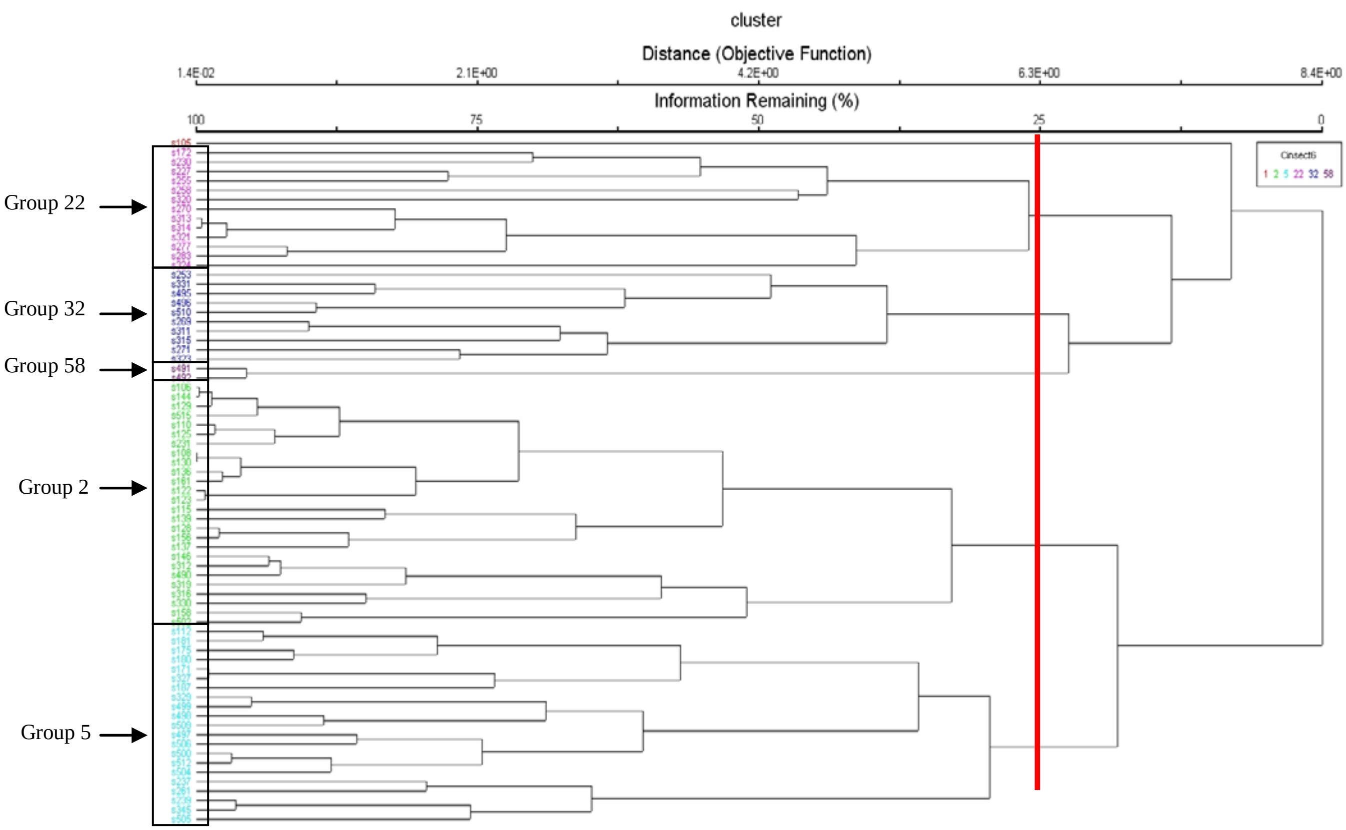
3. RESULTS

3.1 Biological group identification

Two data matrices were prepared showing sample identifiers and measurements of the distinct taxa. One matrix showed taxa presence / absence information. The second was prepared to relate environmental data to the biological samples. For the taxa matrix, identification levels in the Chironomidae family were variable across samples. Further, at the Family level, Chironomids were ubiquitous across samples so we removed this taxon from the matrix. Non-insect taxa occurred with low frequency and at highly variable taxonomic levels. For these reasons, we also removed this taxon from the matrix.

The cluster analysis based on the taxa matrix with beta set at -0.25 resulted in a cluster dendrogram with 2.14% chaining. The beta value of -0.25 was selected based on visual inspection of alternative cluster dendrograms. Cluster analysis using other beta values resulted in less cluster definition (greater chaining with higher beta) or less individual site distinctions (with lower beta). Six biologic groups were defined when using a cut line with 25 percent of the information remaining (Figure 2). One group included a single site that was considered an outlier and subsequently eliminated from further analyses.

Figure 2. Cluster dendrogram of sites arranged by taxonomic similarity. The “cut line” (thick vertical red line) was at the point where approximately 25% of information was remaining. Distinct biological groups are defined by numbers to the left and include sites contained in the respective box. The group with a single member was eliminated from analysis as an outlier.



Another group had only two samples, but was retained because there was a credible defining environmental factor associated with this cluster (See Variable distributions below). The remaining four groups had between 10 and 26 samples in each. A five group clustering scheme was initially retained during the classification analysis because the groups were clearly separated when superimposed on the ordination diagrams (see Ordination results below).

3.2 Indicator taxa identification

Taxa that were indicative to the four of the five cluster groups (2, 5, 22, 32) provided a biological identity to the natural invertebrate assemblages. Group 58 was eliminated from the indicator taxa analysis because its group size (N=2) was too small to give meaningful results. The number of indicator taxa ranged from three for groups 2 and 5 to eleven for group 32 (Table 2). Indicator taxa from groups 2, 5, and 22 were either in the Orders Ephemeroptera, Trichoptera, or Plecoptera. For group 22, two Dipteran taxa also proved to be solid indicators. In contrast, taxa from the Orders Coleoptera, Diptera, Megaloptera, and Ondonta were important in comprising group 32. Trichopteran taxa also helped define this group with four taxa not commonly found in the other groups. Not surprisingly, major Orders of taxa that were good indicators of groups 2, 5, and 22 were not found in group 32. Similarly, Dipterans and Odonates were not found in groups 2 and 5.

The results of the indicator analysis were helpful in identifying the taxa that were most exclusive to or absent from each cluster group. The ability to identify notable taxa that were present or absent from each cluster group lends additional credence to their identity as separate natural assemblages.

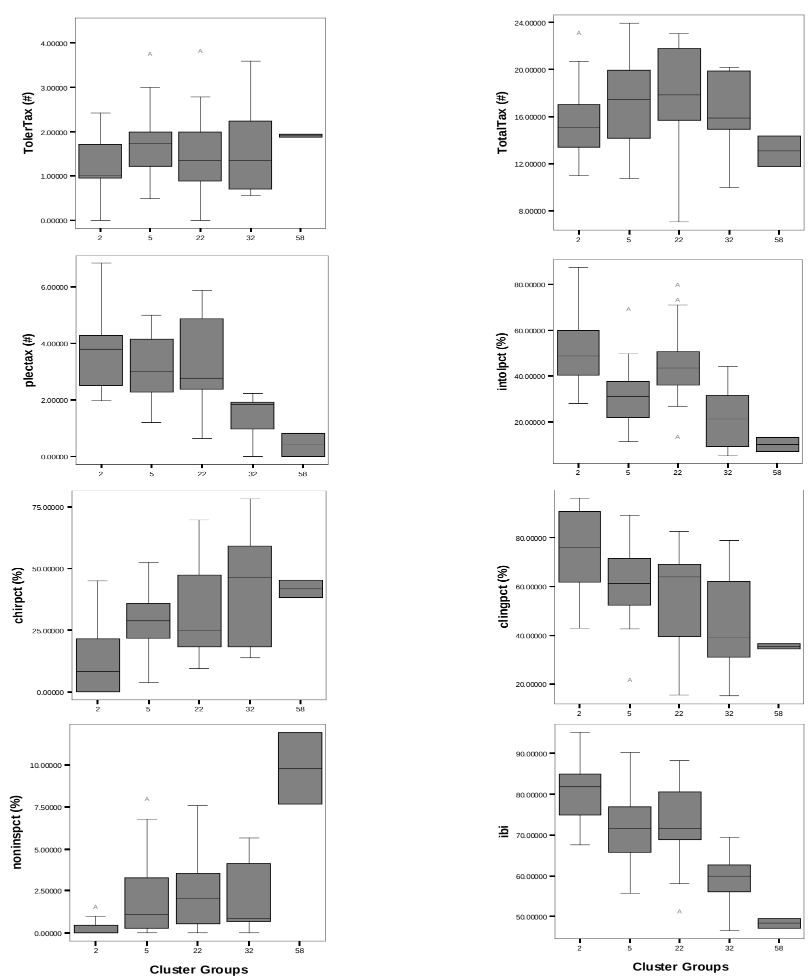
Table 2. Indicator taxa for natural biological groups of macroinvertebrates for reference sites.

Group 2		Group 5		Group 22		Group 32	
Indicator Taxa							
Taxa	*Order*	*Taxa*	*Order*	*Taxa*	*Order*	*Taxa*	*Order*
Rhithrogena	Ephemeroptera	Leucrocuta	Ephemeroptera	Antocha	Diptera	Psephenidae	Coleoptera
Brachycentrus	Trichoptera	Paragnetina	Plecoptera	Dicranota		Clinocera	Diptera
Arctopsyche		Chimarra	Trichoptera	Nemouridae	Plecoptera	Chelifera	
				Apatania	Trichoptera	Megaloptera	Megaloptera
				Hydroptila		Aeshnidae	Odonata
				Lepidostomatidae		Calopterygidae	
						Coenagrionidae	
						Oecetis	Trichoptera
						Limnephilidae	
						Molanna	
						Nyctiophylax	
Taxa Not Present in Group							
Psephenidae	Coleoptera	Clinocera	Diptera	Leucrocuta	Ephemeroptera	Antocha	Diptera
Clinocera	Diptera	Chelifera		Coenagrionidae	Odonata	Epeorus	Ephemeroptera
Chelifera		Calopterygidae	Odonata	Molanna	Trichoptera	Leucrocuta	
Leucrocuta	Ephemeroptera	Arctopsyche	Trichoptera	Chimarra		Rhithrogena	
Calopterygidae	Odonata	Molanna				Paragnetina	Plecoptera
Coenagrionidae		Nyctiophylax				Pteronarcys	
Hydroptila	Trichoptera					Apatania	Trichoptera
Oecetis						Arctopsyche	
Limnephilidae							
Molanna							
Chimarra							
Nyctiophylax							

3.3 Metric Distributions

Distributions of biological metrics from the groups identified through cluster analysis were examined to determine if the groupings were valid in a bioassessment context (Figure 3). K-W tests indicated that significant differences occurred for 5 of 7 metrics and B-IBI scores across the cluster groups (Table 3). Multiple comparisons between cluster groups across individual metrics using the Mann-Whitney U test determined that the number of differences was greatest (6 total; 5 of 7 metrics, plus B-IBI score) between groups 2 and 5, groups 2 and 32, and groups 2 and 58 (Table 4). The fewest differences occurred between groups 5 and 22, and 32 and 58, respectively (1 total; 1 of 7 metrics). When cluster groups were compared within B-IBI scores, 8 of 10 possible combinations were significantly different. Only group combinations 5 / 22 and 32 / 58 had B-IBI scores that were not significantly different. Within metrics, the highest level of differentiation between groups was observed in the percentage of intolerant individuals with 7 of 10 significantly different group combinations. The total taxa metric was not significantly different between any of the cluster groups.

Figure 3. Metric and IBI distributions for natural macroinvertebrate groups. Upper and lower extent of boxes are 75th and 25th percentile, respectively. Upper and lower extent of whiskers are 90th and 10th percentile, respectively. Circles are greater than or less than the 90th and 10th percentiles, respectively. See Appendix C for metric abbreviations and raw data.



In most cases, comparisons based on B-IBI score and individual metrics confirm that the biological groupings established through cluster analysis using taxa presence / absence were meaningful from a bioassessment perspective. The lack of significant differences in B-IBI scores between cluster groups 5 / 22 and 32 / 58 were potential exceptions to this conclusion. For groups 32 and 58 the lack of significant differences among B-IBI scores could have been related to the low number of sites in group 58 (n = 2). Coupled with the environmental uniqueness of group 58, namely naturally low pH conditions, we felt it important to preserve these as separate groups.

Table 3. Kruskal-Wallis (K-W) test result across natural macroinvertebrate groups for individual B-IBI metrics and overall B-IBI score. See Appendix C for metric abbreviations and raw data.

Kruskal-Wallis Test Results								
	TotalTax	PlecTax	ChirPct	NonInsPct	TolerTax	IntolPct	ClingPct	IBI
Chi-Square	5.71	22.51	24.04	22.46	4.97	31.62	21.56	34.34
df	4	4	4	4	4	4	4	4
Significance	0.2218	0.0002	0.0001	0.0002	0.2907	0.0000	0.0002	0.0000

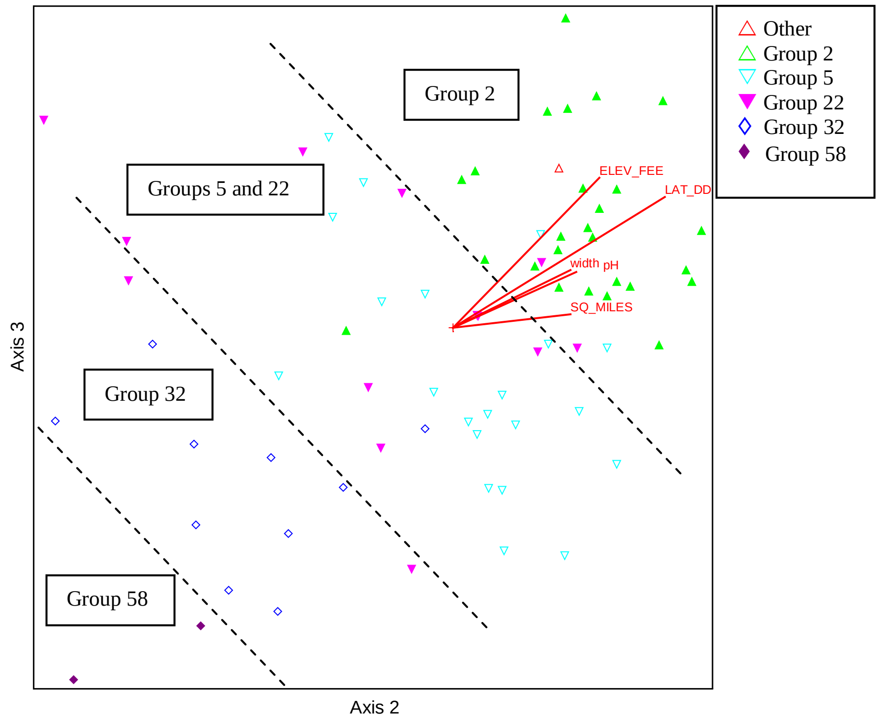
Table 4. Multiple comparisons test results using the Mann-Whitney U test between natural macroinvertebrate groups for B-IBI metrics and overall B-IBI score. See Appendix C for metric abbreviations and raw data.

Mann-Whitney Test Results [Sig = p<0.05)									
Groups	TotalTax	PlecTax	ChirPct	NonInsPct	TolerTax	IntolPct	ClingPct	B-IBI	Total
2,5	NS	NS	Sig	Sig	Sig	Sig	Sig	Sig	6
2,22	NS	NS	Sig	Sig	NS	NS	Sig	Sig	4
2,32	NS	Sig	Sig	Sig	NS	Sig	Sig	Sig	6
2,58	NS	Sig	Sig	Sig	NS	Sig	Sig	Sig	6
5,22	NS	NS	NS	NS	NS	Sig	NS	NS	1
5,32	NS	Sig	NS	NS	NS	NS	Sig	Sig	3
5,58	NS	Sig	NS	Sig	NS	Sig	Sig	Sig	5
22,32	NS	Sig	NS	NS	NS	Sig	NS	Sig	3
22,58	NS	Sig	NS	Sig	NS	Sig	NS	Sig	4
32,58	NS	NS	NS	Sig	NS	NS	NS	NS	1
Total	0	6	4	7	1	7	6	8	

3.4 Ordination results

NMS ordination based on taxa presence / absence data compared well with the cluster analysis in that the five biological groups showed a greater association (nearness) for sites within the same group and separation from sites of alternative groups (Figure 4). Overall, 69.1 percent of the taxonomic variation was accounted for on axes two and three (cumulative $R^2 = 0.691$). The clearest distinction between groups was between sites within group numbers 2, 5, 32, and 58. Sites placed in group 22 were less distinct in their grouping and shared the taxonomic characteristics of all the other groups. Nevertheless, NMS ordination provided a solid confirmation of the existence of at least four distinct macroinvertebrate community assemblages.

Figure 4. NMS ordination diagram for reference sites within biological groups identified through cluster analysis. Diagonal dashed line provides approximate separation between four of five biological groups. Red vectors provide relative importance of individual environmental parameters and association with ordination axes.



The environmental variables that were most strongly associated with the biological ordination axes through NMS (r > 0.40) included latitude, elevation, pH, stream width, and drainage area (Table 5). Of these, the static environmental variables that had correlation coefficients in excess of 0.40 with both axes were latitude and elevation, while pH, width, and drainage area met the same criteria with axis 2. Predicted nitrogen concentrations had the second highest correlation coefficient to ordination axes two and three. Predicted phosphorus concentrations were the highest ranked correlate with axis three. However, nutrients were discounted as possible determinants of stream class because predictions were based on many factors including watershed landuse patterns. Landuse patterns are subject to human alternation and, thus, could not be used as reliable, static *a priori* predictors of stream class. Collection date (year), the fifth and eighth highest ranked correlate with axis three and two, respectively, was discounted because it was confounded by sample location such that samples collected during early years of the biological sampling program at NHDES targeted northern regions. Variables that showed little to no relationship with the ordination axes included measures of geologic types, substrate particle sizes, flow, alkalinity, specific conductance, and land cover / land use.

Table 5. Environmental variable correlation coefficients (Pearson) with NMS ordination axis two and three. Grey boxes refer to top 10 environmental correlates. Bold text refers to environmental variables displayed on the NMS ordination diagram. See Appendix A for abbreviations.

NMS Axis →	2		3	
Variable ↓	r	rank	r	rank
collDate	-0.377	8	-0.367	5
ELEV_FEE	**0.469**	**3**	**0.475**	**3**
SQ_MILES	**0.421**	**6**	**0.146**	**15**
LAT_DD	**0.566**	**1**	**0.445**	**4**
%decid_a	0.338	10	0.121	20
pred_p_c	-0.386	7	-0.646	1
pred_n_c	-0.518	2	-0.598	2
%sp_r_sl	-0.227	15	0.243	9
depth_sd	0.178	19	0.238	10
Vel_sd_v	0.249	13	0.249	8
width	**0.423**	**5**	**0.295**	**6**
flow	0.342	9	0.194	13
pH	**0.432**	**4**	**0.291**	**7**

To evaluate the ability of an ecoregionally-based classification to discern patterns in taxonomic composition, current NHDES ecoregions and EPA Level IV ecounits were overlayed onto taxa presence / absence ordination diagrams. For the NHDES ecoregions, a clear and distinct gradient was visible on the ordination diagram. Sites located in the upper right quadrant of the diagram were predominately part of the northern bioregion whereas sites in the lower left were part of the southern bioregion (Figure 5). Thus, the current bionomial classification system utilized by NHDES appears to have merit in that natural taxonomic differences are present between sites in the northern and southern bioregions. However, when compared to the cluster analysis results, sites in the northern bioregion belonged to groups 2, 5, and 22, while sites in the southern bioregion were distributed across all groups (Table 6). Therefore, while some distinction between the cluster groups was possible using the current classification system, the limited number of classes (north, south) into which sites could be placed represents a relatively coarse classification system that does not fully account for natural differences in macroinvertebrate assemblage taxonomy. Further, the fact that the southern bioregion contains all of the cluster groups confirms the need for a more refined system that accounts for these observed natural differences in taxonomic composition.

Figure 5. NMS ordination diagram for reference sites overlayed with current NHDES B-IBI (IBI region 1, IBI region 2) ecoregions. Dashed line provides visual separation between sites in the northern and southern ecoregions.

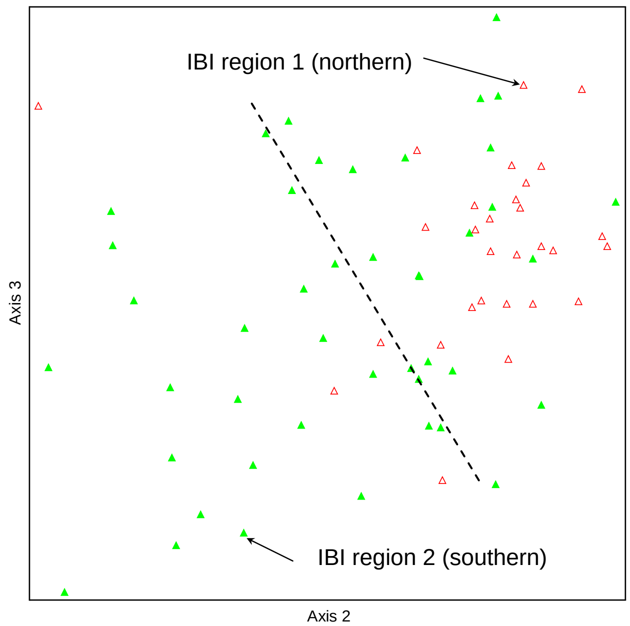


Table 6. Number of reference sites in new cluster groups and NHDES ecoregions.

	NHDES Ecoregion		
Cluster Group	North	South	Total
2	17	9	26
5	7	14	21
22	5	8	13
32	0	10	10
58	0	2	2
Total	29	43	72

In contrast, Level IV ecounits were overly specific in that they were too numerous and unrelated to a site's taxonomic composition, resulting in only weak groupings of sites on the ordination diagrams (Figure 6). These groupings included sites in the northern, mountainous ecoregions (58m, 58p) and the southern, coastal plain ecoregions (58g, 59h) (Figure 6)**.** Other ecoregions were indistinct in that samples collected within cluster groups were scattered across the ordination diagram and overlapped with the mountain and coastal Level IV ecoregions. However, to some extent Level IV ecoregions, similar to the NHDES bioregions, may be useful as a preliminary, coarse filter based on geographic location (northern, mountain sites vs. southern lowland sites) in a refined natural classification system.

A strict breakdown of cluster group assignment to Level IV ecoregions, confirmed the visual observations from the ordination diagram with sites falling in ecoregions 58m (Quebec/New England Boundary Appalachains), 58n (White Mountain Foothills), and 58p (White/Blue Mountains) belonging to cluster groups 2, 5, or 22, while sites within the remaining ecoregions were distributed across all cluster groups (Table 7). While the alignment of cluster groups and Level IV ecoregions was not complete, consolidated Level IV ecoregions were further considered as part of a refined classification system for the purposes of separating northern, mountainous sites from southern plains sites.

Figure 6. NMS ordination diagram for reference sites overlayed with EPA Level IV ecoregions. Dashed line provides visual separation between sites in the northern / mountain and southern / lowland ecoregions.

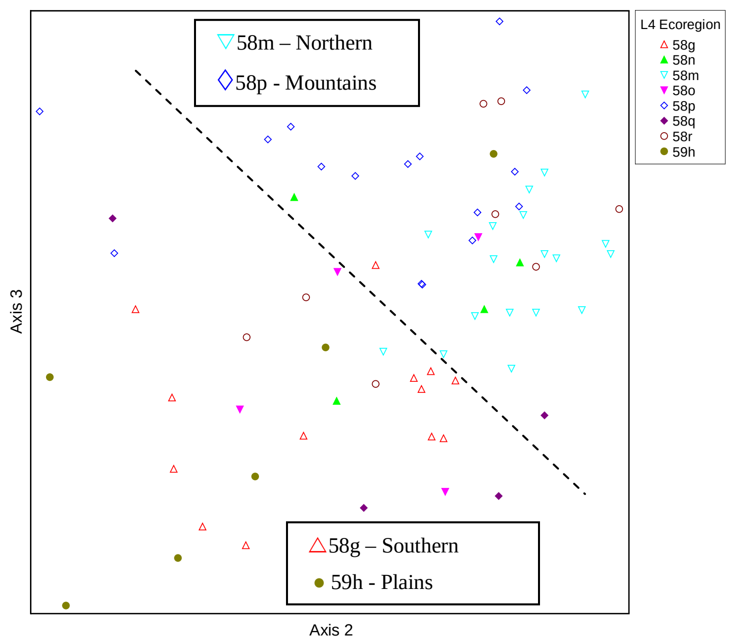


Table 7. Number of reference sites in new cluster groups and EPA Level IV ecoregions.

	Level IV ecounits								
Cluster Group	58g	58m	58n	58o	58p	58q	58r	59h	Total
2		12	1		7		6		26
5	7	4	2	2	3	2	1		21
22		2	1	1	6	2		1	13
32	6			1			1	2	10
58								2	2
Total	13	18	4	4	16	4	8	5	72

3.5 Variable Distributions

Relationships between cluster groups and the most important environmental variables discovered through taxa presence / absence ordinations were further explored to discern their utility as independent predictors of site class. Initially, K-W tests provided a basic interpretation of the level of distinction that could be made between the cluster groups using each environmental variable. Based on K-W test results, 14 of 24 (58.3%) of the environmental variables were significantly ($p<0.05$) different between the cluster groups (Table 8). Of these, and similar to the NMS ordination, elevation, latitude, width, and pH proved to be important environmental variables useful for discriminating between the cluster groups. While additional environmental variables were significantly different across cluster groups, only longitude was considered as a potentially useful predictor of the biological groupings.

Table 8. Kruskal-Wallis (K-W) test results for environmental variables across biological cluster groups. Grey boxes indicate variables that were significantly different ($p<0.05$). K-W rank based on Chi-square value. NMS axis ranks from table 3. See appendix A for abbreviations.

Variable	Chi-Square	Significance	K-W rank	NMS Axis 1 rank	NMS Axis 2 rank
ELEV_FEET	29.05	7.64E-06	1	3	3
LAT_DD	27.65	1.47E-05	2	1	4
pre_n_conc	27.23	1.79E-05	3	7	1
pre_p_conc	22.66	0.000	4	2	2
flow	18.32	0.001	5	9	
SQ_MILES	17.37	0.002	6	6	
width	16.35	0.003	7	5	6
dom%_fix	15.72	0.003	8		
%water_all	13.31	0.010	9		
spcond	11.50	0.022	10		
LONG_DD	10.86	0.028	11		
avg_V	10.53	0.032	12		
pH	9.91	0.042	13	4	7
v_stdev	9.63	0.047	14		8
d_stdev	8.78	0.067	15		10
%sp_r_slp	7.61	0.107	16		9
alk	6.98	0.137	17		
avg_D	6.55	0.162	18		
%decid_all	6.27	0.180	19	10	
per_dom_3	6.06	0.195	20		
measure_grd	5.52	0.238	21		
per_dom_p	3.22	0.522	22		
dom_p	0.78	0.941	23		
%conif_all	0.71	0.950	24		

The rationale for excluding the remaining variables as useful independent predictors of the biological groupings was threefold. First, we attempted to avoid variable redundancy by excluding stream flow and width which were significantly correlated with drainage area with Pearson correlation coefficients of 0.83 and 0.67, respectively. Second, we excluded variables that had narrow ranges of values across the cluster groups, were coarse estimates of watershed characteristics, or were represented by a limited number of field measurements from a given site. These included percentage of surface water in the upstream drainage area, average stream velocity, and variability in stream velocity. Finally, predicted nitrogen and phosphorus concentrations, as well as measured specific conductance were excluded as they represent non-static environmental variables subject to human influence.

The distributions of environmental variables retained as useful predictors showed a separation across biological groups and suggested possible thresholds for defining site classes (Figure 7). First, it was evident that a clear distinction of group 58 from the remaining groups could be made based on pH. As noted above, group 58 had limited membership (2 sites), but was retained as a distinct biological group and, based on pH, can be used to described an assemblage type that is associated with waters that have a naturally low pH (<6.0). Such conditions are uncommon, but can occur in streams or rivers that drain areas with an abundance of wetlands where natural processes produce organics acids that reduce the water's pH. The identification of naturally low pH sites should not, however, be confused with sites where regional atmospheric deposition is responsible for the observed condition (See discussion for further clarification).

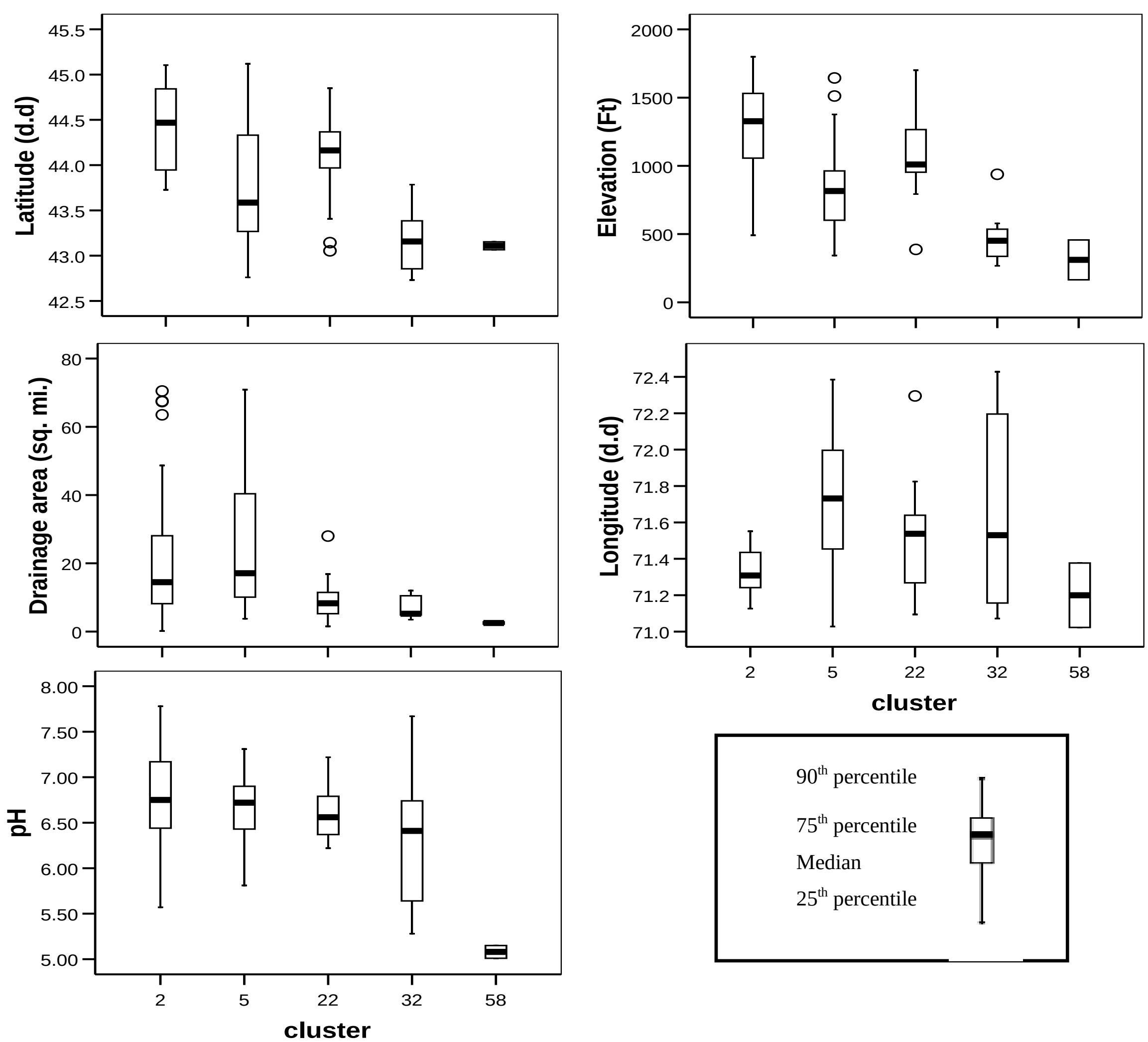
Second, groups 2, 5, and 22 could be distinguished from group 32 based on latitude and elevation (Figure 7) with sites from group 32 having a more southern locations (<43.5^{o} north) and at a lower elevation (<600ft). Geographically speaking we termed sites belonging to group 32 as "plains" sites since they were most commonly associated with southern lowland areas.

Third, group 5 was separated from groups 2 and 22 based on elevation, with group 5 sites occurring at lower elevations (<1000ft) than groups 2 or 22. Based on this observation, group 5 sites were termed "hills" sites. Lastly, to a limited extent, groups 2 and 22 were differentiated by drainage area, with group 2 sites draining slightly larger areas (>15 sq. mi.) than group 22 sites. However, this distinction was not complete, therefore, based the overlap in the distribution of the environmental characters for these two groups, we concluded it would difficult to predict class membership of these sites to their respective cluster group. However, the distinction of groups 2 and 22 from groups 5, 32, and 58 based on elevation led us to term these sites, collectively, as "mountain" sites.

The ability to distinguish four of the five cluster groups based on environmental variables provided a framework for constructing a refined classification system. We opted to emphasize a site's specific environmental characteristics as the basis for classification over a pure ecoregionally-based system since it appeared that basic qualitative rules could be constructed to predict a site's class membership. These rules were:

1) Naturally low pH sites belong to group 58 (acidic);
2) Southerly sites belong to group 32 (plains);
3) Northern sites at lower elevations belong to group 5 (hills);
4) Northern sites at higher elevations belong to groups 2 / 22 (mountains)

Figure 7. Distributions of important environmental variables by natural macroinvertebrate groups.



3.6 Discriminant Function Analysis (DFA)

DFA was completed using the continuous environmental variables as potential predictors of site classification. Cluster group was used as the predictive class. For DFA, clusters 2 and 22 were combined into a single biological group (collectively termed "mountains") due to their similar environmental characters, namely northerly latitudes and higher elevations. Based on the framework outlined above that used the distribution of independent variables, a DFA model that included latitude, elevation, pH, drainage area, and longitude was constructed. Overall the model accounted for 92 percent of the variation in the structure of the data on the first two discriminate functions with each of the variables as significant components ($p<0.05$) of the model (Table 9).. The placement of sites on the ordination diagram based on their respective scores for discriminant functions 1 and 2 displayed marginally distinct groupings for the mountains (groups 2 and 22 = group 24), hills (group 22); plains (group 32), and naturally acidic sites (group 58) (Figure 8). The model correctly classified 65 percent of the sites into their original cluster group (Table 10).

Table 9. Results of discriminate function analysis used for site assignment to a predictive class using 10 environmental variables.

Variable	Wilks' Lambda	F	df1	df2	Sig.	Function 1	Function 2
Elevation	0.624	13.641	3	68	<0.001	0.002	0.001
Drainage Area	0.884	2.963	3	68	0.038	0.004	0.025
Longitude	0.817	5.082	3	68	0.003	0.276	1.378
Latitude	0.622	13.774	3	68	<0.001	0.522	-1.218
pH	0.737	8.074	3	68	<0.001	0.662	1.256
Constant						-48.852	-54.438
Eigenvalue						0.775	0.373
% of variance						62.0	29.9

Figure 8. Ordination of sites for discriminate functions 1 and 2. Cluster refers to original natural biological group. Cluster 24 (2+22) = Mountains; Cluster 5 = Hills; Cluster 32 = Plains; Cluster 58 = naturally acidic.

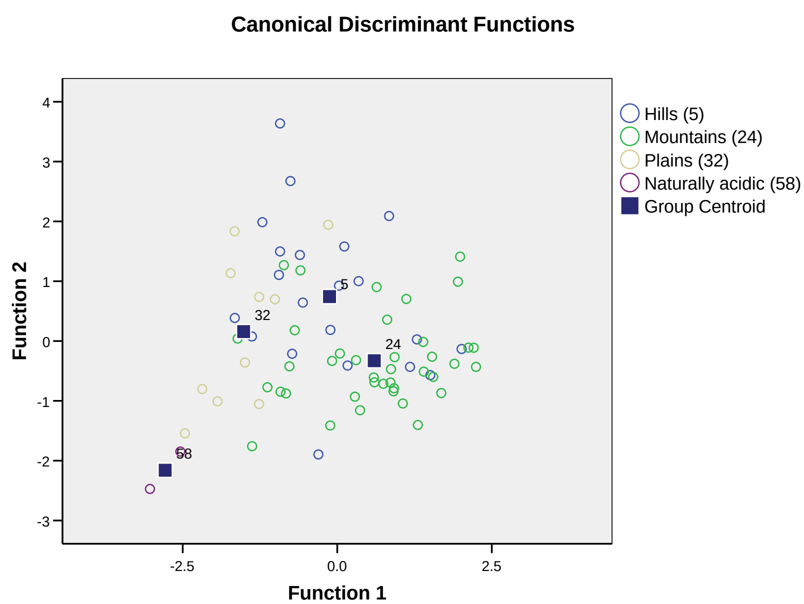


Table 10. Number and percent of sites from original cluster group assigned to each predictive class using the 5 variable discriminate function model. Grey boxes indicate number and percentage of sites correctly classified.

			Predicted Class				
		Cluster	**5**	**24**	**32**	**58**	**Total**
Original Class	**Count**	**5**	10	5	5	1	21
		24	5	29	6	1	13
		32	2	0	4	2	10
		58	0	0	0	2	2
	Percent	**5**	47.6	23.8	23.8	4.8	100
		24	12.8	74.4	10.3	2.6	100
		32	20.0	0.0	60.0	20.0	100
		58	0.0	0.0	0.0	100.0	100

3.7 Prediction of natural classes

The non-linear predictive process for placing sites into the four discernable natural classes [mountains (cluster groups 2 and 22) , hills (cluster group 5), plains (cluster group 32, naturally acidic (cluster group 58)] based on environmental characteristics included iterative review of the results from NMS, individual environmental variable distributions, and DFA. Evidence from these analyses defined the most important environmental characteristics by which we attempted to define a set of logical decision rules for complete or partial class membership. Logical rules were transformed into calculable equations in order to assign a relative likelihood of class membership.

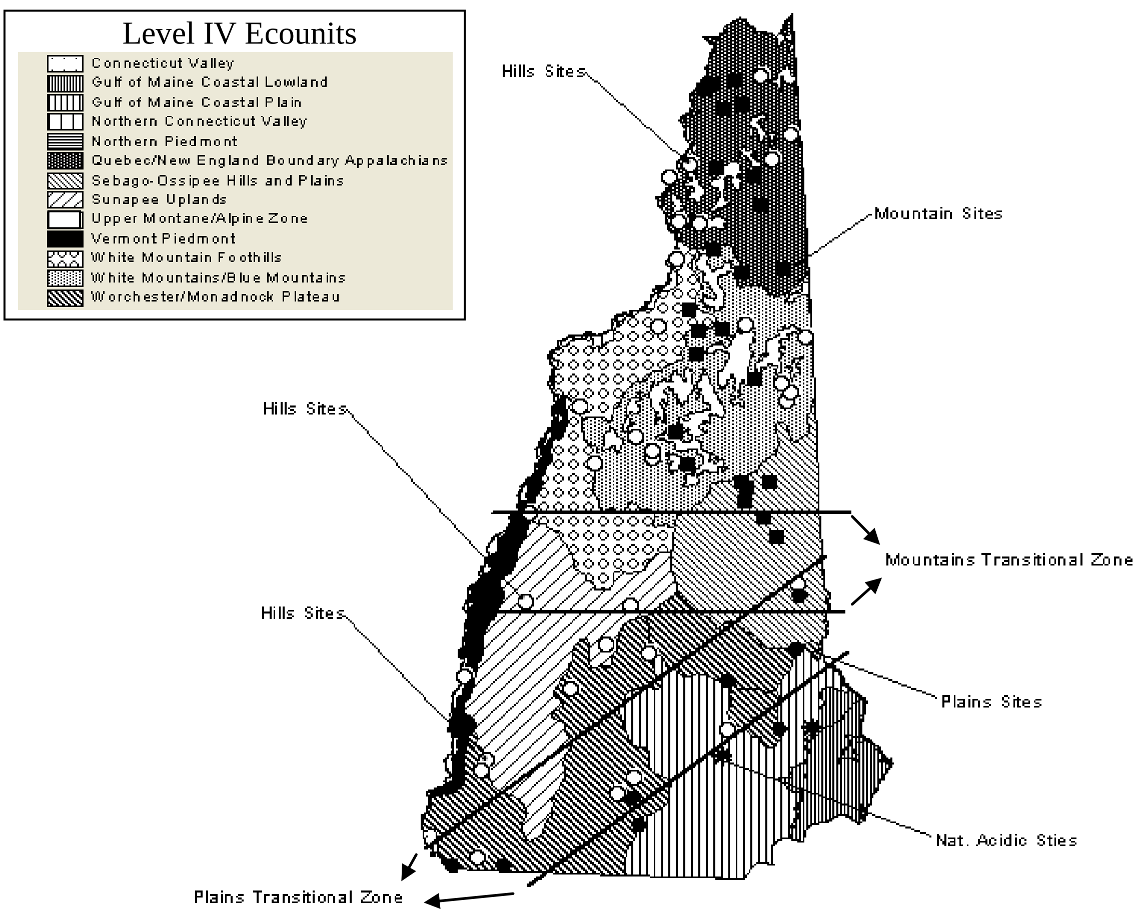
From the outset we attempted to classify sties through the use of four basic environmental categories; natural level of acidity, geographic location, elevation, and stream size. The critical thresholds of the most important classification variables that separated biological groups were identified through the placement of samples locations on a statewide map and the distribution of environmental characteristics using box and whisker plots (See Figure 7). The thresholds were not crisp, but included ranges of values that could be used to predict no, partial, or complete class membership.

First, for sites with naturally acidic conditions a range of acidic severity was developed such that sites with a pH of 5.0 or less would have complete membership to this class and sites with a pH greater than 6.0 would have no membership. For sites between 5.0 and 6.0, membership would be partial and based on a linear relationship.

Next, geographic location was utilized as a partial determinant of class membership to mountains or plains sites. Because latitude, and to a lesser extent longitude, were both important, spatial site distributions by cluster groups was further reviewed on a map with overlays of the Level IV ecoregional schema (Map 2). Mountain and plains sites could be identified based on Level IV ecoregion such that mountain sites almost always fell in northern ecoregions (Quebec/New England boundary Appalachains, upper montaine / alpine zone, White Mountains / Blue Mountains) and plains sites in southern ecoregions

(Gulf of Maine coastal plain, Worchester / Monadonack Plateau, Gulf of Maine coastal lowland). We used this as a partial rule for location. However, the use of latitude was employed as a second identifier within a narrow transitional zone between mountain and hills sites between 43.8 and 43.5 degrees latitude (Map 2). A simple linear regression equation was developed that described the likelihood of membership from 0 to 1 to the mountains class with an increasing likelihood of membership as latitude increased. A third locational rule was developed to describe the transitional zone between plains and hills sites (Map 2). This diagonal zone was located such that sites above the upper border were definitely in the hills whereas sites below the lower border were definitely in the plains. Sites between borderlines were partial members of both classes. Similar to the mountains transitional zone a linear regression equation was developed using latitude and longitude that described the likelihood of membership from 0 to 1 to the plains class such that at a given longitude, southern sites had a greater likelihood of membership. Similarly, given latitude, eastern sites were more likely to be members of the plains class.

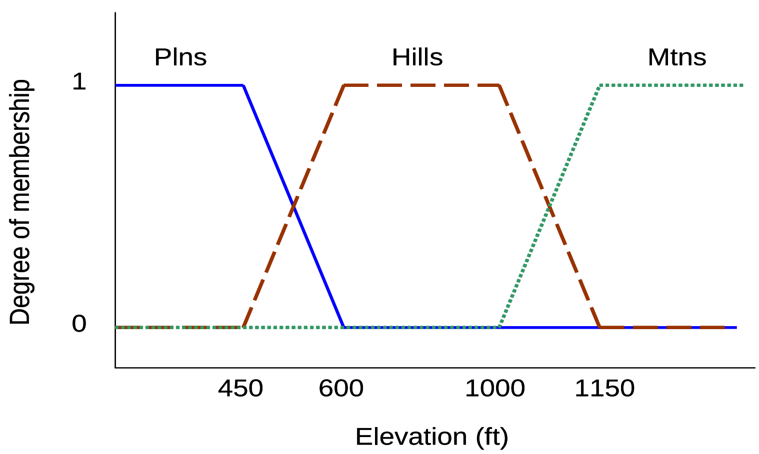
Map 2. Mountain sites (black squares), hills sites (open circles), plains sites (closed circles), and naturally acidic sites (black asterisks) as determined through original cluster analysis. Locations of EPA Level IV ecoregions and mountains and plains transitional zones as partial predictors of class assignment.



The use of elevation as a meaningful variable for differentiating between the cluster groups was approached in similar fashion as above such that pure membership to either the mountains or plains classes was identified through the establishment of logical thresholds. The box and whisker plot of elevation by cluster group (Figure 7) indicated that elevations greater than 1,150 feet were inclusive of sites that belonged to the mountain group. A transitional zone down to 1,000 was identified to define a site's partial class membership to the mountain class as our results indicated that several sites in this range also belonged in the hills group. For sites that fell below 1,000 feet, a zero likelihood of mountain class membership was assigned.

A plains-based elevational relationship was established which related the likelihood of plains class membership between 450 and 600 feet with sites below 400 feet in elevation having pure membership to the plains class, based solely on elevation. Sites over 600 feet in elevation were considered to be non-members of the plains class. As an example, Figure 9 provides a simple graphical display, using elevation, of the non-linear relationship that was developed for use in assigning the degree of class membership of a site the mountains, hills, or plains classes.

Figure 9. Graphical depiction of the site class membership rule for elelvation.



A final rule based on drainage area was developed to distinguish large from small sites that fell in the mountains location. Box and whisker plots indicated that few sites from the mountain group had drainage areas in excess of 50 square miles and greater than 75 percent of these sites had drainage areas less than 30 square miles (Figure 7). From this observation a transitional zone was established between 30 and 50 square miles with a likelihood of membership to the mountain class from one to zero as drainage area increases within this range. The rule was developed to account for sites with large drainage areas within northern ecoregions that were placed in the hills group through cluster analysis.

The final step in predicting degrees of membership for sites to each of the classes was the combination of rules. In the proposed model, an acidic rule was used as a flag, independent of the other rules. Mountain rules were applied such that the location and elevation rules were averaged so that effect of a northern location or high elevation position carries the same weight in determining the likelihood of membership to this class. The final degree of membership to the mountains class was determined as the minimum of either the location and elevation average or the watershed size rule. Linguistically, this says that if a site is large it is less like mountain sites, regardless of location and elevation. Plains sites were identified using the average of the location and elevation membership rules. Hills sites were identified as those that were neither plains nor mountains sites, in degrees inverse to partial memberships to those classes. The mathematical details for determination the likelihood of membership to each of the four classes are outlined in Table 11.

Table 11. Non-linear predictive rules for identifying likelihood of site class membership for New Hampshire wadeable streams.

Acidity If the natural pH is below 5 - 6, the site should be flagged as having effects related to low pH in degrees equal to condition severity. Severity is defined from 0 to 1 (likelihood of class membership) and calculated as: $\textbf{severity}_{\textbf{pH}} = -pH + 6$
Location **Mountains**: If site is in the Upper montaine/alpine zone, boundary Appalachians, or White Mountains/Blue Mountains, then degree of membership in the mountains is 1. If the site is in the Sebago-Ossipee Hills and Plains, then membership is defined from 0 to 1 between the latitudes of 43.5 and 43.8, calculated as: $\mu_{mtn:loc} = 3.3333 * \text{Latitude} - 145$ **Plains**: Degree of membership from 0 to 1 is based on deviation from the upper southwest – northeast diagonal borderline such that (longitude is negative): $\mu_{pln:loc} = 2.5641*((0.5884 * \text{Longitude} + 85.456) - \text{Latitude})$
Elevation **Mountains**: Degree of membership is defined from 0 to 1 between the elevations of 1000 and 1150 feet, calculated as: $\mu_{mtn:elev} = \mathbf{0.006667 * Elevation - 6.66667}$ **Plains**: Degree of membership is defined from 1 to 0 between the elevations of 450 and 600 feet, calculated as: $\mu_{plns:elev} = -0.006667 * \text{Elevation} + 4$
Catchment size **Mountains**: Degree of membership is defined from 1 to 0 between the catchment sizes of 30 and 50 square miles, calculated as: $\mu_{mtn:size} = -0.05 * \text{Catchment Size} + 2.5$
Combination **Mountains**: Minimum of $\mu_{mtn:size}$ and the average of $\mu_{mtn:loc}$ and $\mu_{mtn:elev}$ **Plains**: Average of $\mu_{pln:loc}$ and $\mu_{pln:elev}$ **Hills**: $1 - \mu_{mtn} - \mu_{pln}$

The rules outlined in Table 11 were used to determine each site's level of membership to each of the classes. Of the 72 reference sites, 36 had pure membership to their cluster group, 11 had pure membership to a class other than their cluster group, and 25 were assigned partial membership to multiple classes (hereafter termed "hybrid" sites) (Table 12, Appendix E). Overall, the rule-based classification system classified 77 percent (36 out of 47) of sites with pure membership (non-hybrid) to the respective cluster group. The rule-based classification system recognized 35 percent of the sites as hybrid sites that did not fit crisply into any one single class.

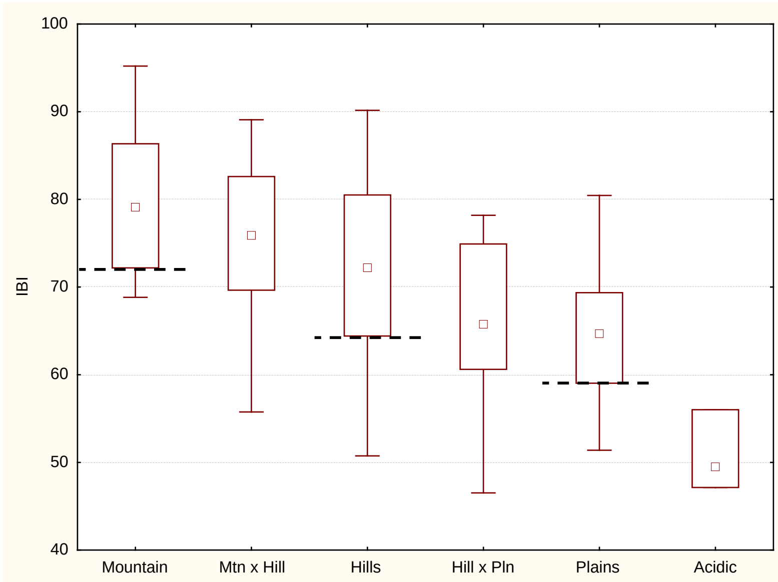
Table 12. Correspondence between cluster groups and predicted class membership using the non-linear logic model for New Hampshire wadeable stream reference sites. Grey boxes indicate number and percent of sites with matching cluster groups and predicted classes.

			Predicted Class					
		Cluster	**Mountain**	**Hills**	**Plains**	**Acidic**	**Hybrid**	**Total**
Cluster Group	**Count**	**Mountain**	19	8	2	0	10	39
		Hills	0	11	0	0	10	21
		Plains	0	0	4	1	5	10
		Acidic	0	0	0	2	0	2
	Percent	**Mountain**	48.7	20.5	5.1	0.0	25.6	100
		Hills	0.0	52.4	0.0	0.0	47.6	100
		Plains	0.0	0.0	40.0	10.0	50.0	100
		Acidic	0.0	0.0	0.0	100.0	0.0	100

3.8 Applications in bioassessment

Using the metrics included in the B-IBI, new impairment thresholds were established based on distributions of index scores for sites with pure class membership (i.e., those with 100% degree of membership to a single predicted class). For reference sites included in this analysis and based on the 25th percentile of the distribution of B-IBI scores, the thresholds were 72 points (out of 100 points) in the mountains class, 64.5 points in the hills class, and 59 points in the plains class (Figure 10). For those sites that were partial members of multiple site classes, a site-specific threshold was calculated by weighting thresholds with the respective degrees of membership in each class. By definition, thresholds for hybrid sites would always fall between 59 and 72.

Figure 10. Distributions of B-IBI scores in pure and hybrid predicted site classes, with proposed impairment thresholds displayed as dashed lines for the pure classes.



To illustrate site classification and threshold development for a hybrid site, we use a site from the Moosilauke River (01M-07) as an example. We calculated the likelihood of class membership based on site information and the classification rules in Table 11, as follows:

Class	Variable	μ	μ (class)
Acidity	pH = 6.72	0, pH > 6.0	0
Mountains	Location (Ecoregion) = White Mtns / Blue Mtns	1 (in the White Mountains/Blue Mountains Level IV ecoregions)	0.5
	Elevation = 815.4 ft	0, elevation < 1000 ft.	
	Catchment Size = 17.1 sq mi	1 (catchment size is less than 30 sq mi.)	
Plains	Location (Lat/Long) = 44.029158 / -71.711912	0 [2.5641*((0.5884 * Long + 85.456) - Lat) < 0]	0
	Elevation = 815.4 ft	0 (Elev > 600 ft)	
Hills			0.5 =(1 - Mountains – Plains)

The example site was similar to mountain sites in location and size, but was like hills sites in elevation, giving it a degree of membership of 0.5 for each of the two site classes, with a zero degree of membership in the plains site class and no natural pH stress. The site-specific impairment threshold was calculated as its degree of membership to each predicted class times the threshold for the respective class, or:

$$\text{Threshold} = \underset{\text{(mountains)}}{(0.5 * 72)} + \underset{\text{(hills)}}{(0.5 * 64.5)} + \underset{\text{(plains)}}{(0.0 * 59)} = 68.25.$$

The B-IBI score for the sample was 64.17, which is below the class-specific threshold of 68.25, indicating biological impairment.

Overall, 66 of the 72 reference sites analyzed achieved or exceeded the respective B-IBI threshold under the previous ecoregional classification system. Under the new classification system, 13 (18 percent) of these reference sites would no longer meet the B-IBI threshold and therefore be subject to a change in assessment status from full support to non-support for aquatic life use (See Appendix E for a full listing of reference sites). None of the sites that did not meet the threshold under the previous classification system achieved the threshold under the refined classification system. When compared to the previous B-IBI classification system, the incidence of assessment status change was greater for sites in the southern (11 of 43 sites) than the northern (2 of 29 sites) ecoregion. On average, the B-IBI threshold increased by 8 points in a paired site comparison of B-IBI thresholds under the previous versus the new classification system.

4. DISCUSSION

The proposed classification scheme recognizes four distinct predictive classes, three (mountains, hills, and plains) of which can be associated with class-specific impairment thresholds. The fourth class, the naturally acidic sites, were not associated with an impairment threshold because this class was underrepresented (n = 2). Sites that do not fall crisply into a single class due to proximity to a class criterion can be associated with multiple classes by infinite degrees through fuzzy classification (i.e. partial likelihood of membership). The degree of membership in a predicted class allows for site specific impairment thresholds that are responsive to site location, elevation, and catchment size.

The advantage of the fuzzy classification is that it allows flexibility for the investigator to use subjective insights and decisions in developing the rules that control the level of predicted class membership. In our case, we identified multiple environmental variables that were related to natural biological groups based on taxonomic composition. The distributions of the most important environmental variables to the respective biological group indicated that logical, non-linear thresholds could be assigned to predict the likelihood of class membership to each group. This approach was in contrast to all-or-nothing class memberships based on a single variable such as location, as with ecoregions or the use of multiple environmental variables in linear mathematical algorithms as with the strict use of DFA.

While DFA could have been used to define probabilities of site membership in individual predictive classes, the fuzzy set procedure was preferred for several reasons. First, the algorithms used to assign probabilities to individual predictive classes in DFA are not transparent to the user as they are typically assigned by statistical software packages. As a result it is difficult to easily apply the model to newly sampled sites. Second, DFA assumes linear relationships between the predictive variables and the resulting discriminate functions. As we demonstrated, the individual environmental variables used to predict class membership had distinctive thresholds whereby above or below the assigned thresholds class membership was nearly complete. In conjunction with these thresholds we defined transitional zones where a site's predicted class membership was incomplete but could be estimated using linear relationships (See figure 9). Finally, DFA derivation is empirical and limits expert input into the selection of classification variables and the definition of breakpoints within those variables. For example, we utilized previous experience in defining fish assemblages in New Hampshire (see below), in part, during the development process for the current classification system.

Our framework and specific rules for the revised classification system was based on multiple lines of evidence (NMS ordination vectors, independent variable distributions, DFA model results) that indicated latitude, longitude, elevation, and drainage area were the most important environmental variables that contributed to a site's biological grouping. However, we also recognized that a single group, however small in sample size, was biologically distinct from all others and was best described through pH. Further, we discovered that ecoregions were useful, to a limited extent, to distinguish mountain sites from plains sites, but that gradients of membership to either of these classes existed that were best evaluated through the use of latitude, longitude, and elevation.

Under the previous classification system utilized by NHDES, B-IBI scores were calculated identically across the state and were compared to the respective threshold values established for two geographically defined site classes (northern, southern ecoregion; see Map 2). The reference site data set used in this study indicated that the previous classification scheme was insufficient for recognizing the biological groups identified in the cluster analysis and NMS ordination. Like the current analysis, the previous classification system indirectly recognized latitude as a dominant determinant of natural macroinvertebrate assemblage types. However, it did not recognize the effects of latitude in the lower two thirds of the state nor did it account for the effects of elevation, catchment size, or pH. The revised classification system takes these environmental characteristics into account while maintaining the coarse utility of ecoregions as a useful component for partial identification of natural macroinvertebrate assemblages.

We recognize that the inclusion of additional reference sites is important for model validation. Assignment of such validation sites to biological groups based on taxonomy alone followed by site class assignment based on environmental characteristics using the logic model is necessary to test the accuracy of the logic model. Model validation, in this manner, will be completed by NHDES as new data becomes available in order to improve the rigor of the revised classification system.

Our results here, based on macroinvertebrates, are confirmed in earlier work by NHDES in classifying fish assemblages (NHDES 2007a, NHDES 2007b, NHDES 2010a). In both cases, the findings indicated that aquatic communities in wadeable streams in New Hampshire are determined, in large part, by latitude, longitude, elevation, and drainage area. Therefore, we feel the revised classification system for the B-IBI is justified in its predictive components. Future adjustments to the revised classification system for the B-IBI are expected to be implemented, where necessary, primarily through minor changes in the mathematical rules that control the likelihood of class membership. Finally, while class membership will always be subject to potential error, the ability to assign partial class membership will, to some extent, correct for these errors through the application of weighted B-IBI thresholds. In this manner, errors will only reflect the extent to which a site was misclassified as a partial, not a pure, member of a particular class.

Ultimately in the refined classification system we combined initial cluster groups 2 and 22 into a single group termed "mountains" because we were unsuccessful at identifying the environmental determinants necessary to predict their occurrence *a-prioi*. The biological differences were evident not only in ordinations, but also in box plots of metric and index distributions. Failure to isolate group 22 as a unique class could result in false positive impairment designations (type I error) as the distributions of B-IBI scores were slightly lower for group 22 (25^{th} percentile = 69) than group 2 (25^{th} percentile = 75) (Figure 3). We attempted to offset this problem by combining sites from group 2 and 22 in the final determination of the mountain class threshold (25^{th} percentile = 72). In this manner we balanced potential impairment decision errors between type I errors for group 22 sites and type II errors (false negative impairment decisions) for group 2 sites. Future investigations with additional environmental variables may allow environmental distinction of the two groups.

The revised classification system also recognizes naturally acidic streams as containing a unique macroinvertebrate assemblage. However, this group was represented by just two reference sites. Given the limited availability of data for this group we felt it was premature to assign a B-IBI threshold to this class for making aquatic life use determinations. Nevertheless, the logic model provides a simple means for determining the severity of pH stress. In the future, for sites that fall into the naturally acidic class (pH <6.0), NHDES will make a site-specific evaluation of the macroinvertebrate data in order to make aquatic life use decisions.

The implementation of the revised classification system represents an overall increase the B-IBI score necessary to achieve full support aquatic life use assessment status. Under the previous classification system, naturally distinct macroinvertebrate assemblages were lumped together and evaluated against two coarse ecoregions. The higher frequency of assessment status change for sites originally assessed using the southern ecoregional B-IBI threshold is in agreement with NHDES' reasoning for developing a more refined classification system. That is, NHDES suspected that under the previous ecoregional classification system sites that fell towards the northern extent of the southern ecoregion (foothills of the White Mountains) were assessed against an unreasonably low B-IBI threshold. Conversely, the use of reference sites at the foothills of the White Mountains as partial determinants of the B-IBI threshold for the southern ecoregion, in effect, led to an artificially high threshold by which sites from "true" southern areas of New Hampshire were compared against. Under this hypothesis, type II assessment errors were more likely for sites at the foothills of the White Mountains while “true” southern sites were more prone to type I assessment errors.

Under the revised classification system we attempted to reduce the amount of variation in the final B-IBI scores used to create the respective class assessment thresholds through the identification of more specific assemblages of macroinvertebrates. Overall B-IBI score variation decreased by just 2 percent based on mean B-IBI score coefficients of variation (CV) for the ecoregional-based classification (CV=14.0; n=72) compared to the revised classification system (CV=13.8; n=72). While this decrease was negligible, the B-IBI thresholds associated with each predictive class in the revised system were more reflective of their respective natural biological condition reducing the likelihood of assessment errors. In addition, the implementation of continuous B-IBI thresholds weighted towards a site’s partial class membership will allow for future sites to be assessed against their expected degree of similarity to multiple natural biological assemblages.

The application of the new classification system in New Hampshire is specific to wadeable streams. Formally, in its 2010 Comprehensive Assessment and Listing Methodology (CALM) NHDES defines these as first through fourth order streams (NHDES 2010b). The data set used herein contained streams throughout the state that had drainage areas that ranged from less than one square mile up to ninety-five square miles. It is recommended that the utilization of the revised classification remain within these limits. Applications of the classification system and current B-IBI to large rivers or intermittent streams may lead to incorrect assessments. Further, the data set analyzed herein is based on streams with naturally firm substrates. Sections of streams with extremely low gradients (<0.05 percent) and naturally soft bottoms (muck) are to be avoided in the application of the B-IBI. Finally, as with any deterministic outcome, best professional judgment by a trained professional is required for a final accurate assessment of biological condition.

In summary, the revised logic-based classification system represents an improvement over the previous ecoregional-based classification system. It was able to recognize four of the five distinct macroinvertebrate assemblages identified through presence or absence of aquatic insect taxa. Partial class membership provided a means to assign sites which did not fit crisply into any one “pure” class to multiple classes based on environmental characteristics. Subsequently, class-specific B-IBI thresholds were set based the 25th percentile of reference sites. Continuous B-IBI thresholds can be computed for future sites based on a site’s likelihood of membership to each of the classes. The revised classification system increased B-IBI thresholds compared to the previous system and is believed to provide an increased and more appropriate level of protection to aquatic communities in wadeable streams.

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Appendix A. Site and Environmental Metadata

Variable	Variable abbreviation	Variable description
Station ID	StationID	Corresponds to EDAS station ID
River Name	River	Name of river or stream sampled
Benthic Sample ID	BenSampID	Corresponds to EDAS benthic sample ID
Assessment Unit ID	AUID	Hydrologic catalog number assigned to river reach sampled
Sparrow model reach ID	Sparrow_rch_ID	Corresponds to USGS Sparrow model stream reach identification number
Major River Basin	BASIN	Major New Hampshire River Basin: 1=Androscoggin; 2=Connecticut; 3=Merrimack; 4=Piscataqua; 5=Saco
Level 4 ecounit	lev4_units	EPA Level IV ecological units; 58m=boundary Appalachians; 59a=Connecticut Valley; 59h= Gulf of Maine Coastal Plain; 59f Gulf of Maine Coastal lowland; 58l= Northeast Kingdom; 58o= Northern CT Valley; 58r=Sebago-Ossipee Hills and Plains; 58q=Sunapee-Cardigan Uplands; 58j=Upper montaine/alpine zone; 58f=Vermont piedmont; 58p=White Mountains/Blue Mountains; 58g=Worchester/Monadonock Plateau
Ecological Drainage Unit (EDU)	TNC_BIOREG	Major ecological units identified by The Nature Conservancy: 1=Androscoggin; 2=Coastal-Merrimack; 3=Lower Connecticut; 4=Upper Connecticut
Benthic IBI bioregion	B_IBI_BIOR	Bioregions previously used by NHDES benthic IBI (based on TNC EDUs): 1=North; 2=South
Stream order	ORDER	Stream order taken from NHDES biomonitoring GIS coverage
Elevation	ELEV_FEET	Elevation of stream at point of sampling (feet)
Drainage Area	SQ_MILES	Area of land draining to point of sampling (square miles); taken from NHDES biomonitoring GIS coverage
Longitude	LONG_DD	Longitude at point of sampling (degree decimals)
Latitude	LAT_DD	Latitude at point of sampling (degree decimals)
Percent surface water	%water	Percent of surface water contained within Sparrow catchment containing sample point; (surface water area / drainage area)*100; surface water area obtained from USGS Sparrow model
Percent coniferous forest	%conif	Percent of coniferous forest contained within Sparrow catchment containing sample point (coniferous forest area / drainage area)*100; coniferous forest area obtained from USGS Sparrow model
Percent deciduous forest	%decid	Percent of deciduous forest contained within Sparrow catchment reach containing sample point (deciduous forest area / drainage area)*100; coniferous forest area obtained from USGS Sparrow model
Predicted total phosphorus concentration	pre_p_conc	Predicted total phosphorus concentration (mg/L) in Sparrow catchment containing sample point; predictions based on USGS Sparrow model
Predicted total nitrogen concentration	pre_n_conc	Predicted total nitrogen concentration (mg/L) in Sparrow catchment containing sample point; predictions based on USGS Sparrow model
Alkalinity	alk	Alkalinity (mg/L $CaCO_3$)
pH	pH	pH (standard units)
Specific conductance	spcond	Specific conductance (μmhos)
Percent slope (sparrow)	%sp_r_slp	River / stream slope (%) in USGS Sparrow reach containing sample point
Dominant particle size category	dom_p	Dominant bottom substrate particle size category; based on 100 particle pebble count; 1=boulder; 2=bedrock; 3=cobble; 4=coarse gravel; 5=sand
Percent dominant size particle category	per_dom_p	Percentage of particles contained in the dominant particle size category
Percent dominance top 3 particle size categories	per_dom_3	Percentage of particles contained in the 3 most dominant particle size categories

Appendix A. con't.

Variable	Variable abbreviation	Variable description
Standard deviation of depth	d_stdev	Standard deviation of cross-sectional water depth measures (ft)
Standard deviation of velocity	v_stdev	Standard deviation of cross-sectional water velocity measures (ft/sec)
Wetted stream width	width	Cross-sectional wetted width of stream/river (ft)
Average water depth	avg_D	Average cross-sectional stream/river depth (ft)
Streamflow	flow	Field measured streamflow (ft^3/sec)
Average water velocity	avg_V	Average cross-sectional stream/river velocity (ft/sec)
Percent dominant geologic division	dom%	Percent of land area consisting of the most dominant geologic division (area dominant geologic division / total drainage area)*100; geologic divisions based on NHDES NHGS lith GIS coverage (Gregg Barker)
Diversity of geologic divisions	geo_div	Categorical rating of drainage area geologic diversity; based on percentage of most dominant geologic division (high percentage=low diversity; low percentage=high diversity); percentages used to create categories as follows: High diversity (hd) <35% area in dominant geologic division; moderate diversity (md) 35-51.25%; intermediate diversity (id) 51.25-67.5%; low diversity (ld) 67.5-83.75%; restricted diversity (rd) >83.75%
Dominant geologic division (watershed)	geo_dom	Geologic division with greatest land area within drainage area A_G (Alkali Granite); CF (Calcgranofels); F_V (Felsic Volcanics); G_O (Granite, Other); M_O (Metamorphic Rocks, other); M_R (Mafic Rocks), P_G (Peraluminous Granite), P_L (Pelitic Rocks); S_S (Sulfidic Schists)
Dominant geologic division (sample point)	geo_pt	Geologic division at point of sample collection A_G (Alkali Granite); CF (Calcgranofels); F_V (Felsic Volcanics); G_O (Granite, Other); M_O (Metamorphic Rocks, other); M_R (Mafic Rocks), P_G (Peraluminous Granite), P_L (Pelitic Rocks); S_S (Sulfidic Schists)

Appendix B. Site and environmental raw data.

StationID	River	BenSampID	AUID	Sparrow_rch_ID	BASIN	lev4_units	TNC_BIOREG	B_IBI_BIOR
97C-167	ASHUELOT	s83	NHRIV802010104-13	30560	Connecticut	58g	3	South
01M-03	Baker River	s237	NHRIV700010301-03	26880	Merrimack	58p	2	South
05P-03	Bean River	s495	NHRIV600030705-05	17097	Coastal	59h	2	South
99M-44	Bear Brook	s172	NHRIV700060503-16	28105	Merrimack	59h	2	South
98S-55	Bearcamp River	s136	NHRIV600020605-09	36457	Saco	58r	2	South
98S-65	Beech River	s161	NHRIV600020701-06	36484	Saco	58r	2	South
98C-9	Bog Brook	s130	NHRIV801010104-02	14147	Connecticut	58m	4	North
NH HEX 10.02	Bog Brook	s277	NHRIV801030101-04	14556	Connecticut	58l	4	North
99M-8	Bradley Brook	s182	NHRIV700030403-09	29784	Merrimack	58q	2	South
NH HEX 11.01	Bumpus Brook	s258	NHRIV400020101-06	40844	Androscoggin	58p	1	North
01S-15	Burnt Knoll Brook	s227	NHRIV600020301-02	35595	Saco	58p	2	South
98C-26	Cherry Mill Brook	s144	NHRIV801010802-01	14520	Connecticut	58p	4	North
98P-79	Churchill Brook	s135	NHRIV600030401-05	16161	Coastal	58r	2	South
98A-13	Clear Stream	s106	NHRIV400010502-01	40721	Androscoggin	58m	1	North
04c-15	Cone Brook	s321	NHRIV801010405-01	14279	Connecticut	58m	4	North
98C-6	Connecticut River	s127	NHRIV801010102-02	14080	Connecticut	58m	4	North
99C-59	Dart Brook	s177	NHRIV802010104-08	30781	Connecticut	58q	3	South
05A-13	Dead Diamond River	s499	NHRIV400010404-01	40710	Androscoggin	58m	1	North
98C-7	Dead Water Stream	s128	NHRIV801010104-05	14152	Connecticut	58m	4	North
05C-09	Dean Brook	s498	NHRIV801010902-01	14412	Connecticut	58o	4	North
98C-39	Deception Brook	s146	NHRIV801030402-01	14677	Connecticut	58p	4	North
98S-57	Deer River	s137	NHRIV600020801-01	36654	Saco	58r	2	South
SP04M-200	Dinesmore Pond Brook	s345	NHRIV700030103-02	30048	Merrimack	58g	2	South
NH HEX 16.02	East Branch Saco River	s283	NHRIV600020301-01	35596	Saco	58p	2	South
98C-90	East Branch Stratford Bog Brook	s156	NHRIV801010602-02	14281	Connecticut	58m	4	North

Appendix B. Con't.

StationID	River	BenSampID	AUID	Sparrow_rch_ID	BASIN	lev4_units	TNC_BIOREG	B_IBI_BIOR
NH HEX 18.01	Eastman Brook	s270	NHRIV700010204-02	26983	Merrimack	58p	2	South
01S-23	Ellis River	s231	NHRIV600020105-02	35612	Saco	58p	2	South
NH HEX 21.05	Grant Brook	s261	NHRIV801040204-02	36958	Connecticut	58q	3	South
05M-17	Hancock Branch East	s502	NHRIV700010104-02	26745	Merrimack	58p	2	South
NH HEX 26.05	Hewes Brook	s271	NHRIV801040402-04	36951	Connecticut	58o	3	South
98C-1	Indian Stream	s122	NHRIV801010201-04	14089	Connecticut	58m	4	North
98C-2	Indian Stream	s123	NHRIV801010202-04	14089	Connecticut	58m	4	North
98C-24	Isreal River	s142	NHRIV801010801-01	14526	Connecticut	58p	4	North
98C-36	Isreal River	s145	NHRIV801010806-06	14515	Connecticut	58l	4	North
NH HEX 23.01	Johnson Brook	s255	NHRIV700010204-03	26983	Merrimack	58p	2	South
03P-02	Lamprey River	s269	NHRIV600030701-01	17167	Coastal	59h	2	South
06c-13	Little Sugar River	s506	NHRIV801060701-12	37372	Connecticut	58o	3	South
05M-01	Lord's Brook	s496	NHRIV700060606-03	29007	Merrimack	58g	2	South
98S-60	Lovell River	s139	NHRIV600020802-04	36577	Saco	58r	2	South
05M-21	M. Branch Piscataquog	s497	NHRIV700060605-12	29314	Merrimack	58g	2	South
06m-01	Mad River	s490	NHRIV700010401-09	26924	Merrimack	58p	2	South
98P-48	Mad River	s147	NHRIV600030601-08	17255	Coastal	58r	2	South
98C-8	Middle Branch Cedar Stream	s129	NHRIV801010104-03	14148	Connecticut	58m	4	North
98C-16	Mill Brook	s113	NHRIV801010705-02	14185	Connecticut	58p	4	North
99C-4	Mirey Brook	s171	NHRIV802010402-04	30947	Connecticut	58g	3	South
98A-20	Moose Brook	s107	NHRIV400010602-07	40763	Androscoggin	58m	1	North
06m-05	Moose Meadow Brook	s491	NHRIV700060701-05	28322	Merrimack	59h	2	South
01M-07	Moosilauke River	s239	NHRIV700010202-06	26784	Merrimack	58p	2	South
98C-15	Nash Stream	s112	NHRIV801010706-05-04	14202	Connecticut	58m	4	North
99M-6	Needle Shop Broo	s181	NHRIV700010803-09	27070	Merrimack	58q	2	South
06p-07	Oyster River	s492	NHRIV6000030902-02	17234	Coastal	59h	2	South
04c-07	Pauchaug Brook	s315	NHRIV802010501-03	31124	Connecticut	58g	3	South

Appendix B. Con't.

StationID	River	BenSampID	AUID	Sparrow_rch_ID	BASIN	lev4_units	TNC_BIOREG	B_IBI_BIOR
98S-44	Paugus Brook	s158	NHRIV600020603-12	36591	Saco	58r	2	South
98P-70	Pawtuckaway River	s105	NHRIV600030703-14	17126	Coastal	59h	2	South
98C-4	Perry Stream	s125	NHRIV801010103-03	14138	Connecticut	58m	4	North
99P-19	Pike Brook	s187	NHRIV600030401-01	16156	Coastal	58r	2	South
NH HEX 53.01	Purgatory Brook	s253	NHRIV700060904-07	28834	Merrimack	59h	2	South
99m-33	Rand Brook	s512	NHRIV700060604-11	28997	Merrimack	58g	2	South
03M-TREND01	Sanborn Brook	s284	NHRIV700060501-22	27760	Merrimack	59h	2	South
98C-10	Simms Stream	s109	NHRIV801010403-02	14227	Connecticut	58m	4	North
98C-11	Simms Stream East Branch	s110	NHRIV801010403-01	14284	Connecticut	58m	4	North
99C-35	Skinner Brook	s175	NHRIV801060401-21	37285	Connecticut	58q	3	South
01S-14	Slippery Brook	s230	NHRIV600020301-03	35657	Saco	58p	2	South
98A-21	Sterns Brook	s108	NHRIV400010604-04	40750	Androscoggin	58m	1	North
99M-5	Stirrup Iron Bro	s180	NHRIV700060101-11	27763	Merrimack	58r	2	South
98C-14	Stratford Bog Brook	s100	NHRIV801010602-03	14209	Connecticut	58o	4	North
05A-15	Swift Diamond River	s500	NHRIV400010405-03	40707	Androscoggin	58m	1	North
98S-43	Swift River	s153	NHRIV600020603-12	36589	Saco	58r	2	South
04c-03	Tully Brook	s311	NHRIV802020203-05	38555	Connecticut	58g	3	South
98C-18	Upper Ammonoosuc River	s115	NHRIV801010702-01	14193	Connecticut	58m	4	North
06m-11	West Branch Warner River	s509	NHRIV700030302-12	29699	Merrimack	58g	2	South
05C-05	Wild Ammonoosuc	s504	NHRIV801030505-08	14423	Connecticut	58l	4	North
06a-03	Wild River	s505	NHRIV400020201-03	25456	Androscoggin	58p	1	North

Appendix B. (con't)

StationID	River	BenSampID	ORDER	ELEV_FEET	SQ_MILES	LONG_DD	LAT_DD	%water	%conif	%decid	pre_p_conc	pre_n_conc	alk	pH	spcond
97C-167	ASHUELOT	s83	6	528	95.3	72.3145	43.0220	2.53	21.68	45.59	0.026	0.494	4.20	6.40	53
01M-03	Baker River	s237	3	963	19.5	71.8723	43.9484	0.01	27.58	34.69	0.016	0.411	4.10	6.99	35
05P-03	Bean River	s495	3	276	4.1	71.1570	43.1460	2.03	14.99	27.82	0.030	0.661	4.80	5.64	46
99M-44	Bear Brook	s172	3	388	9.9	71.3511	43.1441	0.88	37.35	24.13	0.028	0.615	3.30	6.51	43
98S-55	Bearcamp River	s136	4	491	67.6	71.2836	43.8313	0.80	19.37	32.20	0.018	0.395	5.00	6.94	34
98S-65	Beech River	s161	3	520	15.0	71.1565	43.7271	3.83	11.01	26.82	0.022	0.474	8.40	6.47	35
98C-9	Bog Brook	s130	3	1435	8.8	71.2778	45.0298	0.06	22.31	52.19	0.017	0.204	16.20	7.17	44
NH HEX 10.02	Bog Brook	s277	3	953	12.4	71.6181	44.3651	0.33	28.21	20.87	0.030	0.526	7.90	6.77	55
99M-8	Bradley Brook	s182	1	853	2.2	71.8247	43.4067	0.03	8.99	57.16	0.024	0.493	4.20	6.27	24
NH HEX 11.01	Bumpus Brook	s258	2	1359	1.5	71.2681	44.3670	0.00	53.83	17.77	0.020	0.414	1.70	6.79	19
01S-15	Burnt Knoll Brook	s227	3	1023	3.0	71.1096	44.1358	0.44	21.09	36.18	0.015	0.278	4.50	6.39	25
98C-26	Cherry Mill Brook	s144	4	1349	0.2	71.4610	44.3497	0.00	19.12	39.69	0.021	0.404	6.10	6.67	24
98P-79	Churchill Brook	s135	2	578	4.9	71.0714	43.5472	5.61	9.17	48.54	0.024	0.586	7.20	6.34	34
98A-13	Clear Stream	s106	3	1348	19.7	71.2418	44.8119	0.10	30.50	42.53	0.018	0.331	9.30	7.11	77
04c-15	Cone Brook	s321	2	989	7.3	71.5720	44.8132	0.00	14.26	51.85	0.020	0.350	10.10	7.22	39
98C-6	Connecticut River	s127	4	1644	59.9	71.2071	45.1190	4.18	26.66	29.30	0.004	0.257	7.10	6.78	26
99C-59	Dart Brook	s177	5	793	6.1	72.2950	43.0531	2.07	21.03	47.25	0.029	0.551	4.70	6.23	34
05A-13	Dead Diamond River	s499	4	1512	40.4	71.1610	44.8664	1.03	25.17	39.11	0.016	0.229	13.60	6.70	34
98C-7	Dead Water Stream	s128	3	1531	13.3	71.3674	45.0143	0.02	8.71	61.96	0.018	0.220	23.50	7.52	60
05C-09	Dean Brook	s498	2	882	6.1	71.5473	44.5652	0.04	11.61	38.52	0.033	0.607	11.10	5.95	44
98C-39	Deception Brook	s146	3	1739	4.2	71.4702	44.2789	0.15	18.64	35.56	0.016	0.469	8.00	6.65	21
98S-57	Deer River	s137	2	515	4.3	71.1855	43.8894	1.06	24.29	9.69	0.020	0.444	8.00	6.03	28
SP04M-200	Dinesmore Pond Brook	s345	4	950	25.7	71.9964	42.9103	8.78	15.30	46.63	0.018	0.368	8.00	5.81	45
NH HEX 16.02	East Branch Saco River	s283	3	1701	9.9	71.1299	44.1905	0.01	26.54	31.29	0.015	0.281	2.20	6.22	18
98C-90	East Branch Stratford Bog Brook	s156	2	1548	2.4	71.4960	44.6817	0.00	13.52	40.40	0.020	0.362	8.00	6.88	38

Appendix B. (con't)

StationID	River	BenSampID	ORDER	ELEV_FEET	SQ_MILES	LONG_DD	LAT_DD	%water	%conif	%decid	pre_p_conc	pre_n_conc	alk	pH	spcond
NH HEX 18.01	Eastman Brook	s270	3	992	11.5	71.6395	43.9892	0.10	32.30	29.26	0.015	0.363	1.90	6.37	19
01S-23	Ellis River	s231	3	1180	13.6	71.2378	44.2020	0.04	38.60	32.53	0.013	0.263	1.70	6.40	55
NH HEX 21.05	Grant Brook	s261	2	743	11.9	72.1339	43.8010	0.42	16.32	46.33	0.036	0.617	13.70	7.29	111
05M-17	Hancock Branch East	s502	4	1532	11.9	71.5516	44.0419	0.00	55.41	8.95	0.014	0.312	2.10	5.57	38
NH HEX 26.05	Hewes Brook	s271	3	459	10.6	72.1956	43.7851	0.15	24.01	35.79	0.037	0.523	25.85	7.67	170
98C-1	Indian Stream	s122	4	1307	63.5	71.4097	45.0924	0.13	9.20	46.82	0.018	0.249	16.30	7.53	49
98C-2	Indian Stream	s123	4	1229	67.4	71.4354	45.0744	0.13	9.20	46.82	0.018	0.249	17.20	7.78	76
98C-24	Isreal River	s142	4	1457	8.2	71.3699	44.3550	0.00	29.08	39.96	0.019	0.416	6.65	6.44	28
98C-36	Isreal River	s145	5	1057	70.5	71.4989	44.4125	0.07	19.48	41.19	0.024	0.462	8.50	6.69	46
NH HEX 23.01	Johnson Brook	s255	3	1010	5.2	71.6440	43.9690	0.00	15.38	52.16	0.015	0.363	4.15	6.56	25
03P-02	Lamprey River	s269	3	452	4.9	71.2298	43.1688	1.35	32.07	15.02	0.028	0.638	2.20	5.90	34
06c-13	Little Sugar River	s506	4	343	29.3	72.3853	43.3057	0.25	26.26	36.31	0.037	0.566	12.70	7.26	81
05M-01	Lord's Brook	s496	2	536	3.5	71.7279	42.9388	0.24	41.51	15.39	0.030	0.710	2.80	5.28	39
98S-60	Lovell River	s139	3	655	14.0	71.2083	43.7850	1.37	6.22	55.41	0.017	0.405	3.50	6.36	19
05M-21	M. Branch Piscataquog	s497	4	496	15.8	71.7181	43.0035	2.46	22.36	33.50	0.043	0.626	9.90	6.07	71
06m-01	Mad River	s490	4	1435	24.7	71.5108	43.9467	0.08	37.09	19.74	0.015	0.559	3.50	6.52	35
98P-48	Mad River	s147	3	343	10.5	71.0841	43.3852	0.19	13.22	29.15	0.038	0.589	2.40	6.73	37
98C-8	Middle Branch Cedar Stream	s129	3	1433	12.6	71.2778	45.0261	0.07	4.19	67.46	0.017	0.216	25.70	7.48	68
98C-16	Mill Brook	s113	3	1063	15.5	71.4051	44.5946	0.02	15.71	50.96	0.019	0.349	5.70	6.81	24
99C-4	Mirey Brook	s171	4	591	13.8	72.3297	42.7606	0.81	24.76	38.33	0.029	0.599	9.10	6.43	62
98A-20	Moose Brook	s107	2	1550	3.6	71.2043	44.7277	1.09	14.31	63.88	0.024	0.455	11.50	6.58	30
06m-05	Moose Meadow Brook	s491	2	457	2.8	71.3769	43.0661	0.31	18.83	25.33	0.039	0.825	11.50	5.01	43
01M-07	Moosilauke River	s239	4	815	17.1	71.7119	44.0292	0.06	16.40	48.36	0.015	0.339	1.90	6.72	76
98C-15	Nash Stream	s112	4	1377	38.4	71.4539	44.6758	0.47	27.69	36.96	0.018	0.299	5.00	6.78	25
99M-6	Needle Shop Broo	s181	3	686	6.5	71.7317	43.5200	0.16	18.48	35.60	0.037	0.697	6.43	6.85	38
06p-07	Oyster River	s492	2	165	2.2	71.0227	43.1529	0.08	16.57	16.30	0.026	0.605	0.80	5.15	48
04c-07	Pauchaug Brook	s315	3	337	5.1	72.4281	42.7313	0.50	13.62	46.20	0.035	0.663	5.30	6.48	72

Appendix B. (con't)

StationID	River	BenSampID	ORDER	ELEV_FEET	SQ_MILES	LONG_DD	LAT_DD	%water	%conif	%decid	pre_p_conc	pre_n_conc	alk	pH	spcond
98S-44	Paugus Brook	s158	3	743	25.3	71.2968	43.8934	0.54	28.40	32.39	0.016	0.415	3.00	6.07	21
98P-70	Pawtuckaway River	s105	3	133	24.6	71.1304	43.0452	6.48	18.28	24.31	0.006	0.419	8.00	6.58	152
98C-4	Perry Stream	s125	3	1579	24.4	71.3200	45.1043	0.12	11.92	52.62	0.016	0.228	19.20	7.41	52
99P-19	Pike Brook	s187	2	601	3.8	71.0687	43.5864	0.11	20.59	27.66	0.028	0.526	9.60	7.06	51
NH HEX 53.01	Purgatory Brook	s253	3	267	12.0	71.6993	42.8554	1.12	27.31	24.53	0.040	0.674	4.60	6.74	57
99m-33	Rand Brook	s512	3	615	10.1	71.7855	42.9566	1.02	19.07	36.26	0.033	0.587	7.60	6.89	49
03M-TREND01	Sanborn Brook	s284	2	450	10.1	71.3605	43.2927	1.86	24.68	27.75	0.050	0.580	6.80	7.13	65
98C-10	Simms Stream	s109	4	1266	28.0	71.4933	44.8494	0.28	22.10	34.94	0.023	0.298	18.95	7.14	54
98C-11	Simms Stream East Branch	s110	2	1799	2.3	71.3866	44.8419	0.00	32.54	17.45	0.026	0.296	21.15	7.16	60
99C-35	Skinner Brook	s175	4	1225	5.1	72.1425	43.5319	1.41	20.35	45.25	0.032	0.631	5.50	7.31	45
01S-14	Slippery Brook	s230	3	1369	8.3	71.0939	44.1622	1.44	12.66	47.25	0.016	0.294	2.00	6.70	21
98A-21	Sterns Brook	s108	3	1225	34.7	71.1263	44.5331	0.00	35.31	28.68	0.018	0.347	8.00	6.92	32
99M-5	Stirrup Iron Bro	s180	3	417	5.9	71.6610	43.3759	2.55	18.39	29.14	0.044	0.645	5.27	6.49	40
98C-14	Stratford Bog Brook	s100	3	1011	16.9	71.5379	44.6783	0.30	15.07	44.41	0.022	0.396	9.00	6.81	33
05A-15	Swift Diamond River	s500	5	1360	70.9	71.0853	44.9426	0.12	14.94	51.14	0.017	0.263	15.60	6.43	34
98S-43	Swift River	s153	3	645	28.1	71.2748	43.8733	0.54	28.40	32.39	0.016	0.361	3.50	6.43	28
04c-03	Tully Brook	s311	2	938	5.3	72.2322	42.7365	0.92	15.28	56.30	0.025	0.535	7.50	5.51	48
98C-18	Upper Ammonoosuc River	s115	5	1157	48.7	71.2879	44.5235	0.21	14.97	52.77	0.018	0.366	8.00	7.26	32
06m-11	West Branch Warner River	s509	4	670	10.9	71.9674	43.2678	0.34	18.34	54.15	0.030	0.490	9.00	6.90	45
05C-05	Wild Ammonoosuc	s504	4	848	48.3	71.9318	44.1218	0.45	23.56	34.71	0.018	0.392	4.70	6.51	51
06a-03	Wild River	s505	4	954	44.6	71.0278	44.3313	0.03	34.08	35.99	0.016	0.281	0.90	6.66	15

Appendix B. (con't)

StationID	River	BenSampID	%sp_r_ slp	dom_p	per_ dom_p	per_ dom_3	d_stdev	v_stdev	width	avg_D	flow	avg_V	dom%	geo_div	geo_dom	geo_pt
97C-167	ASHUELOT	s83	0.35	coarse gravel	64.0	100.0	0.36	0.77	27	0.87	47.5	1.77	40.3	md	G_O	P_G
01M-03	Baker River	s237	2.36	boulder	51.0	95.0	0.46	0.61	36	0.75	22.7	0.72	51.1	md	P_L	G_O
05P-03	Bean River	s495	0.64	boulder	65.0	95.0	0.36	0.36	20	0.58	3.9	0.24	39.4	md	P_L	P_G
99M-44	Bear Brook	s172	0.59	coarse gravel	52.0	84.0	0.51	0.36	24	1.04	7.6	0.29	47.4	md	M_R	M_R
98S-55	Bearcamp River	s136	0.57	cobble	59.0	94.0	0.25	0.75	58	0.73	74.9	1.66	52.1	id	P_G	P_L
98S-65	Beech River	s161	0.57	coarse gravel	50.6	80.2	0.29	0.63	24	0.63	14.0	0.72	64.1	id	M_R	M_R
98C-9	Bog Brook	s130	2.17	cobble	45.0	100.0	0.29	0.29	12	0.89	3.7	0.33	62.9	id	P_L	C_F
NH HEX 10.02	Bog Brook	s277	0.69	coarse gravel	37.0	85.0	0.21	1.11	20	0.71	31.3	1.98	95.1	rd	G_O	M_R
99M-8	Bradley Brook	s182	2.32	boulder	58.0	88.0	0.39	0.05	22	1.15	1.4	0.04	58.2	id	G_O	G_O
NH HEX 11.01	Bumpus Brook	s258	14.29	boulder	62.0	98.0	0.24	0.47	19	0.58	2.0	0.40	88.1	rd	G_O	G_O
01S-15	Burnt Knoll Brook	s227	2.36	cobble	38.0	87.0	0.44	0.44	14	1.01	0.3	0.18	99.6	rd	G_O	G_O
98C-26	Cherry Mill Brook	s144	2.12	boulder	51.2	91.9	0.33	0.50	19	0.74	4.9	0.67	100.0	rd	S_S	P_L
98P-79	Churchill Brook	s135	1.62	cobble	52.0	99.0	0.22	0.09	11	0.79	2.0	0.16	29.3	hd	A_G	P_L
98A-13	Clear Stream	s106	0.85	boulder	33.3	72.0	0.43	0.65	27	0.92	10.4	0.43	46.6	md	P_L	P_R
04c-15	Cone Brook	s321	7.39	cobble	36.8	97.1	0.19	0.40	11	0.43	3.5	0.66	51.7	id	P_G	P_L
98C-6	Connecticut River	s127	0.53	boulder	52.4	96.8	0.39	0.97	22	0.76	45.3	2.27	51.2	md	P_L	P_L
99C-59	Dart Brook	s177	2.37	cobble	72.0	100.0	0.24	0.13	10	0.60	0.8	0.13	41.7	md	G_O	G_O
05A-13	Dead Diamond River	s499	0.53	boulder	44.0	90.0	0.25	0.24	40	1.02	19.3	0.48	32.5	hd	G_O	CF
98C-7	Dead Water Stream	s128	2.02	boulder	52.0	97.0	0.33	0.35	20	0.43	4.1	0.32	89.1	rd	G_O	G_O
05C-09	Dean Brook	s498	0.91	coarse gravel	36.0	87.0	0.32	0.22	19	0.44	2.6	0.17	52.0	id	A_G	P_L
98C-39	Deception Brook	s146	2.54	boulder	51.2	91.9	0.33	0.50	20	0.74	7.2	0.67	68.0	ld	P_G	F_V
98S-57	Deer River	s137	0.88	coarse gravel	45.0	87.7	0.33	0.50	20	0.74	7.2	0.67	94.3	rd	G_O	P_R
SP04M-200	Dinesmore Pond Brook	s345	0.20	coarse gravel	45.0	87.7	0.33	0.50	27	0.74	19.6	0.67	71.5	ld	G_O	G_O
NH HEX 16.02	East Branch Saco River	s283	3.22	boulder	41.0	97.0	0.23	0.66	16	1.04	16.2	0.99	49.4	md	P_L	P_L

Appendix B. (con't)

StationID	River	BenSampID	%sp_r_ slp	dom_p	per_ dom_p	per_ dom_3	d_stdev	v_stdev	width	avg_D	flow	avg_V	dom%	geo_div	geo_dom	geo_pt
98C-90	East Branch Stratford Bog Brook	s156	4.36	boulder	46.1	95.1	0.33	0.50	20	0.74	6.1	0.67	64.3	id	P_L	P_L
NH HEX 18.01	Eastman Brook	s270	1.43	cobble	54.0	92.0	0.23	0.64	30	0.48	10.9	0.75	43.1	md	P_L	M_R
01S-23	Ellis River	s231	4.11	boulder	40.0	97.0	0.46	1.05	34	0.76	31.1	0.98	92.8	rd	A_G	A_G
NH HEX 21.05	Grant Brook	s261	2.54	cobble	41.6	88.3	0.38	0.58	23	0.56	12.9	0.81	34.9	hd	P_L	G_O
05M-17	Hancock Branch East	s502	2.62	cobble	38.8	88.8	0.31	0.54	48	0.52	16.2	0.53	100.0	rd	P_G	P_L
NH HEX 26.05	Hewes Brook	s271	2.27	bedrock	54.6	90.9	0.44	1.10	20	0.47	15.9	1.24	53.7	id	A_G	A_G
98C-1	Indian Stream	s122	0.36	boulder	55.0	94.0	0.51	0.41	36	1.05	22.8	0.65	94.3	rd	P_G	P_G
98C-2	Indian Stream	s123	0.36	cobble	47.0	95.0	0.45	0.23	52	1.05	26.5	0.47	94.7	rd	M_R	M_R
98C-24	Isreal River	s142	2.41	boulder	60.0	89.0	0.23	0.30	16	0.92	6.4	0.36	38.7	md	S_S	P_L
98C-36	Isreal River	s145	0.18	cobble	35.0	84.0	0.33	0.56	19	0.90	64.2	0.94	69.3	ld	P_L	G_O
NH HEX 23.01	Johnson Brook	s255	1.43	boulder	63.0	100.0	0.32	0.19	20	0.66	3.7	0.22	62.3	id	M_O	P_L
03P-02	Lamprey River	s269	0.82	cobble	57.3	96.9	0.23	0.11	19	0.52	0.8	0.06	63.4	id	CF	CF
06c-13	Little Sugar River	s506	2.09	cobble	51.0	94.0	0.42	0.41	23	1.07	51.8	0.61	23.6	hd	G_O	G_O
05M-01	Lord's Brook	s496	0.72	coarse gravel	32.5	86.3	0.09	0.37	9	0.32	1.2	0.44	96.3	rd	G_O	G_O
98S-60	Lovell River	s139	1.47	boulder	57.1	89.3	0.32	0.42	30	0.65	21.9	0.90	87.6	rd	A_G	A_G
05M-21	M. Branch Piscataquog	s497	0.97	coarse gravel	35.0	73.0	0.29	0.28	30	0.73	10.3	0.41	43.1	md	A_G	A_G
06m-01	Mad River	s490	1.54	boulder	61.0	99.0	0.48	0.82	39	0.87	44.8	1.10	58.8	id	P_L	G_O
98P-48	Mad River	s147	1.38	cobble	48.0	93.0	0.16	0.72	39	1.04	44.8	0.85	37.5	md	P_L	M_R
98C-8	Middle Branch Cedar Stream	s129	1.52	cobble	49.5	87.0	0.23	0.42	20	0.72	6.1	0.44	57.8	id	P_L	P_L
98C-16	Mill Brook	s113	3.54	boulder	54.7	92.3	0.45	1.05	24	1.26	24.0	0.79	100.0	rd	A_G	P_L
99C-4	Mirey Brook	s171	0.08	cobble	46.0	93.0	0.30	0.47	24	0.51	7.6	0.56	60.2	id	A_G	A_G
98A-20	Moose Brook	s107	3.60	coarse gravel	38.9	87.6	0.15	0.45	14	0.20	1.3	0.30	57.7	id	P_L	M_R
06m-05	Moose Meadow Brook	s491	1.06	boulder	49.0	92.0	0.34	0.23	20	0.62	3.4	0.27	100.0	rd	G_O	G_O
01M-07	Moosilauke River	s239	2.25	boulder	42.0	89.0	0.45	0.43	20	1.09	18.2	1.75	98.6	rd	G_O	G_O
98C-15	Nash Stream	s112	1.41	cobble	48.0	92.0	0.42	0.25	53	1.14	27.1	0.40	62.4	id	P_G	P_L
99M-6	Needle Shop Broo	s181	1.94	boulder	51.2	91.9	0.33	0.50	21	0.74	8.5	0.67	45.2	md	G_O	G_O

Appendix B. (con't)

StationID	River	BenSampID	%sp_r_ slp	dom_p	per_ dom_p	per_ dom_3	d_stdev	v_stdev	width	avg_D	flow	avg_V	dom%	geo_div	geo_dom	geo_pt
06p-07	Oyster River	s492	0.94	coarse gravel	54.0	96.0	0.27	0.14	12	0.84	2.0	0.17	99.5	rd	P_G	P_G
04c-07	Pauchaug Brook	s315	2.37	boulder	47.0	88.0	0.10	0.35	4	0.25	1.0	0.86	65.4	id	A_G	A_G
98S-44	Paugus Brook	s158	1.90	boulder	40.4	89.9	0.40	0.89	40	0.94	48.4	1.10	88.2	rd	G_O	G_O
98P-70	Pawtuckaway River	s105	0.42	coarse gravel	45.0	87.7	0.33	0.50	27	0.74	18.9	0.67	37.1	md	P_L	P_G
98C-4	Perry Stream	s125	0.41	cobble	31.0	78.0	0.30	0.40	28	0.66	9.8	0.44	94.3	rd	G_O	G_O
99P-19	Pike Brook	s187	0.09	coarse gravel	45.0	87.7	0.33	0.50	20	0.74	6.9	0.67	66.4	id	P_L	P_L
NH HEX 53.01	Purgatory Brook	s253	1.73	cobble	42.0	85.0	0.34	0.40	20	0.54	3.4	0.27	79.9	ld	G_O	G_O
99m-33	Rand Brook	s512	0.81	boulder	30.0	83.0	0.35	0.25	23	0.53	5.5	0.40	41.7	md	G_O	G_O
03M-TREND01	Sanborn Brook	s284	1.11	cobble	55.6	90.1	0.31	0.16	18	0.63	2.2	0.11	91.7	rd	G_O	G_O
98C-10	Simms Stream	s109	1.30	boulder	62.8	95.8	0.37	0.52	27	0.60	13.5	0.65	41.8	md	S_S	S_S
98C-11	Simms Stream East Branch	s110	2.15	coarse gravel	37.0	74.0	0.19	0.51	15	0.36	3.1	0.52	77.9	ld	P_L	P_L
99C-35	Skinner Brook	s175	2.38	boulder	51.2	91.9	0.33	0.50	21	0.74	7.7	0.67	38.4	md	P_L	P_L
01S-14	Slippery Brook	s230	4.03	cobble	49.0	97.0	0.35	0.73	30	0.59	13.8	0.65	83.0	ld	G_O	G_O
98A-21	Sterns Brook	s108	0.64	coarse gravel	44.0	85.0	0.17	0.76	20	0.90	18.3	1.40	71.6	ld	P_L	M_R
99M-5	Stirrup Iron Bro	s180	2.99	boulder	51.2	91.9	0.33	0.50	21	0.74	8.1	0.67	62.5	id	M_O	M_R
98C-14	Stratford Bog Brook	s100	2.84	boulder	52.0	100.0	0.40	0.72	11	0.84	12.6	0.96	33.9	hd	G_O	P_L
05A-15	Swift Diamond River	s500	0.11	coarse gravel	39.8	80.6	0.32	0.94	36	0.76	35.7	1.07	65.7	id	A_G	C_F
98S-43	Swift River	s153	1.29	boulder	45.0	82.0	0.32	0.57	32	0.84	25.5	0.72	79.3	ld	F_V	F_V
04c-03	Tully Brook	s311	2.43	cobble	31.0	86.0	0.20	0.08	24	0.65	0.4	0.01	99.9	rd	P_L	G_O
98C-18	Upper Ammonoosuc River	s115	0.58	cobble	37.0	82.0	0.36	0.45	40	0.68	19.6	0.61	62.7	id	S_S	P_L
06m-11	West Branch Warner River	s509	1.29	boulder	72.0	99.0	0.36	0.22	30	1.03	13.5	0.42	93.8	rd	G_O	G_O
05C-05	Wild Ammonoosuc	s504	1.47	boulder	68.7	100.0	0.47	1.20	47	0.47	25.8	0.92	45.5	md	A_G	P_L
06a-03	Wild River	s505	0.95	boulder	44.0	94.0	0.76	0.76	48	1.17	19.6	1.04	73.6	ld	M_O	S_S

Appendix C. Reference site raw biological metrics. Cluster_grp = cluster group; TotalTax = total number taxa; PlecTax= Plecopteran taxa; ChirPct= Percentage of Chironomid individuals; NonInsPct= Percentage of Non-insect individuals; ToterTax= Number of tolerant taxa; IntolPct= Percentage of intolerant individuals; ClingPct= Percentage of clinger individuals.

StationID	Name	LONG_DD	LAT_DD	Cluster_grp	TotalTax	PlecTax	ChirPct	NonInsPct	TolerTax	IntolPct	ClingPct
98A-13	Clear Stream	71.241844	44.81188	2	12.54	3.75	0.00	0.00	0.29	40.41	95.64
98A-21	Sterns Brook	71.126322	44.53313	2	13.61	3.85	1.53	0.00	0.98	46.29	94.72
98C-11	East Branch Simms Stream	71.386635	44.84194	2	18.00	4.27	6.44	0.00	0.00	57.06	76.99
98C-15	Nash Stream	71.453938	44.67577	5	12.91	4.16	8.78	0.00	1.49	19.44	89.23
98C-18	Upper Ammonoosuc River	71.287915	44.5235	2	11.73	1.98	0.00	0.00	1.60	32.53	96.39
98C-1	Indian Stream	71.409699	45.09244	2	13.99	2.31	0.00	0.00	1.72	69.90	90.86
98C-2	Indian Stream	71.43539	45.07435	2	13.91	1.98	0.00	0.18	1.22	77.33	80.49
98C-4	Perry Stream	71.319965	45.10431	2	20.14	4.15	21.52	0.26	2.36	58.01	58.01
98C-7	Dead Water Stream	71.367413	45.01431	2	14.00	5.00	20.44	0.00	0.88	37.23	64.60
98C-8	Middle Branch Cedar Stream	71.277789	45.02614	2	17.00	4.86	0.00	0.00	1.00	53.89	91.82
98C-9	Bog Brook	71.277765	45.02977	2	14.74	4.29	32.77	0.00	0.33	50.59	56.04
98S-55	Bearcamp River	71.283582	43.83133	2	13.08	1.97	2.89	0.00	1.31	34.42	92.50
98S-57	Deer River	71.185548	43.88935	2	15.68	3.94	9.09	0.00	0.00	42.42	75.00
98S-60	Lovell River	71.208345	43.785	2	13.08	2.69	12.71	0.00	1.00	47.46	83.05
98C-26	Cherry Mill Brook	71.461023	44.34972	2	16.91	4.60	0.00	0.00	0.00	85.26	83.24
98C-39	Deception Brook	71.470205	44.27895	2	16.20	2.50	3.93	0.44	1.43	59.83	82.97
98C-90	East Branch Stratford Bog Brook	71.495988	44.68175	2	18.00	6.85	7.72	0.93	2.00	73.46	61.73
98S-44	Paugus Brook	71.296772	43.89342	2	11.00	4.00	0.00	0.00	1.00	87.65	65.43
98S-65	Beech River	71.156478	43.7271	2	13.39	3.37	1.44	0.00	1.00	40.96	93.44
99C-4	Mirey Brook	72.3297	42.7606	5	22.24	4.67	33.74	3.27	1.83	40.29	49.49
99M-44	Bear Brook	71.351054	43.14407	22	18.73	4.00	17.22	0.66	2.00	50.33	65.56
99C-35	Skinner Brook	72.1425	43.5319	5	12.00	3.00	52.45	7.84	2.00	27.94	21.08
99M-5	Stirrup Iron Brook	71.661003	43.37589	5	21.86	5.00	32.68	1.63	1.00	36.93	46.73
99M-6	Needle Shop Brook	71.7317	43.52	5	21.00	4.00	3.88	4.26	2.00	68.22	88.37

Appendix C. Con't.

StationID	Name	LONG_DD	LAT_DD	Cluster_grp	TotalTax	PlecTax	ChirPct	NonInsPct	TolerTax	IntolPct	ClingPct
99P-19	Pike Brook	71.068657	43.58638	5	15.27	1.98	24.40	0.27	3.00	24.67	71.62
01S-15	Burnt Knoll Brook	71.109584	44.1358	22	15.66	0.95	27.62	0.00	0.00	31.43	63.81
01S-14	Slippery Brook	71.093913	44.16218	22	17.87	5.50	19.82	0.00	0.99	71.17	72.07
01S-23	Ellis River	71.237811	44.20204	2	16.91	3.49	24.90	0.91	0.95	41.89	60.83
01M-03	Baker River	71.872307	43.94844	5	13.66	1.70	19.29	1.07	0.94	44.29	71.79
01M-07	Moosilauke River	71.711912	44.02916	5	13.41	1.82	28.70	6.78	0.49	20.00	59.13
NH HEX 53.01	Purgatory Brook	71.699339	42.85545	32	20.20	1.93	16.91	4.41	3.59	44.12	65.44
NH HEX 23.01	Johnson Brook	71.643993	43.96903	22	17.41	2.57	9.42	2.74	0.61	72.64	82.67
NH HEX 11.01	Bumpus Brook	71.268065	44.36701	22	7.03	0.63	10.13	7.59	0.00	79.11	81.65
NH HEX 21.05	Grant Brook	72.133903	43.80098	5	17.96	4.28	13.77	0.93	1.22	37.75	63.97
03P-02	Lamprey River	71.22983	43.1688	32	15.00	1.00	18.24	5.66	1.00	26.42	62.26
NH HEX 18.01	Eastman Brook	71.63954	43.98917	22	21.79	5.01	49.62	0.57	1.06	43.58	39.62
NH HEX 26.05	Hewes Brook	72.19559	43.78508	32	19.89	2.24	52.27	2.50	2.24	22.95	39.55
NH HEX 10.02	Bog Brook	71.61806	44.36511	22	23.03	2.40	19.19	5.93	3.78	38.97	62.41
NH HEX 16.02	East Branch Saco River	71.12989	44.19045	22	19.69	4.88	47.44	1.75	1.84	36.20	39.08
04c-03	Tully Brook	72.23223	42.7365	32	13.08	0.96	78.42	0.72	0.72	13.67	15.11
98S-43	Swift River	71.274765	43.87328	2	18.71	2.94	12.31	0.00	1.00	57.69	73.08
98C-14	Stratford Bog Brook	71.537864	44.67831	22	22.07	5.87	18.44	2.08	1.35	50.75	67.44
98C-10	Simms Stream	71.493265	44.84944	22	12.25	1.50	25.01	0.36	0.89	43.78	69.19
04c-07	Pauchaug Brook	72.42809	42.73129	32	19.14	1.89	59.22	0.71	0.71	31.56	31.56
98A-20	Moose Brook	71.204276	44.72765	2	22.95	5.13	45.00	1.43	2.42	42.14	42.86
98C-24	Israel River	71.369944	44.35496	2	15.37	2.24	17.31	1.92	1.58	35.58	74.68
99C-59	Dart Brook	72.295	43.0531	22	16.21	2.78	55.36	3.57	2.78	26.79	34.82
04c-15	Cone Brook	71.57199	44.81316	22	22.13	3.35	42.69	6.10	2.36	38.25	45.49
98P-79	Churchill Brook	71.07144	43.54723	32	20.17	2.19	49.73	4.12	2.88	19.23	39.01
99M-8	Bradley Brook	71.8247	43.4067	22	13.31	2.78	69.66	2.43	1.88	12.38	15.53
97C-167	Ashuleot River	72.314531	43.02202	5	17.44	2.27	36.56	3.92	3.71	12.14	54.40
98C-6	Connecticut River	71.207107	45.11903	5	10.71	1.21	35.85	1.31	1.71	11.17	61.47
98C-16	Mill Brook	71.405131	44.5946	2	17.00	5.00	10.65	0.30	1.98	80.47	82.84
03M-TREND01	Sanborn brook	71.3605	43.29271	32	14.93	1.92	43.55	0.70	0.70	36.24	31.01

Appendix C. Con't.

StationID	Name	LONG_DD	LAT_DD	Cluster_grp	TotalTax	PlecTax	ChirPct	NonInsPct	TolerTax	IntolPct	ClingPct
SP04M-200	Dinesmore Pond Brook	71.99635	42.91025	5	23.97	4.21	25.12	5.39	4.39	35.59	61.17
06m-01	Mad River	71.5108	43.94666	2	20.71	3.44	39.95	5.03	1.94	38.36	48.41
06m-05	Moose Meadow Brook	71.37691	43.06609	58	14.34	0.83	45.33	7.65	1.95	7.08	34.28
06p-07	Oyster River	71.02271	43.15291	58	11.77	0.00	38.21	11.94	1.87	13.13	36.42
05P-03	Bean River	71.15696	43.14595	32	9.96	0.00	13.89	0.00	0.56	5.00	78.89
05M-01	Lords Brook	71.72787	42.93883	32	16.11	0.96	36.47	0.46	2.01	5.17	54.86
05M-21	M. Br. Piscataquog	71.71814	43.00352	5	17.04	2.96	40.00	0.00	1.67	16.67	55.56
05C-09	Dean Brook	71.54728	44.56524	5	19.75	3.32	28.83	0.46	0.67	49.66	64.07
05A-13	Swift Diamond River	71.16102	44.8664	5	17.99	3.85	41.42	1.60	1.35	29.80	52.33
05A-15	Dead Diamond River	71.08533	44.94257	5	19.94	2.86	30.70	0.18	0.86	31.06	61.22
05M-17	Hancock Br. E. Br. Pemigewasset	71.55162	44.04189	2	13.35	3.85	40.19	0.47	1.00	50.00	44.39
05C-05	Wild Ammonoosuc River	71.93184	44.1218	5	14.16	2.56	21.83	0.18	1.73	35.04	74.47
06a-03	Wild River	71.02778	44.33127	5	14.20	1.63	13.92	2.35	2.90	33.53	80.00
06c-13	Little Sugar River	72.38526	43.30566	5	19.83	4.40	40.24	0.98	2.76	21.95	42.68
06m-11	Warner River - West Branch	71.9674	43.26778	5	15.62	2.67	26.33	0.79	1.84	30.28	63.07
98P-48	Mad River	71.084132	43.38522	32	15.70	1.79	66.90	0.99	1.69	9.19	27.58
99m-33	Rand Brook	71.7855	42.95656	5	20.16	4.10	31.60	0.00	1.80	38.21	51.42
98C-36	Israel River	71.498855	44.41253	2	14.09	2.30	23.91	0.99	2.06	27.88	72.98

Appendix D. Metadata for B-IBI scores, past and newly developed classification systems

Variable	Variable abbreviation	Variable description
Station ID	StationID	Corresponds to EDAS station ID
River Name	River	Name of river or stream sampled
Benthic Sample ID	BenSampID (1st)	Corresponds to EDAS first benthic sampling effort
Second Benthic Sample ID	BenSampID (2nd)	Corresponds to EDAS second benthic sampling effort
Benthic Index of biologic integrity (B-IBI) score	B_IBI_score	Index score for benthic sample
Previous B-IBI bioregion	Old_B_IBI_BIOR	Past benthic classification (1=North, 2=South)
Previous B-IBI threshold	old_thres	Past B-IBI threshold used to make aquatic life use (ALU) determination
Previous B-IBI assessment outcome	old_assess	Past ALU assessment (p=equal to or above threshold; f=below threshold)
Level of membership to naturally acidic stream class	pAcid	Estimated likelihood of membership to the naturally acidic stream class (0=no membership; 1 = complete membership)
Level of membership to the plains stream class	pPlns	Estimated likelihood of membership to the plains stream class (0=no membership; 1=complete membership)
Level of membership to the mountains stream class	pMtns	Estimated likelihood of membership to the mountains stream class (0=no membership; 1=complete membership)
Level of membership to the hills stream class	pHLL	Estimated likelihood of membership to the hills stream class (0=no membership; 1=complete membership)
Stream class assigned using new classification system	new_class	HLL=Hills; mtn=mountain; plns=plains; acid=naturally acidic
Assessment threshold applied using new stream classification system	new_thres	B-IBI threshold used to make ALU determination
B-IBI assessment outcome	new_assess	ALU assessment (p=equal to or above threshold; f=below threshold)
ALU outcomes agreement	old_new_agree	ALU outcome agreement using old and new classification system (0=yes; 1=no)

Appendix E. Site B-IBI classification, scores, and outcomes for previous and revised classification system.

StationID	River	BenSampID (1st)	BenSampID (2nd)	B_IBI_score	Old_B_IBI_ BIOR	old_ thres	old_ assess	pAcid	pPlns	pMtns	pHLL	new_ class	new_ thres	new_ assess	old_new _agree
98A-13	Clear Stream	s106	s106	84.57	1	65	p	0.00	0.00	1.00	0.00	Mtn	72.00	P	0
98A-21	Sterns Brook	s108	s108	84.91	1	65	p	0.00	0.00	0.77	0.23	Mtn	70.25	P	0
98C-11	Simms Stream East Branch	s110	s110	90.09	1	65	p	0.00	0.00	1.00	0.00	Mtn	72.00	P	0
98C-15	Nash Stream	s112	s112	77.37	1	65	p	0.00	0.00	0.58	0.42	MtnXHil	68.84	P	0
98C-18	Upper Ammonoosuc River	s115	s115	73.77	1	65	p	0.00	0.00	0.07	0.93	HLL	65.00	P	0
98C-1	Indian Stream	s122	s122	82.54	1	65	p	0.00	0.00	0.00	1.00	HLL	64.50	P	0
98C-2	Indian Stream	s123	s123	82.14	1	65	p	0.00	0.00	0.00	1.00	HLL	64.50	P	0
98C-4	Perry Stream	s125	s125	80.81	1	65	p	0.00	0.00	1.00	0.00	Mtn	72.00	P	0
98C-7	Dead Water Stream	s128	s128	78.23	1	65	p	0.00	0.00	1.00	0.00	Mtn	72.00	P	0
98C-8	Middle Branch Cedar Stream	s129	s129	90.12	1	65	p	0.00	0.00	1.00	0.00	Mtn	72.00	P	0
98C-9	Bog Brook	s130	s130	79.10	1	65	p	0.00	0.00	1.00	0.00	Mtn	72.00	P	0
98S-55	Bearcamp River	s136	s136	74.94	2	54	p	0.00	0.36	0.00	0.64	PlnXHil	62.51	P	0
98S-57	Deer River	s137	s137	84.08	2	54	p	0.00	0.28	0.50	0.22	Mtnx	66.69	P	0
98S-60	Lovell River	s139	s139	77.63	2	54	p	0.00	0.00	0.47	0.53	MtnXHil	68.06	P	0
98C-26	Cherry Mill Brook	s144	s144	95.23	1	65	p	0.00	0.00	1.00	0.00	Mtn	72.00	P	0
98C-39	Deception Brook	s146	s146	81.59	1	65	p	0.00	0.00	1.00	0.00	Mtn	72.00	P	0
98C-90	East Branch Stratford Bog Brook	s156	s156	86.37	1	65	p	0.00	0.00	1.00	0.00	Mtn	72.00	P	0
98S-44	Paugus Brook	s158	s158	85.02	2	54	p	0.00	0.00	0.50	0.50	MtnXHil	68.25	P	0
98S-65	Beech River	s161	s161	81.98	2	54	p	0.00	0.27	0.38	0.36	x	65.87	P	0
99C-4	Mirey Brook	s171	s171	76.97	2	54	p	0.00	0.20	0.00	0.80	HLL	63.38	P	0
99M-44	Bear Brook	s172	s172	80.47	2	54	p	0.00	0.92	0.00	0.08	plns	59.43	P	0
99C-35	Skinner Brook	s175	s175	55.78	2	54	p	0.00	0.00	0.50	0.50	MtnXHil	68.25	F	1
99M-5	Stirrup Iron Bro	s180	s180	78.21	2	54	p	0.00	0.50	0.00	0.50	PlnXHil	61.75	P	0
99M-6	Needle Shop Broo	s181	s181	90.18	2	54	p	0.00	0.00	0.00	1.00	HLL	64.50	P	0
99P-19	Pike Brook	s187	s187	64.44	2	54	p	0.00	0.07	0.14	0.79	HLL	65.20	F	1
01S-15	Burnt Knoll Brook	s227	s227	67.95	2	54	p	0.00	0.00	0.58	0.42	MtnXHil	68.83	F	1
01S-14	Slippery Brook	s230	s230	88.15	2	54	p	0.00	0.00	1.00	0.00	Mtn	72.00	P	0

Appendix E. Con't.

StationID	River	BenSampID (1st)	BenSampID (2nd)	B_IBI_score	Old_B_IBI_ BIOR	old_ thres	old_ assess	pAcid	pPlns	pMtns	pHLL	new_ class	new_ thres	new_ assess	old_new _agree
01S-23	Ellis River	s231	s231	76.61	2	54	p	0.00	0.00	1.00	0.00	Mtn	72.00	P	0
01M-03	Baker River	s237	s237	71.53	2	54	p	0.00	0.00	0.50	0.50	MtnXHil	68.25	P	0
01M-07	Moosilauke River	s239	s239	64.17	2	54	p	0.00	0.00	0.50	0.50	MtnXHil	68.25	F	1
NH HEX 53.01	Purgatory Brook	s253	s253	69.38	2	54	p	0.00	1.00	0.00	0.00	plns	59.00	P	0
NH HEX 23.01	Johnson Brook	s255	s255	85.76	2	54	p	0.00	0.00	0.53	0.47	MtnXHil	68.50	P	0
NH HEX 11.01	Bumpus Brook	s258	s258	73.67	1	65	p	0.00	0.00	1.00	0.00	Mtn	72.00	P	0
NH HEX 21.05	Grant Brook	s261	s261	80.53	2	54	p	0.00	0.00	0.00	1.00	HLL	64.50	P	0
03P-02	Lamprey River	s269	s269	64.71	2	54	p	0.10	0.97	0.00	0.03	plns	59.14	P	0
NH HEX 18.01	Eastman Brook	s270	s270	75.98	2	54	p	0.00	0.00	0.50	0.50	MtnXHil	68.25	P	0
NH HEX 26.05	Hewes Brook	s271	s271	60.64	2	54	p	0.00	0.47	0.00	0.53	PlnXHil	61.91	F	1
NH HEX 10.02	Bog Brook	s277	s277	69.37	1	65	p	0.00	0.00	0.00	1.00	HLL	64.50	P	0
NH HEX 16.02	East Branch Saco River	s283	s283	71.66	2	54	p	0.00	0.00	1.00	0.00	Mtn	72.00	F	1
04c-03	Tully Brook	s311	s311	46.54	2	54	f	0.49	0.28	0.00	0.72	PlnXHil	62.96	F	0
98S-43	Swift River	s153	s312	82.63	2	54	p	0.00	0.00	0.50	0.50	MtnXHil	68.25	P	0
98C-14	Stratford Bog Brook	s100	s313	85.09	1	65	p	0.00	0.00	0.04	0.96	HLL	64.76	P	0
98C-10	Simms Stream	s109	s314	68.86	1	65	p	0.00	0.00	1.00	0.00	Mtn	72.00	F	1
04c-07	Pauchaug Brook	s315	s315	62.21	2	54	p	0.00	0.64	0.00	0.36	PlnXHil	60.99	P	0
98A-20	Moose Brook	s107	s316	73.60	1	65	p	0.00	0.00	1.00	0.00	Mtn	72.00	P	0
98C-24	Isreal River	s142	s319	71.90	1	65	p	0.00	0.00	1.00	0.00	Mtn	72.00	F	1
99C-59	Dart Brook	s177	s320	58.09	2	54	p	0.00	0.00	0.00	1.00	HLL	64.50	F	1
04c-15	Cone Brook	s321	s321	69.67	1	65	p	0.00	0.00	0.50	0.50	MtnXHil	68.25	P	0
98P-79	Churchill Brook	s135	s323	58.54	2	54	p	0.00	0.19	0.08	0.73	PlnXHil	64.05	F	1
99M-8	Bradley Brook	s182	s324	50.76	2	54	f	0.00	0.00	0.00	1.00	HLL	64.50	F	0
97C-167	ASHUELOT	s83	s327	57.97	2	54	p	0.00	0.24	0.00	0.76	HLL	63.18	F	1
98C-6	Connecticut River	s127	s329	56.04	1	65	f	0.00	0.00	0.00	1.00	HLL	64.50	F	0
98C-16	Mill Brook	s113	s330	89.11	1	65	p	0.00	0.00	0.71	0.29	MtnXHil	69.82	P	0
03M-TREND01	Sanborn Brook	s284	s331	62.58	2	54	p	0.00	0.72	0.00	0.28	PlnXHil	60.52	P	0
SP04M-200	Dinesmore Pond Brook	s345	s345	72.26	2	54	p	0.19	0.23	0.00	0.77	HLL	63.21	P	0
06m-01	Mad River	s490	s490	71.41	2	54	p	0.00	0.00	1.00	0.00	Mtn	72.00	F	1

Appendix E. Con't.

StationID	River	BenSampID (1st)	BenSampID (2nd)	B_IBI_score	Old_B_IBI_ BIOR	old_ thres	old_ assess	pAcid	pPlns	pMtns	pHLL	new_ class	new_ thres	new_ assess	old_new _agree
06m-05	Moose Meadow Brook	s491	s491	49.54	2	54	f	0.99	0.98	0.00	0.02	acid	59.13	F	0
06p-07	Oyster River	s492	s492	47.16	2	54	f	0.85	1.00	0.00	0.00	acid	59.00	F	0
05P-03	Bean River	s495	s495	59.06	2	54	p	0.36	1.00	0.00	0.00	plns	59.00	P	0
05M-01	Lord's Brook	s496	s496	56.04	2	54	p	0.72	0.61	0.00	0.39	acid	61.12	F	1
05M-21	M. Branch Piscataquog	s497	s497	65.75	2	54	p	0.00	0.67	0.00	0.33	PlnXHil	60.81	P	0
05C-09	Dean Brook	s498	s498	80.03	1	65	p	0.05	0.00	0.00	1.00	HLL	64.50	P	0
05A-13	Dead Diamond River	s499	s499	71.56	1	65	p	0.00	0.00	0.48	0.52	MtnXHil	68.11	P	0
05A-15	Swift Diamond River	s500	s500	74.05	1	65	p	0.00	0.00	0.00	1.00	HLL	64.50	P	0
05M-17	Hancock Branch East	s502	s502	72.21	2	54	p	0.43	0.00	1.00	0.00	Mtn	72.00	P	0
05C-05	Wild Ammonoosuc	s504	s504	71.27	1	65	p	0.00	0.00	0.00	1.00	HLL	64.50	P	0
06a-03	Wild River	s505	s505	66.93	1	65	p	0.00	0.00	0.27	0.73	PlnXHil	66.51	P	0
06c-13	Little Sugar River	s506	s506	68.64	2	54	p	0.00	0.50	0.00	0.50	PlnXHil	61.75	P	0
06m-11	West Branch Warner River	s509	s509	68.99	2	54	p	0.00	0.00	0.00	1.00	HLL	64.50	P	0
98P-48	Mad River	s147	s510	51.42	2	54	f	0.00	0.81	0.00	0.19	plns	60.02	F	0
99m-33	Rand Brook	s512	s512	75.83	2	54	p	0.00	0.33	0.00	0.67	PlnXHil	62.66	P	0
98C-36	Isreal River	s145	s515	67.64	1	65	p	0.00	0.00	0.00	1.00	HLL	64.50	P	0