Development of Biocriteria for Vermont and New Hampshire Lakes

Criteria Development for Macroinvertebrates for Three Lake Classes

and

Implementation Procedure for Biological Assessment of Vermont Lakes

Final Report

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Introduction and Acknowledgments

This report summarizes efforts made during 2004-2006 to further develop biological criteria for Vermont lakes. This report follows from a 2003 report entitled *Development of Biocriteria for Vermont and New Hampshire Lakes Criteria Development for Phytoplankton and Macroinvertebrate Assemblages for Three Lake Classes* (VTDEC 2003). In that document, efforts to develop biological criteria for lakes and ponds, using phytoplankton and macroinvertebrate information, have been described. The macroinvertebrate analyses in the 2003 document were an initial attempt at deriving a benthic index of biotic integrity. The analyses presented in the present document revisits the 2003 analyses for all lake macroinvertebrate data collected over the course of Vermont's efforts since 1996.

This document presents a macroinvertebrate assessment framework applicable to Vermont lakes that is transferable to other north-temperate lakes. Importantly, it should be understood that development of a biological index for lakes is an evolving process. The present document provides the second analytical "iteration" of index development. The index developed from the presently available data is highly refined from earlier attempts (VTDEC 2003), but is one of many potential avenues for index development (e.g., Blocksom et al., 2002). It can reasonably be expected that with additional lakes assessed, the opportunity to revisit the analyses outlined in the following will arise, and new techniques may at such a time be applied to the dataset. A critical consideration in assuring integrity of assessments in the face of evolving biological indices is the maintenance of a stable set of well-selected reference lakes. In the present analysis, the reference lakes comprise a set of least-disturbed, and in many cases, highly conserved waterbodies.

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Background

The objective of this project was to determine the range of biological characteristics of the macroinvertebrate community that constitute reference conditions for lakes of differing types, and to use this information to develop a bioassessment procedure for Vermont lakes. The overall approach along with a complete description of field methods is presented in detail by VTDEC (2003), and is restated in brief in the following.

There are typically four steps involved in developing biological criteria for lakes. These are classification, determination of the reference condition, determination of sensitive biological indicators, and multi-metric index construction (e.g., USEPA, 1997; USEPA, 1998; Gerritsen et al., 2000). For this project, a-priori classification of lakes was conducted using lake physico-chemical attributes that are not typically affected by anthropogenic factors, and is reported by VTDEC (2003). This classification was subsequently corroborated using the biological measurements of macroinvertebrates inhabiting five specific habitat types. The biological reference condition of three lake classes was then defined, and was used to assess known-impaired lakes and lakes of unknown biological condition. Between 1996 and 2004, biological and chemical sampling was conducted on 61 lakes; 49 in Vermont and 12 in New Hampshire.

Evaluation and preliminary use of the trial macroinvertebrate index presented by VTDEC (2003) highlighted that certain lake types were under-represented by the initial selection of reference and test lakes. Specifically, the need for additional lakes with water-level manipulations and lakes that may be naturally eutrophic was evident. In this instance, water-level manipulations are meant to describe periodic or seasonal drawdowns for the purpose of flood control, ice-damage control, or power generation, while potentially naturally-eutrophic lakes describe those waters that exhibit high nutrient concentrations but have little evidence of anthropogenic impacts. The analysis presented herein includes biological assessment data from six newly-sampled lakes that presently or historically experienced water level manipulation, and four lakes that were considered potentially naturally eutrophic (Table 1). The resulting dataset comprises 26 reference lakes across three lake classes (see below). The remaining assessed lakes are considered "test" lakes that are subject to a variety of known or suspected stressors (Table 2).

Table 1. Study lakes visited in conjunction with the Bioassessment of Vermont and New Hampshire Lakes Project, 1996-2004.

Lake Id	Year assessed	Town	State	Lake Id	Year assessed	Town	State
Bald Hill	1997	Newark	VT	Little Elmore	1996	Elmore	VT
Beaver	1996	Derry	NH	Long (Grnsbo)	1997	Greensboro	VT
Beebe (Hubdtn)	2000	Hubbardton	VT	Long (Shefld)	1997	Sheffield	VT
Bliss *	2004	Calais	VT	Lyford *	2004	Walden	VT
Branch	1998	Sunderland	VT	Maidstone	1998	Maidstone	VT
Burr (Sudbry)	2000	Sudbury	VT	Mcconnell	1996	Brighton	VT
Butternut	1996	Grantham	NH	Nathan	1996	Dixville	NH
Carmi	1996	Franklin	VT	Ninevah ‡	2004	Wallingford	VT
Caspian	1997	Greensboro	VT	North (Brkfld)	2001	Brookfield	VT
Chittenden ‡	2003	Chittenden	VT	North St. Albans	2000	Fairfax	VT
Clyde ‡	2004	Newport	VT	Parker	1999	Glover	VT
Colchester *	2004	Colchester	VT	Pleasant Valley	2002	Brattleboro	VT
Cole	1998	Jamaica	VT	Russell	1997	Woodstock	NH
Crystal (Barton)	1997	Barton	VT	Sessions	1997	Dummer	NH
Curtis	1998	Woodbury	VT	Shadow (Glover)	1998	Glover	VT
Danby *	2004	Danby	VT	Silver (Barnrd) ‡	2004	Barnard	VT
Dudley	1996	Deering	NH	Smith	1997	Washington	NH
Dunmore	1998	Leicester	VT	South St. Albans	2000	Fairfax	VT
Eden ‡	1998[A]	Eden	VT	Spring (Shrwby)	1997	Shrewsbury	VT
Ewell	1997	Peacham	VT	St. Catherine	1998	Wells	VT
Fairfield	1998	Fairfield	VT	Stiles	2001	Waterford	VT
French	1997	Henniker	NH	Stratton	1998	Stratton	VT
Gilman	1996	Alton	NH	Sugar Hill	2002	Leicester	VT
Great Hosmer	1997	Craftsbury	VT	Sunrise	2001	Benson	VT
Hatch	1996	Eaton	NH	Ticklenaked	1999	Ryegate	VT
High (Sudbry)	1997	Sudbury	VT	Turtlehead	1996	Marshfield	VT
Hinkum	1997	Sudbury	VT	Wallingford	1996	Wallingford	VT
Indian Brook (Essex)	2003	Essex	VT	Wheeler (Brunwk)	1996	Brunswick	VT
Intervale	1997	Sandwich	NH	Willard	1997	Antrim	NH
Joes (Danvll) ‡	2004	Danville	VT	Wolcott	1996	Wolcott	VT
[A] Assessed 1998 for phytoplankton, 2004 for macroinvertebrates * Newly assessed as a potentially naturally eutrophic lake ‡ Newly assessed as a water-level managed lake				Woodward	1998	Plymouth	VT

Table 2. Counts of reference and test lakes, and lakes with known or suspected stressors, by lake class.

Lake class	Count of reference lakes	Count of test lakes	Stressor type*				
			Eutrophication/ cumulative development	Acidification	Aquatic herbicide application	Water level management	Other
Well buffered	10	18	10	0	2	3	1
Low alkalinity	12	9	2	6	0	2	1
Large	4	8	6	0	0	3	0

*Since some lakes are affected by multiple stressors, the sum of lakes within stressor categories does not equal the sum of test lakes.

Reanalysis of Macroinvertebrate-Based Criteria

Overview

The analytical approach for macroinvertebrate criteria development was similar to that employed for the phytoplankton assemblage that is described by VTDEC 2003. However, the analysis was more complex owing to the evaluation of five separate community types within each lake. These community types are named based on the habitats sampled, as follows: **rocky-littoral**, consisting of rocky, cobbled and/or shale shorelines and associated coarse woody debris; **macrophyte beds**; **muddy littoral**, consisting of shallow littoral muds and fines; **subblitoral**, consisting of organic fines from four meters in depth or deeper, but above the thermocline; and, **profundal**, consisting of deep-lake organic sediments composed of gyttja or dy.

After reviewing canonical correspondence analyses of several of the community types (one such analysis is presented below), it was decided to retain the physicochemical classification initially inferred using the phytoplankton community data, and validated with independent physicochemical data using discriminant function analysis (VTDEC 2003). This general classification of well buffered lakes, low alkalinity lakes, and large lakes was the starting point for the macroinvertebrate analysis. Following taxonomic identifications, metrics were calculated, and analyses performed on these metrics were segregated by lake class. The process was structured to iteratively cull metrics that did not contribute towards determining whether biota occupying one habitat of an individual lake might deviate from the reference expectation for lakes in that class. These retained metrics were then used to construct a multimetric index, called the Vermont Lake Condition BioIndex.

There were many candidate metrics from which to derive macroinvertebrate criteria, not all of which contributed meaningfully to a multimetric index. Accordingly, the first step in reducing the metric set was to generate simple spread-location plots to visualize distributions of metrics across classes, and between reference and test lakes within classes. These plots were reviewed, and metrics which appeared to show discrimination either across classes, or between reference and test lakes were retained for further evaluation. This was done within each of the five community types.

In the next step, the distributions of the retained metrics were quantified, and the retained metrics were subjected to a Spearman non-parametric analysis, to identify metrics which were highly correlated. The information quantity contained by a given metric was calculated using the interquartile coefficient; a measure of the ability for a metric to detect deviations from the reference expectation based on distributional statistics. Metrics that were identified as redundant (e.g. Spearman $R \geq 0.75$), and contained a lower quantity of original information were rejected from the dataset. Metrics with excessively high interquartile coefficients were not retained in the final metric set.

The next step in the analysis was to evaluate the statistical significance of observed differences in the joint distributions of metrics across classes, and between reference and test lakes. To accomplish this, multivariate analysis of variance (MANOVA) was used.

Finally, scoring algorithms were developed from the final metric set using the EPA 'bi-section' scoring method, to develop the Vermont Lake Condition BioIndex for macroinvertebrates. For well buffered lakes, the index is comprised of 15 metrics. For the low alkalinity lakes, six metrics are needed to assess a lake using the index. For large lakes, 12 metrics are needed. Using the Vermont Lake Condition BioIndex, overall assessments were derived for each lake, by averaging the scores for metrics representing each habitat type. Overall mean score thresholds are proposed for water management types.

Lake Classification

The physico-chemical lake classification developed using canonical correspondence analysis and discriminant function analysis presented by VTDEC (2003) was used for development of macroinvertebrate criteria. The validity of this approach was reassessed using the full 1996-2004 dataset by performing several CCA analyses, with the two criteria for accepting the phytoplankton-inferred classification being a similar clustering of sites within classes based on biometric scores, and a reasonably high percent variance explained within the ordination. Figure 1 shows a CCA ordination diagram where reference lakes, biometrics, and physico-chemical variables are arranged by their relative positions in ordination space, with 23.7 percent of the total dataset variance explained. A similar analysis performed using combined reference and test lakes yielded similar site, and explained 12.4 percent of the total dataset variance on the first three axes. These analyses suggest that the pre-established physicochemical classification is valid for macroinvertebrates, although many other factors also influence the occurrence of macroinvertebrates.

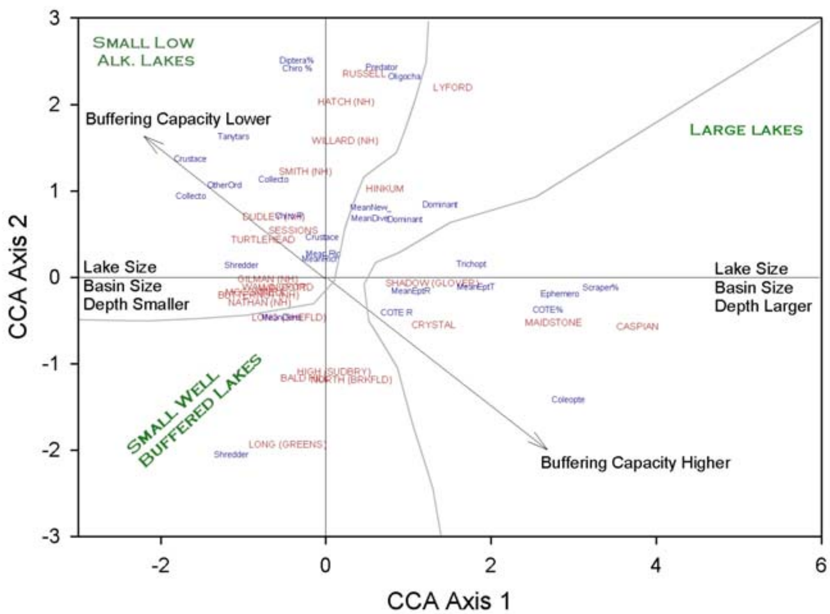

Figure 1. Canonical correspondence triplot of 25 reference lakes (red lettering) as weighted averages of 28 macroinvertebrate biometrics (blue lettering) collected from the rocky-littoral community, in relation to 5 environmental variables (vectors). Sites are plotted as linear combinations of environmental variables. This ordination explains 23.7% of the total dataset variance on the first three axes. The term "buffering capacity" refers to the covarying effects of pH and conductivity. Boundaries are inscribed to separate sites which were identified by discriminant function analysis as belonging to one of three lake classes (identified in uppercase).

Candidate Metrics

Given the level of taxonomic precision within this project's data, numerous candidate macroinvertebrate biometrics were available for evaluation. The VTDEC "Biology" database is a Microsoft Access-based data management utility which automatically calculates a large number of biometrics which are relevant to stream bioassessment and are described by VTDEC (2001). Several additional metrics which are thought to be relevant to lake systems were also calculated. The roster of 32 trial metrics was adapted from various sources (e.g. USEPA 1997, USEPA 1998, VTDEC 2001, VTDEC 2003), and is presented in Table 3.

Table 3. Roster of candidate macroinverterbate biological metrics used to derive trial biological criteria for Vermont and New Hampshire Lakes.

Metric	Metric type	Description
MeanDensity	Structural	Average density of individuals
MeanRichness	Structural	Average taxa richness
DominantTaxa%	Structural	Percent of organisms in most dominant taxa
Dominant3Taxa%	Structural	Percent of organisms in three most dominant taxa
Ept/Ept+Chiro	Structural	Proportion of Ephemoptera, Plecoptera, Tricoptera to EPT+Chironomidae
EptRichness	Structural	Mean number EPT taxa
MeanNew_BI	Structural	Hilsenhoff biotic index, rescaled to a max. value of 10
MeanDiversity	Structural	Shannon-Weiner index of diversity
%Dips as intol. chiros	Structural	Proportion of dipteran community (Chironomidae + chaoboridae + Oligocheata) as non-*Chironomus chironomus* (e.g. intolerant) chironomidae
COTE/COTE+ CHIRO+ OLI	Structural	Proportion of Coleoptera, Odonata, Tricoptera, Ephemoptera to COTE+Chironomidae+Oligochaeta
Hydropsychidae%	Compositional	self explanatory
Coleoptera%	Compositional	self explanatory
Diptera%	Compositional	self explanatory
Ephemeroptera%	Compositional	self explanatory
Plecoptera%	Compositional	self explanatory
Trichoptera%	Compositional	self explanatory
Oligochaeta%	Compositional	self explanatory
OtherOrders%	Compositional	self explanatory
Crust - Moll % and R	Compositional	Crustaceans and molluscs, sum, expressed as percent of community and as numeric richness
COTE% and R	Compositional	Sum, expressed as percent of community and as numeric richness
Tanytarsus sp. %	Compositional	self explanatory
Chiro % and R	Compositional	Expressed as percent of community and as numeric richness
Chaoboridae%	Compositional	self explanatory
Collector Gatherer%	Functional	self explanatory
Collector Filterer%	Functional	self explanatory
Predator%	Functional	self explanatory
Shredder Detritivore%	Functional	self explanatory
Shredder Herbivore%	Functional	self explanatory
Scraper%	Functional	self explanatory

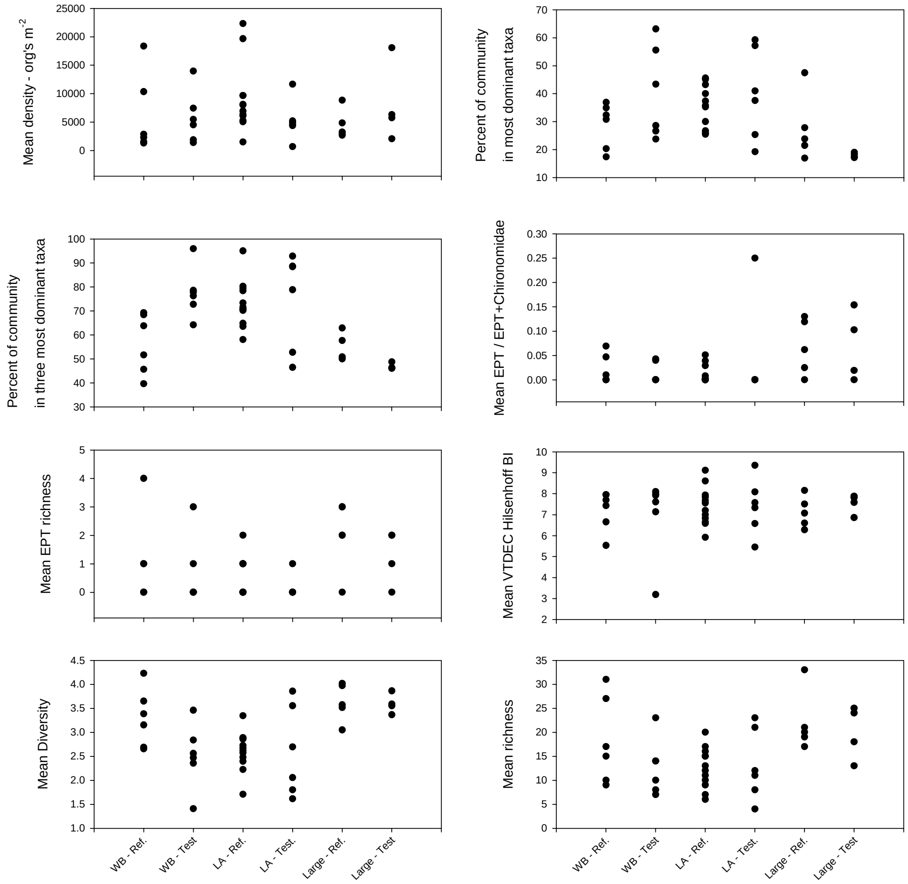

Figure 2. Spread-location plot of eight macroinvertebrate metrics separated by lake class and reference status, for samples collected from the sublittoral habitat. WB: well buffered reference lakes. LA: low alkalinity lakes. Large: large lakes. Ref.: reference status lakes. Test: lakes of impaired or unknown status.

Initial Metric Evaluation

The initial evaluation of metrics, by habitat type, was accomplished by developing spread-location and Tukey plots of each metric, by lake class and reference status. Presentation of each individual such plot is beyond the scope of this report. By means of example, Figure 2 shows a spread-location plot for eight metrics within the sublittoral habitat, while Figure 3 provides one example Tukey plot, superimposed over a spread-location plot. Plots such as these are useful for discerning whether a metric should be retained as a candidate for index development, based simply on the raw distribution of the data.

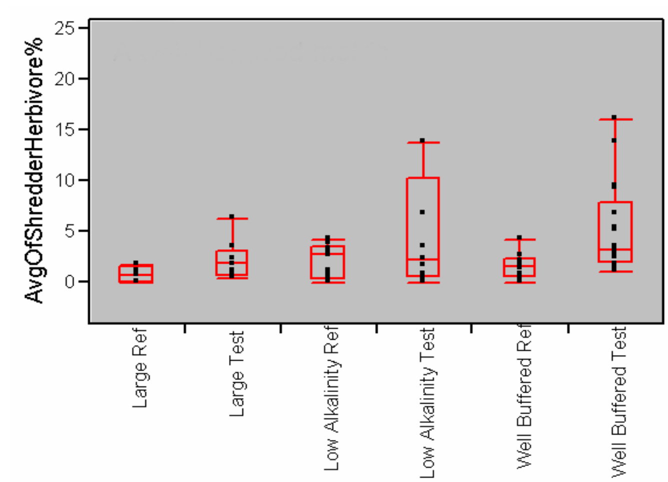

Figure 3. Mean shredder herbivore composition (%) in the rocky-littoral habitat, for three lake classes.

Figure 3 shows an apparent difference in the median and distribution of the *percent shredder herbivores* metric between reference and test lakes, for well-buffered and large lakes. These initial comparisons are not statistically-based, but serve as a guide for retaining or rejecting candidate metrics for further evaluation. Similar plots were prepared for all metrics within all community types.

Evaluation of metric sensitivity and redundancy

From the collective set of box-plots, metrics which appeared to show promising discrimination between reference and test lakes were evaluated for information content using the interquartile coefficient. The interquartile coefficient is defined as the *interquartile range* of reference lake metric distributions, divided by the *scope for detection* (Figure 4). The interquartile range is calculated as the 75th percentile of the reference metric range, minus the 25th percentile of the reference metric range. For metrics where larger values indicate impact, the scope for detection is calculated as the maximum test-lake metric value minus the 75th percentile of the reference lake distribution. Conversely, for metrics where impact is noted at low values, the scope for detection is calculated as the 25th percentile of reference metric range, minus the minimum test-lake metric value. Where interquartile coefficients exceed one, the metric is considered overly variable (USEPA 1998).

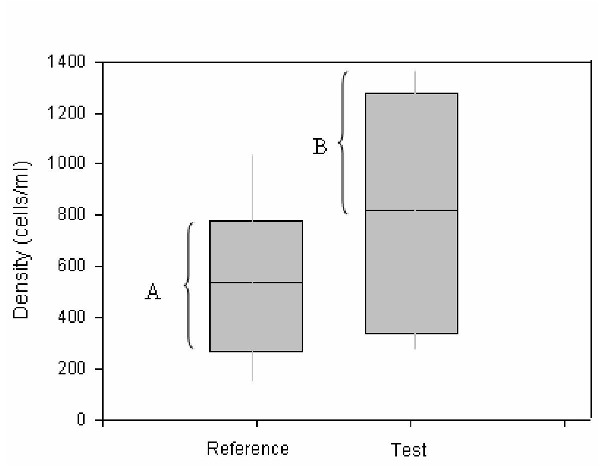

Figure 4. Illustration of interquartile range (A) and scope for detection (B), using total phytoplankton cell density.

Metrics were subjected to a redundancy analysis, using Spearman correlations. In this analysis, metrics were considered redundant if the Spearman correlation for the pair was statistically significant ($p \leq 0.05$), and exceeded a Spearman's "R" value of 0.75. When selecting metrics to retain from among redundant metric pair, the metric with the lowest interquartile coefficient was retained. This approach maximized the information content inherent in the metrics. Table 4 provides a trimmed roster of candidate metrics that result from the analysis described above.

*Table 4. Subset of candidate macroinvertebrate metrics retained for multivariate statistical evaluation and index development. Metrics preceded by * were determined by Spearman correlation analysis to be redundant and were not used subsequently, as were metrics with interquartile coefficients in excess of one. Habitat codes: ML, muddy littoral; RL, rocky littoral; MA, macrophytes; SL, sublittoral; PF, profundal.*

Habitat	Metric	Class	Interquartile Coefficient	Habitat	Metric	Class	Interquartile Coefficient
Muddy littoral	%Diptera	Well Buffered	0.99	**←Macrophytes**	DOM%	Well Buffered	0.61
	%Diptera	Low Alkalinity	1.20		Oligochaeta%	Large	0.24
	Mean Richness	Low Alkalinity	1.16		Oligochaeta%	Low Alkalinity	0.31
	Mean Richness	Well Buffered	0.54		Oligochaeta%	Well Buffered	0.07
	%Oligochaetes	Low Alkalinity	0.82		COTE/ COTE+CHI+OLI	Well Buffered	0.67
	%Oligochaetes	Well Buffered	0.74		DOM%	Large	1.58
	%Scrapers	Low Alkalinity	0.68		DOM%	Well Buffered	0.61

Habitat	Metric	Class	Interquartile Coefficient	Habitat	Metric	Class	Interquartile Coefficient
	%Scrapers	Well Buffered	0.08		COTE-R	Low Alkalinity	1.17
	COTE-R	Well Buffered	1.33		COTE-R	Well Buffered	1.33
Rocky littoral	Dominant 3 taxa %	Large	1.18		ChiroR	Low Alkalinity	0.20
	Dominant 3 taxa %	Well Buffered	0.71		ChiroR	Well Buffered	0.69
	Dominant 3 taxa %	Low Alkalinity	0.60		Mean Richness	Low Alkalinity	1.16
	*EPT/ EPT+Chiro	Large	0.57		EPT/EPT+Chiro	Well Buffered	0.60
	*EPT/ EPT+Chiro	Well Buffered	0.35	**Sublittoral**	EPT_Richness	Large	1.00
	Ephemeroptera%	Large	0.78		Collector Filterer%	Large	0.49
	Ephemeroptera%	Well Buffered	0.98		ChiroR	Large	0.38
	Tricoptera%	Large	0.86		DOM%	Well Buffered	0.20
	Shred. Herbivore%	Large	0.30		EPT/EPT+Chiro	Well Buffered	0.14
	Shred. Herbivore%	Low Alkalinity	0.13		Diversity	Well Buffered	0.40
	Shred. Herbivore%	Well Buffered	0.13	**Profundal**	DOM%	Large	0.14
	COTE%	Large	0.84		DOM%	Low Alkalinity	0.84
	COTE%	Well Buffered	0.35		Diversity	Large	0.14
	Crust. Moll.%	Large	0.55		Diversity	Low Alkalinity	1.20
	Crust .Moll.%	Low Alkalinity	1.18		Collector Gatherer%	Large	0.23
	Diptera%	Well Buffered	0.17		Collector Gatherer%	Well Buffered	0.29
	Chiro%	Low Alkalinity	1.30		*Predator%	Large	0.57
	Chiro%	Well Buffered	0.19		ChiroR	Large	1.00
	COTE/ COTE+CHI+OLI	Large	0.59		Chaoboridae%	Large	0.03
	COTE/ COTE+CHI+OLI	Well Buffered	0.33		Chaoboridae%	Well Buffered	0.52
	Oligochaeta%	Large	0.32				
	Oligochaeta%	Low Alkalinity	0.13				
	Oligochaeta%	Well Buffered	0.04				

Multivariate Analysis of Variance

Multivariate analysis of variance (MANOVA) was employed to determine if the shortened list of metrics (Table 5) statistically detected differences in metric distributions, across classes and between reference and test lakes. MANOVA was preferred to sequential univariate ANOVA's to account for the residual co-variance among many of the metrics, even given the trimming of the metric set. MANOVA is also preferred to maintain control of experiment-wise error.

The MANOVA models tested were designed to assess the degree to which the multivariate distributions of the metrics varied by lake class and reference status. The statistical models were constructed specifically to answer three questions: 1) is there a statistically significant difference in biota, as described by the metrics employed, that can be attributed to the effect of lake class?; 2) is there a statistically significant difference in biota, as described by the metrics employed, that can be attributed to a lakes reference status?; 3) does the response in biota attributable to a lakes reference status depend on the lake class? The reason these questions are posed is that in the development of biological indices, metric ranges are commonly scored as described on page 13. Owing to these scoring algorithms, individual metric scores, and index scores themselves, can artificially inflate the observed difference in biological communities, by partitioning all of the variance in any given metric or array of metric scores into a few categories. This augments the apparent separation between waters that are within the reference set and those that are of sub-reference quality. The point of the MANOVA presented herein is to statistically determine the strength of separation in the biological measurements absent the artificial enhancement to that separation imparted by the metric scoring and index development.

MANOVA requires multivariate normality of the underlying data, and thus where necessary, metrics were rescaled to approximate the normal statistical distribution. Normality was assessed using normal probability plots and the Kolmogorov-Smirnov test (SAS Institute, 2005), with follow-up diagnostic evaluation using normal probability plots. Specifically, in the rocky-littoral habitat, the trichoptera % and shredder-herbivore % metrics were log-transformed, and the oligocheates % metric was root-transformed. In the sublittoral habitat, the collector-filterer % was log-transformed, and the EPT/EPT+chiro metric was root transformed.

These analyses were performed for each community type, and the significance of any interaction effect was taken to mean that the overall direction of change in the metric set between reference and test lakes depended on the lake class. Since the metric sets varied between classes and within habitats, the MANOVA designs were by definition imbalanced (see, for example, the metric roster for macrophytes across the three classes, Table 5). For this reason, where the statistical model indicated significance in the differences between classes, the underlying strength of that differentiation was conservatively estimated by the model. The multivariate distributions of metrics varied significantly among classes and between refrence and test lakes in many cases (Table 6).

Table 5. Metrics used to derive the Vermont Lake Condition Index for macroinvertebrates[1].

Lake class	Habitat				
	Rocky littoral	Muddy littoral	Macrophytes	Sublittoral	Profundal
Large	Tricoptera% Shred. Herbivore% COTE% Crust. Moll.% COTE/ COTE+CHI+OLI Oligochaeta%	No valid metrics	Oligochaeta%	Collector Filt.% ChiroR	DOM% Diversity Collector Gath.% Chaoboridae%
Low Alkalinity	Dom3% Shred. Herbivore% Oligochaeta%	%Scrapers	Oligochaeta% Chiro R	No valid metrics	DOM%

Well Buffered	Dom3% Shred. Herbivore% COTE% Chiro% COTE/ COTE+CHI+OLI Oligochaeta%	%Diptera Mean Richness %Scrapers	DOM% Oligochaeta% COTE/ COTE+CHI+OLI DOM% Chiro R EPT/ EPT+Chiro	DOM% EPT/ EPT+Chiro Diversity	Collector Gath.% Chaoboridae%

[1])Definitions of metric acronyms are provided in Table 2.

Table 6. Results of MANOVA analyses for metric sets for five habitat types.

Habitat	Wilks'Λ, F-statistic, and p-value for ***interaction*** effect			Wilks' Λ, F-statistic, and p-value for ***lake class*** effect			F-statistic, and p-value for ***reference status*** effect	
	Λ	F	*p*	Λ	F	*p*	F	*p*
Rocky littoral	0.69	1.22	0.253	0.54	2.2	0.007	0.42	0.015
Macrophytes	0.08	1.18	0.311	0.71	1.9	0.042	1.36	0.253
Muddy littoral	Not tested			Not tested			2.49	0.094
Sublittoral	N/A	1.8	0.17	N/A	0.48	0.79	0.46	0.79
Profundal	0.69	1.11	0.37	0.62	1.5	0.18	3.11	0.035

These MANOVA highlighted the following. First, no significant interaction terms were evident, meaning that the direction of change in the mean metric observation from reference to test lakes did not depend on class. Second, the mean metric observation varied significantly between lake classes for rocky-littoral epibenthos and macrophyte epibenthos. Third, in the rocky-littoral and profundal habitats, the metric set yielded a highly significant separation between reference and test lakes. A weaker separation was also apparent in the muddy littoral habitat (p=0.094), and the weak response was due to imbalance in the model. The effect of lake class could not be measured using muddy littoral metrics since there were no common metrics across the three classes (Table 5). This information indicates that when considering raw (unscored) metrics, the rocky-littoral and macrophyte communities varied significantly with lake class, and rocky-littoral, muddy-littoral and profundal communities varied significantly between reference and test lakes.

Vermont Lake Condition BioIndex for macroinvertebrates

Scoring algorithms for the final metric set

Individual metric scoring ranges were calculated using the EPA recommended "bisection" method (USEPA, 1998). This algorithm allocates a score of five for all metric values which fell within the best 75 percent of the reference range for that metric. The range of metric values from the lower quartile of the reference range to the worst-case test lake value was then bisected, with a score of three and one being allocated to the ranges of values corresponding to the 'better' and 'worse' of these values, respectively. Table 7 identifies the scoring ranges for the selected metrics, and the direction that each metric varied relative to reference when impact was evident. These scores were summed to arrive at a final macroinvertebrate community index value, which was expressed in proportion to the maximum attainable score within a habitat and lake class.

Table 7. Vermont Lake Condition BioIndex component metrics, with scoring ranges.

Habitat	Lake class	Metric	Departs from reference when:	Threshold for score = 5	Threshold for score = 3	Threshold for score = 1
Macrophytes	Large	Oligochaeta%	High	<11.13	33.54	>33.54
	Low Alkalinity	ChiroR	Low	>9.50	5.75	<5.75
	Low Alkalinity	Oligochaeta%	High	<12.14	31.07	>31.07
	Well Buffered	ChiroR	Low	>9.00	4.50	<4.5
	Well Buffered	COTE/COTE+CHI+OLI	Low	>0.47	0.24	<0.24
	Well Buffered	DOM%	High	<51.89	75.94	>75.94
	Well Buffered	EPT/EPT+Chiro	Low	>0.41	0.21	<0.21
	Well Buffered	Oligochaeta%	High	<5.53	45.53	>45.53
Muddy littoral	Low Alkalinity	%Scrapers	High	<17.59	29.49	>29.49
	Well Buffered	%Diptera	Low	>41.08	20.54	<20.54
	Well Buffered	%Scrapers	High	<2.92	20.32	>20.32
	Well Buffered	MeanRichness	Low	>24.00	12.50	<12.5
Profundal	Large	Chaoboridae%	High	<2.00	40.50	>40.5
	Large	Collector Gatherer%	Low	>50.00	26.14	<26.14
	Large	Diversity	Low	>2.44	1.70	<1.7
	Large	DOM%	High	<30.44	54.71	>54.71
	Low Alkalinity	DOM%	High	<68.42	84.21	>84.21
	Well Buffered	Chaoboridae%	Low	>66.00	33.00	<33
	Well Buffered	Collector Gatherer%	High	<13.33	36.40	>36.4
Rocky littoral	Large	COTE%	Low	>62.22	43.11	<43.11
	Large	COTE/COTE+CHI+OLI	Low	>0.72	0.52	<0.52
	Large	CrustMoll%	High	<13.25	24.63	>24.63
	Large	Oligochaeta%	High	<4.73	11.64	>11.64
	Large	Shred. Herbivore%	High	<1.57	3.94	>3.94
	Large	Tricoptera%	Low	>3.96	1.98	<1.98
	Low Alkalinity	Dom3%	Low	>52.92	45.19	<45.19
	Low Alkalinity	Oligochaeta%	High	<1.32	6.52	>6.52
	Low Alkalinity	Shred. Herbivore%	High	<3.65	16.53	>16.53

Habitat	Lake class	Metric	Departs from reference when:	Threshold for score = 5	Threshold for score = 3	Threshold for score = 1
	Well Buffered	Chiro%	High	<19.50	45.15	>45.15
	Well Buffered	COTE%	Low	>53.63	27.97	<27.97
	Well Buffered	COTE/COTE+CHI+OLI	Low	>0.68	0.36	<0.36
	Well Buffered	Diptera%	High	<19.66	48.03	>48.03
	Well Buffered	Dom3%	High	<59.66	71.18	>71.18
	Well Buffered	Oligochaeta%	High	<2.94	38.89	>38.89
	Well Buffered	Shred. Herbivore%	High	<2.35	9.23	>9.23
Sublittoral	Large	ChiroR	Low	>12.00	8.00	<8
	Large	Collector Filterer%	High	<23.16	39.62	>39.62
	Well Buffered	Diversity	Low	>2.66	1.51	<1.51
	Well Buffered	DOM%	High	<34.75	64.72	>64.72
	Well Buffered	EPT/EPT+Chiro	High	<0.07	0.28	>0.28

For the well buffered lakes, the Vermont Lake Condition BioIndex is comprised of 15 metrics. For the large lakes, the index is comprised of 12 metrics, and the muddy littoral habitat does not yield relevant metrics for assessment. For the low alkalinity lakes, the index is comprised of six metrics and the sublittoral habitat does not yield relevant metrics for assessment.

An overall habitat biotic index value can be calculated by summing the individual metric scores, and dividing by the sum of the maximum possible scores, to arrive at a value expressed on a scale of one to 100. Likewise, an overall lake biotic index value is similarly derived by calculating the mean of the habitat values.

BioIndex scores

For the present dataset, overall biotic index scores ranged from 42 to 100 for test lakes across the three classes, and 58-100 for reference lakes. The mean scores varied significantly with reference status (Table8), with reference lakes having significantly higher mean scores for all lake classes. Distributions of scores for all lakes are shown in Figure 5, and mean overall scores for individual lakes are shown in Table 9.

Table 8. Mean overall Vermont Lake Condition BioIndex scores for 61 lakes across VT and NH. Statistical p-values refer to T-tests between reference and test lakes.

Lake Class	Reference status	N	Mean score	Std err.	p-value
Large	Test	8	60.57	4.31	<0.001
Large	Ref.	4	89.52	6.09	
Low Alkalinity	Test	9	73.64	4.06	0.035
Low Alkalinity	Ref.	12	87.78	3.52	
Well Buffered	Test	10	71.58	2.87	<0.001
Well Buffered	Ref.	18	88.78	3.85	

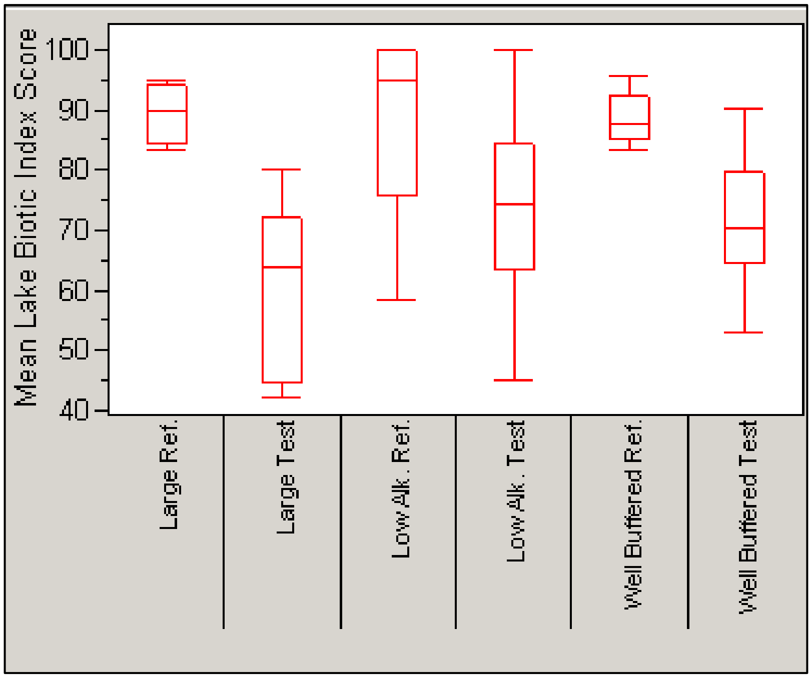

Figure 5. Distribution of mean lake biotic index scores for three lake classes, by reference status. Shown are Tukey plots of 5th, 25th, 50th, 75th, and 95th percentiles.

Table 9. Individual lake mean biotic index scores for 61 VT and NH lakes.

Lake class	Reference status Y/N	Lake name	Mean overall lake biotic index
Large	N	CARMI	61.67
Large	N	CHITTENDEN	44.17
Large	N	DUNMORE	46.67
Large	N	EDEN	80.00
Large	N	FAIRFIELD	71.11
Large	N	JOES (DANVLL)	66.19
Large	N	PARKER	72.50
Large	N	ST. CATHERINE	42.22
Large	Y	CASPIAN	94.76
Large	Y	CRYSTAL (BARTON)	91.67
Large	Y	MAIDSTONE	88.33
Large	Y	SHADOW (GLOVER)	83.33
Low Alkalinity	N	BEAVER (NH)	75.00
Low Alkalinity	N	BRANCH	100.00
Low Alkalinity	N	COLE	82.22
Low Alkalinity	N	FRENCH (NH)	71.67
Low Alkalinity	N	INTERVALE (NH)	45.00
Low Alkalinity	N	LITTLE ELMORE	91.11
Low Alkalinity	N	NINEVAH	64.44
Low Alkalinity	N	STRATTON	73.33

Lake class	Reference status Y/N	Lake name	Mean overall lake biotic index
Low Alkalinity	N	SUGAR HILL	60.00
Low Alkalinity	Y	DUDLEY (NH)	100.00
Low Alkalinity	Y	GILMAN (NH)	73.33
Low Alkalinity	Y	MCCONNELL	88.33
Low Alkalinity	Y	NATHAN (NH)	100.00
Low Alkalinity	Y	RUSSELL (NH)	100.00
Low Alkalinity	Y	SESSIONS (NH)	100.00
Low Alkalinity	Y	SMITH (NH)	100.00
Low Alkalinity	Y	TURTLEHEAD	58.33
Low Alkalinity	Y	WALLINGFORD	78.33
Low Alkalinity	Y	WHEELER (BRUNWK)	95.00
Low Alkalinity	Y	WILLARD (NH)	96.67
Low Alkalinity	Y	WOLCOTT	63.33
Well Buffered	N	BEEBE (HUBDTN)	76.57
Well Buffered	N	BLISS	63.56
Well Buffered	N	BURR (SUDBRY)	70.05
Well Buffered	N	CLYDE	65.00
Well Buffered	N	COLCHESTER	77.33
Well Buffered	N	CURTIS	66.00
Well Buffered	N	EWELL	80.53
Well Buffered	N	GREAT HOSMER	82.67
Well Buffered	N	INDIAN BROOK (ESSEX)	68.67
Well Buffered	N	NORTH ST. ALBANS	53.00
Well Buffered	N	PLEASANT VALLEY	68.53
Well Buffered	N	SILVER (BARNRD)	77.87
Well Buffered	N	SOUTH ST. ALBANS	55.67
Well Buffered	N	SPRING (SHRWBY)	81.60
Well Buffered	N	STILES	79.33
Well Buffered	N	SUNRISE	62.00
Well Buffered	N	TICKLENAKED	90.33
Well Buffered	N	WOODWARD	69.67
Well Buffered	Y	BALD HILL	95.47
Well Buffered	Y	BUTTERNUT (NH)	84.00
Well Buffered	Y	DANBY	93.33
Well Buffered	Y	HATCH (NH)	85.60
Well Buffered	Y	HIGH (SUDBRY)	91.73
Well Buffered	Y	HINKUM	86.67
Well Buffered	Y	LONG (GRNSBO)	86.67
Well Buffered	Y	LONG (SHEFLD)	83.20
Well Buffered	Y	LYFORD	92.27
Well Buffered	Y	NORTH (BRKFLD)	88.89

Application of the Vermont Lake Condition BioIndex

The Vermont Lake Condition BioIndex is designed to assist in the formal assessment of aquatic life use support for lakes and ponds in Vermont, and may be suited to application in other northeastern lakes. It was designed around detection of biological responses to five broad classes of stressors: eutrophication and cumulative development; acidification; nuisance aquatic species infestations; water-level fluctuations; and, chemical treatments to control nuisance aquatic plants. At the current stage of development, the Index is suitable for a determination of aquatic life use attainment in conjunction with parallel data from an assessment of phytoplankton (see VTDEC 2003), littoral habitat quality, and/or water quality. Additional testing should be done prior to relying on the BioIndex to document an impairment without parallel corroborating data. The relationship of BioIndex scores to existing water quality criteria is not yet defined. A proposal below provides one approach to incorporating the BioIndex into water quality criteria attainment decisions.

The Vermont Water Quality Standards (2006) establish water management types as descriptors of Class A and B waters. As pertains to the assessment of aquatic life and habitat, these water management types constitute tiered aquatic life use criteria. The specific language describing the water management types for aquatic biota and habitat is redacted in the following.

Aquatic Biota, Wildlife, and Aquatic Habitat	
Class	**Criteria**
A(1)	Change from the natural condition limited to **minimal impacts** from human activity. Measures of biological integrity for aquatic macroinvertebrates and fish assemblages are **within the range of the natural condition**. Uses related to either the physical, chemical, or biological integrity of the aquatic habitat or the composition or life cycle functions of aquatic biota or wildlife are fully supported. All life cycle functions, including overwintering and reproductive requirements are maintained and protected.
A(2) Reserved for designated water supplies	Biological integrity is maintained, no change from the reference condition that would prevent the full support of aquatic biota, wildlife or aquatic habitat uses. Change from the reference condition for aquatic macroinvertebrates and fish assemblages shall not exceed moderate changes in the relative proportions of taxonomic, functional, tolerant and intolerant components. All expected functional groups are present in a **high quality habitat** and none shall be eliminated. All life cycle functions, including overwintering and reproductive requirements are maintained and protected. Changes in the aquatic habitat shall not exceed **moderate differences from the reference condition** consistent with full support of all aquatic biota and wildlife uses.
B(1)	Change from the reference condition for aquatic macroinvertebrate and fish assemblages shall be limited to **minor changes** in the relative proportions of taxonomic and functional components; relative proportions of tolerant and intolerant components are within the range of the reference condition. Changes in the aquatic habitat shall be limited to **minimal differences from the reference condition** consistent with the full support of all aquatic biota and wildlife uses.
B(2)	Change from the reference condition for aquatic macroinvertebrate and fish assembledges shall be limited to **moderate changes** in the relative proportions of tolerant, intolerant, taxonomic, and functional components. Changes in the aquatic habitat shall be limited to **minor differences from the reference condition** consistent with the full support of all aquatic biota and wildlife uses.
B(3)	Change from the reference condition for aquatic macroinvertebrate and fish assemblages shall be limited to **moderate changes** in the relative proportions of tolerant, intolerant, taxonomic, and functional components. Changes in the aquatic habitat shall be limited to **moderate differences from the reference condition** consistent with the full support of all aquatic biota and wildlife uses. When such habitat changes are a result of hydrological modification or water level fluctuation, compliance may be determined on the basis of aquatic habitat studies.

Within this framework, the Vermont Lake Condition BioIndex is specifically tailored to assess the *aquatic life* component of the above criteria. Overall lake scores can be related to water management types, and while the legal promulgation of specific threshold values is the responsibility of the Vermont Water Resources Panel, preliminary recommendations are provided. These recommendations were developed with the following assumptions. First, the lowest quartile of reference lake scores may not be representive of reference conditions (see Figure 4). This is consistent with the USEPA approach to biocriteria development that accommodates unforseen legacy effects that impact biota even on the most pristine waters. Second, lakes scoring above the 25th percentile of reference exhibit naturally occurring biota consistent with Water Management Type A(1). Third, the BioIndex has the discriminatory precision to segregate so-called "B(1)" biota from B(2)/B(3), and B(2)/B(3) from non-attainment. Based on these fundamental assumptions, the recommendations in Table 10 were derived using the simple algorithm shown by Figure 6.

Table 10. Overall mean Vermont Lake Condition BioIndex score threshold values for the attainment of water management type criteria.

Lake class→ / Water Management type ↓	Well buffered	Low alkalinity	Large
A(1)	>85	>75	>85
B(1)	≥74	≥65	≥71
B(2)/B(3)	≥63	≥55	≥57
Non-attainment	<63	<55	<57

The multi-habitat design of the index is such that it is suited to the assessment of component habitats, or the overall quality of a lake. However, not every habitat is required for a valid lake bioassessment, and in lakes where not all five habitat types are present, BioIndex scores will be comprised of a subset of habitats. For this reason, when comparing lake index scores across multiple types, or where habitats were not sampled, it is important to calculate individual habitat scores normalized to a common denomenator, such that the mean overall lake score reflects only the habitats assessed.

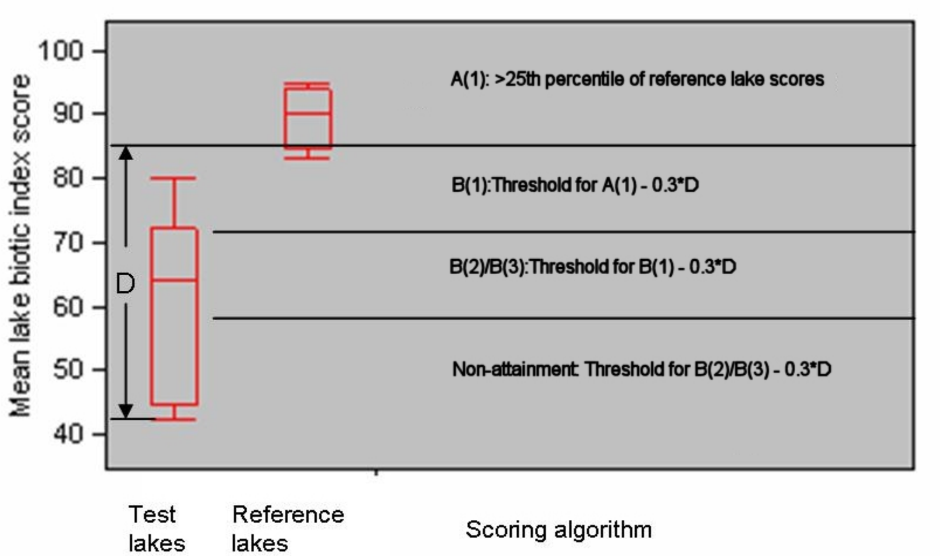

Figure 6. Proposed algorithm to determine threshold Vermont Lake Condition BioIndex scores in relation to Vermont water management type criteria for Class A and B waters. Data shown are for large lakes.

Procedurally, each sampled habitat carries the cost of sampling, picking, sorting, taxonomy, data-entry, and assessment. At first-order, the muddy-littoral habitat should not be sampled for large lakes, and the sublittoral habitat should not be sampled for low-alkalinity lakes (Table 5). Beyond that, the decision to sample habitats with low numbers of useful metrics (e.g., macrophyte habitat in large lakes) should be predicated on the need for information from that habitat, likelihood of stressors to macroinvertebrates utilizing that habitat, and the overall need for bioassessment information from the lake.

Low-alkalinity lakes displayed the smallest differentiation in BioIndex scores between test and reference lakes, the smallest number of applicable metrics, and the largest statistical *p*-values for determining deviation from reference. There is a higher cost to assessing lakes of this type relative to the precision of the obtainable results, and the decision to assess a lake of this type should strongly consider the need for the bioassessment data. In other lake types, specific habitats may or may not need to be assessed, depending on the stressor in question. Considering this, the following framework for deciding which habitats to sample is proposed (Table 11).

Table 11. Recommendations for habitats to sample in relation to certain stressors.

Lake class	Stressors	Higher priority habitats	Lower priority habitats
Large	Eutrophication or Acidification	Rocky littoral Sublittoral Profundal	Macrophytes
	Water level manipulation or Chemical treatment for nuisance plant species[1]	Rocky littoral Macrophytes	Sublittoral Profundal
Low alkalinity	Eutrophication or Acidification	Rocky littoral Macrophytes Profundal	Muddy littoral
	Water level manipulation or Chemical treatment for nuisance plant species	Rocky littoral Muddy littoral Macrophytes	Profundal
Well buffered	Eutrophication or Acidification	Rocky littoral Muddy littoral Macrophytes Sublittoral	Profundal
	Water level manipulation or Chemical treatment for nuisance plant species	Rocky littoral Muddy littoral Macrophytes	Sublittoral Profundal

1) Nuisance species here refers to aquatic macrophytes. For assessment of stress due to chemical treatments to control in-lake algae growth or sediment nutrient release, profundal habitats should be sampled regardless of lake type.

Implementing the BioIndex

This section describes the general steps to follow in order to assess a lake using the Vermont Lake Condition Bioindex for macrionvertebrates. The sequence begins with identification and classification of the target lake. Habitats to sample are selected, based on an understanding of the likely stressor(s) involved. The lake is then sampled, and taxonomy is carried out. Metrics are calculated, and the resultant habitat scores are expressed on a scale of 100. Overall lake scores can be calculated to permit assessment of lake biota in relation to water management types.

Lake selection and classification

VTDEC (2003) describes a linear discriminant classification model to allocate lakes to a class. This model uses five physico-chemical attributes that are considered unaffected by anthropogenic stressors. These are lake area, lake basin area, maximum depth, lake mean alkalinity, and lake mean conductivity. The minimum, maximum, and median values for these parameters are shown in Table 12 by lake class. Lakes selected for evaluation using the BioIndex should conform to the degree possible to the ranges shown to remain within the prediction range of the statistical model. In order to classify a lake, these attributes are entered in three parallel linear functions (Table 13), and the function that yields the largest value indicates the class to which the lake would belong. An example is provided.

Table 12. Attributes used to develop a lake classification model for application of the Vermont Lake Condition BioIndex.

Attribute	Dataset wide values		Median values by class		
	Minimum	Maximum	Large	Low Alkalinity	Well buffered
Lake area (LA, ac)	20	1402	754	87	80
Basin area (BA, ac)	173	14453	4043	1247	1132
BA:LA	4.2	63	11.4	14.7	12.5
Max. depth (m)	3.3	43	39.7	7.0	10.9
ALK mg/l	-0.3	99.8	39.9	6.8	38.5
COND µs/cm^3	9.2	217	95.6	26.0	91.8

Table 13. Classification coefficients and constants which constitute the classification functions used to allocate lakes into one of three classes.

Lakes are classified to the largest solution of each linear function.			
Lake Class →	Well buffered	Large	Low alkalinity
Coefficient ↓			
CONSTANT	-214.12	-240.40	-217.32
Ln{1+Lake area (ac)}	889.09	879.35	884.97
Ln {1+Basin area (ac)}	-871.96	-856.23	-866.60
Ln (1+BA:LA)	938.44	922.31	935.72
Ln {1+Maximum depth (m)}	-0.01	2.08	0.18
Ln {1+Alkalinity (mg/l)}	-22.51	-23.38	-27.55
Ln {1+Conductivity (*u*s/cm^3)}	36.03	34.82	37.22

For Spring Lake in Shrewsbury, VT, the attributes and solutions are:

Lake area: 66 ac Basin area: 275 ac BA:LA: 4.17 Max depth: 24 m Alkalinity: 36.9 mg/l Conductivity: 79.9 µs/cm^3	Solution for well buffered lakes = 241.0 Solution for low alkalinity lakes = 234.0 Solution for large lakes = 233.7 Since the solution for the well buffered lake function is largest, and considerably larger than those for low alkalinity or well buffered lakes, the most likely class for Spring Lake is the well buffered lakes.

Habitat selection, sampling approach, and taxonomy

Once a lake is classified, the selection of target habitats should consider the need for data and total cost of the assessment (Table 11). All sampling activities for macroinvertebrates should be carried out during the index period of late July through early September, and in all cases, prior to erosion of the lakes thermal stratification.

Macroinvertebrate sampling activities should conform to sections 6.4.5 for littoral zone sampling, and 6.4.6 #2 of the Vermont Department of Environmental Conservation, Water Quality Division Field Methods Manual (VTDEC 2006, http://www.vtwaterquality.org/bass/docs/bs_fieldmethodsmanual.pdf).

Sample picking, sorting, and taxonomy should conform to he entirety of Section 6.6 of the Field Methods Manual. Calculation algorithms for metrics are described in detail in VTDEC (2001). VTDEC maintains a data system that houses biological monitoring data and automatically calculates many of the relevant metrics. Other metrics are calculated by hand. Lake specific data and calculated metrics are stored in the VTDEC "LakeBiocriteria" database, which is a Microsoft Access © application.

Assessment

Once metric calculations are completed, the threshold values shown in Table 7 are used to generate habitat-specific scores, and overall lake scores can be calculated as the mean of those scores. The "LakeBiocriteria" application automatically extracts relevant metrics by lake class and habitat type, and a standalone Microsoft Excel© application has been developed to assess lakes by comparing metric results to thresholds. Habitat specific scores are calculated as:

$$\frac{\sum_{(1\rightarrow i)} \text{metric scores for habitat}_j}{5*n}$$

The overall lake score is calculated as the mean of the habitat scores:

$$\frac{\sum_{(1\rightarrow j)} \text{habitat scores}}{j}$$

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