Development of Biocriteria for Vermont and New Hampshire Lakes

Criteria Development for
Phytoplankton and Macroinvertebrate Assemblages
for Three Lake Classes

Final Report

March 2003

prepared by:

Neil Kamman, Environmental Scientist

Vermont Department of Environmental Conservation
103 S Main 10N
Waterbury VT 05671-0408

TABLE OF CONTENTS

Introduction and Acknowledgments

This report summarizes efforts made in developing biological criteria for Vermont and New Hampshire lakes. A brief project overview is presented, followed by a detailed description of bioassessment methods employed. Biological assessments for lakes sampled between 1996 and 1999 are presented. These assessment data are used within a multimetric bioassessment framework (USEPA 1997, Reynoldson et. al., 1997) to generate draft criteria for assessing a lake's overall compliance with standards for aquatic life use support. Results from the paleolimnology component of this project will be provided by New Hampshire DES under separate cover.

Overview

The objective of this project was to determine the range of biological characteristics that constitutes reference conditions for lakes of differing types. Our ultimate aim is the development of lake-specific biological criteria for inclusion in Vermont's Water Quality Standards. A corollary project goal was the evaluation of bioassessment methods presented in the USEPA Lake and Reservoir Bioassessment and Biocriteria Technical Guidance Document (USEPA 1998). Summary results from earlier portions of this project form one of the case studies in that document.

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, 1996; USEPA, 1998; Gerritsen et al., 2000). A-priori classification of lakes was conducted using lake physico-chemical attributes that are not typically affected by anthropogenic factors. This classification was subsequently corroborated using the biological measurements. The biological reference condition of three lake classes was defined, and was used to assess known-impaired lakes and lakes of unknown biological condition. Between 1996 and 1999, biological and chemical sampling was conducted on 43 lakes; 31 in Vermont and 12 in New Hampshire. Lakes sampled during the project period are described in Table 1, and are geographically located on Figure 1.

Table 1. Study lakes visited in conjunction with the Bioassessment of Vermont and New Hampshire Lakes Project, 1996-1999.

Lake Name	Town	State	Year Assessed	Lake Name	Town	State	Year Assessed
BALD HILL	NEWARK	VT	1997	LITTLE ELMORE	ELMORE	VT	1996
BEAVER	DERRY	NH	1996	LONG	GREENSBORO	VT	1997
BRANCH	SUNDERLAND	VT	1998	LONG	SHEFFIELD	VT	1997
BUTTERNUT	GRANTHAM	NH	1996	MAIDSTONE	MAIDSTONE	VT	1998
CARMI	FRANKLIN	VT	1996	MCCONNELL	BRIGHTON	VT	1996
CASPIAN	GREENSBORO	VT	1997	NATHAN	DIXVILLE	NH	1996
COLE	JAMAICA	VT	1998	PARKER	GLOVER	VT	1999
CRYSTAL	BARTON	VT	1997	RUSSELL	WOODSTOCK	NH	1997
CURTIS	WOODBURY	VT	1998	SESSIONS	DUMMER	NH	1997
DUDLEY	DEERING	NH	1996	SHADOW	GLOVER	VT	1998
DUNMORE	LEICESTER	VT	1998	SMITH	WASHINGTON	NH	1997
EDEN	EDEN	VT	1998	SPRING	SHREWSBURY	VT	1997
EWELL	PEACHAM	VT	1997	ST. CATHERINE	WELLS	VT	1998
FAIRFIELD	FAIRFIELD	VT	1998	STRATTON	STRATTON	VT	1998
FRENCH	HENNIKER	NH	1997	TICKLENAKED	RYEGATE	VT	1999
GILMAN	ALTON	NH	1996	TURTLEHEAD	MARSHFIELD	VT	1996
GREAT HOSMER	CRAFTSBURY	VT	1997	WALLINGFORD	WALLINGFORD	VT	1996
HATCH	EATON	NH	1996	WHEELER	BRUNSWICK	VT	1996
HIGH	SUDBURY	VT	1997	WILLARD	ANTRIM	NH	1997
HINKUM	SUDBURY	VT	1997	WOLCOTT	WOLCOTT	VT	1996
HOLLAND	HOLLAND	VT	1998	WOODWARD	PLYMOUTH	VT	1998
INTERVALE	SANDWICH	NH	1997				

For this project, three biological assemblages were measured and their metrics evaluated for criteria development. These were epipelagic phytoplankton, littoral macrophytes, and macroinverterbates within five habitat zones. Trophic measurements were also made for each study lake. A comprehensive listing of candidate biological metrics and chemical measures presented in this report for the all study lakes is provided in Table 2.

Methods

Study Lake Selection

Across the study period, lakes were selected to represent a range of physico-chemical conditions, and to provide an even distribution between candidate reference and test lakes. Lakes sampled during 1996 and 1997 were largely low and moderate alkalinity respectively, and for the most part between 20 and 100 ac in size. Lakes sampled in 1998 and 1999 were larger in many cases (100-800 ac), and had a wide alkalinity range.

Study lakes were delineated into littoral, sublittoral, and profundal zones. Table 3 presents the number of stations and the level of sampling effort used to characterize each biological assemblage within each lake zone.
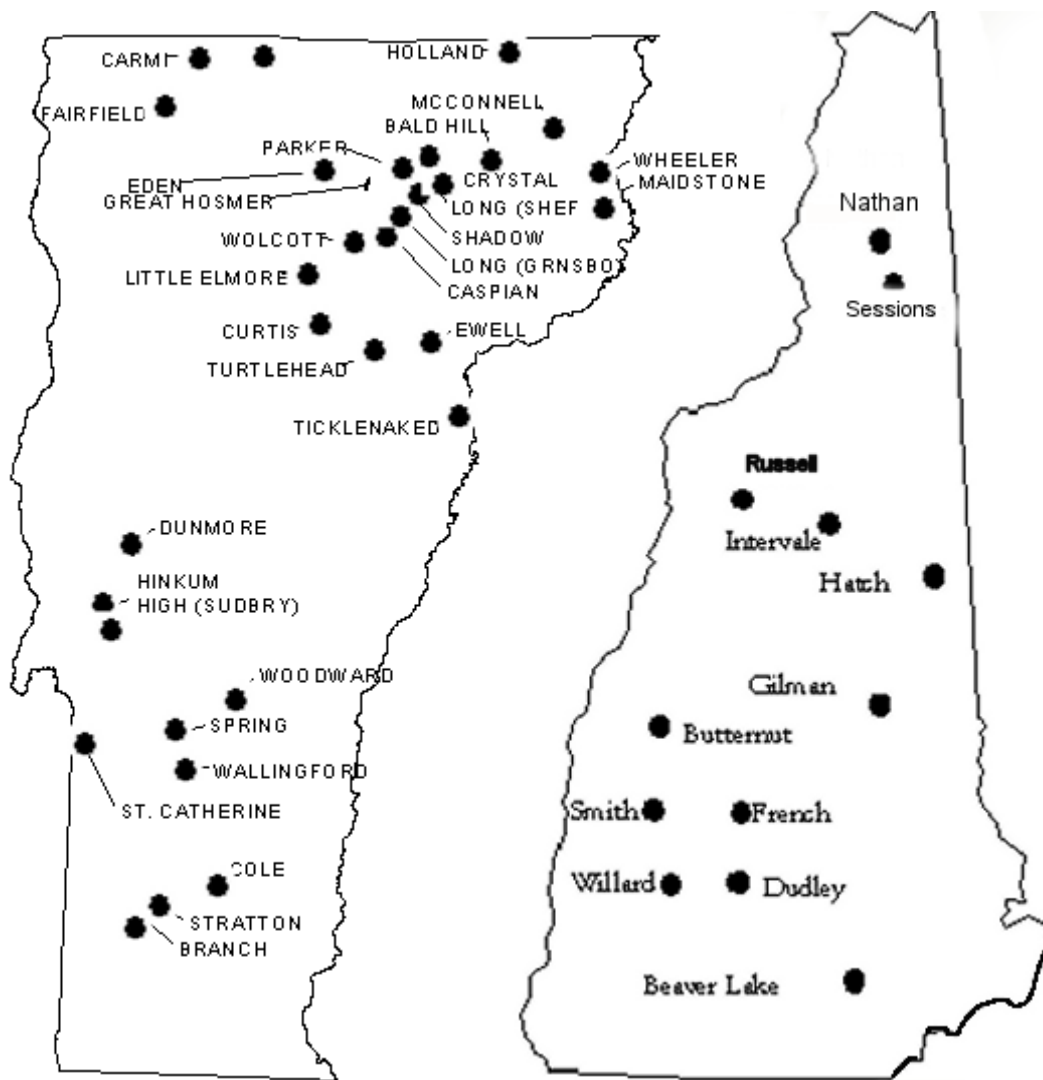
Figure 1. Location of Study Lakes sampled in conjunction with the Bioassessment of Vermont and New Hampshire Lakes Project

Table 2. Tier two chemical parameters and biological metrics evaluated for 1996-1997 Bioassessment and Paleolimnology of Vermont and New Hampshire Lakes Project study lakes.

Physico-chemical and trophic state parameters to be evaluated:	
Alkalinity Conductivity Dissolved oxygen Algal biovolume - Sweet TSI Chlorophyll-a - Carlsons' TSI Secchi transparency - Carlsons' TSI	
Biological Assemblage	**Candidate biometrics to be evaluated:**
Macroinvertebrates	Taxa richness Percent dominants Shannon-Weiner index of diversity Percent intolerant species COTE index (*Coleoptera, Odonata, Ephemeroptera, Trichoptera*) Percent intolerant chironomids Taxa richness - *Crustacea - Mollusca* Functionality (ie. shredder, scrapers...)
Macrophytes	Percent cover - littoral zone Percent cover - littoral zone, nuisance species Species richness Relative percent dominance Richness - rare species Richness - *Potamogeton spp.* Richness - *Utricularia spp.* Percent occurrence by structural morphology
Phytoplankton	Total density Total biovolume Total taxa richness Shannon-Weiner diversity Percent *Anabena* spp., *Aphanizomenon spp.*, *Anacystis spp.* Percent Cyanobacteria (density and biovolume) Percent Diatoms (density and biovolume) Percent Chlorophytes (density and biovolume) Percent Euglenophytes (density and biovolume) Percent Phyrrophytes (density and biovolume) Percent Cryptophytes (density and biovolume)

Table 3. Distribution of sampling effort for study lakes in the Bioassessment and Paleolimnology of Vermont and New Hampshire Lakes Project.

Assemblage:	Lake zone:			Sampling effort
	Littoral	Sublittoral	Profundal	
Macroinvertebrates	3 habitat types	composite of 3 stations	Composite of three dredge samples from the vicinity of the deep station	Synoptic - one visit during the late-summer index period
Macrophytes	Full survey	--	--	
Zooplankton	--	--	Deep-hole, profundal station	
Physico-chemical	--	--		
Phytoplankton	composite of 4 stations			bi-weekly
Chlorophyll-a	--	--		

Sampling Sites

Each study lake has a centrally located, deepwater (hypolimnetic) station from which water chemistry and biological samples were collected. Deepwater station locations are given in AppendiX A. An additional three macroinvertebrate sampling stations were located over the sublittoral zone. Phytoplankton samples were collected from equally spaced stations, located along a transect from inlet to outlet, and which passed through the deepwater station. All stations were sited in the field during the first field visit, and were revisited during any subsequent visits. Figure 2 presents a graphical representation of station layout for a hypothetical study lake.
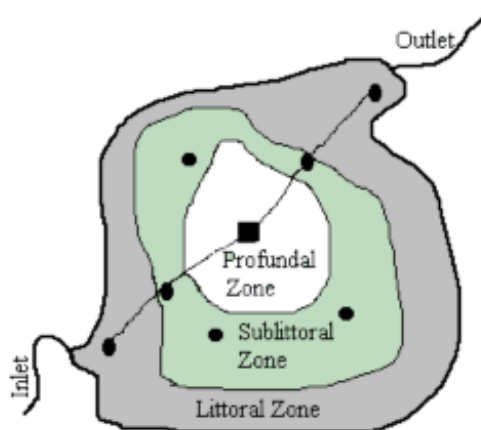
Figure 2. Graphical representation of sampling station locations for each study zone of a hypothetical study lake. The deep station is denoted by a □, sublittoral macroinvertebrate stations are denoted by a □, and phytoplankton transect stations are denoted by an oval.

Sampling Procedures

Macroinvertebrates

Littoral Zone: The lake littoral zone is made up of many microhabitat types which have a strong influence on the macroinvertebrate species composition present. Therefore sample collection was stratified on the following three specific habitat types: rocky/cobble/large woody debris; macrophyte beds; and organic fine muds. Target macroinvertebrate communities for these habitat types were rocky-littoral epibenthos, macrophytic epibenthos, and muddy-littoral epi- and infaunal benthos, respectively. Three sites within each littoral habitat type were sampled, and these composited. Each of the habitat types were qualitatively sampled using a sweep net, forceps, and a strainer (VTDEC, 1990, method 4.4.4). Samples were cleaned using 500*u* mesh netting and/or a #30 sieve to remove sediment, debris, and meiofaunal organisms. All samples were preserved in the field with 75% ETOH.

Sublittoral Zone: The sublittoral zone (where present) is defined as that area of the lake bottom that is below the area of macrophyte growth, but above the thermocline. The target macroinvertebrate community within this habitat zone was epi- and infaunal benthos. An Ekman dredge (15cm*15cm) was used to collect samples into a #30 sieve bucket. Single Ekman dredge grabs from three separate sublittoral zone sites were composited to form a single sample (VTDEC, 1990, method 4.4.5). Each sample was preserved with 75% ETOH and returned to the laboratory.

Profundal Zone: The profundal zone (where present) is defined as that area of the lake below the thermocline. The sediment type targeted for the purpose of this study was gyttja, where available and consistent with the lake type. The target macroinvertebrate community within this habitat zone infaunal benthos, although some epifaunal benthic species were also expected (e.g. Mysis *spp.*).The Ekman dredge was used to collect samples into a #30 sieve bucket, and three single grabs from the area surrounding the profundal station were composited together into a single sample. Each sample was preserved with 75% ETOH and returned to the laboratory.

Taxonomy: Samples were washed of ETOH through a #30 sieve and spread evenly over a white gridded tray by adding a small amount of water to allow the sample to be evenly spread, but not so much as to cause the macroinvertebrates to float freely around the tray. No fewer than 300 animals from no less than one quarter of each sample were picked and sorted into major groups. The animals were then preserved in 75% ETOH and later identified to genus/species except for the Oligochaeta which were identified to family.

Macrophytes

Each lake was comprehensively surveyed for littoral zone macrophyte communities. In summary, the entire littoral zone of each study lake was traversed in a suitable vessel. Species were identified in-situ, or removed from the water using a throw rake as needed. Species with questionable identifications were returned to the laboratory for more thorough taxonomy. The Braun-Blanquet scale was used to semi-quantitatively estimate percent macrophyte cover, by species, for subsections of the littoral zone with similar species associations. These data were then used to calculate an average percent macrophyte cover for the entire littoral zone. Detailed data collection procedures for macrophyte surveys are presented in Warren, 1995, and VTDEC, 1990 (method 4.3.1).

Physico-chemical parameters

Collection procedures for physico-chemical parameters are presented in Table 4. These parameters were collected once during midsummer, with the exception of Secchi transparency and chlorophyll-a, which were measured bi-weekly (June through mid-September), concurrent with collection of phytoplankton samples. Each state laboratory was responsible for analysis of their respective lakes' water chemistry samples, with a minimum of ten percent of all chlorophyll-a samples run as split samples to assess inter-laboratory comparability.

Phytoplankton

Phytoplankton samples were collected by depth-integrating sampler at a depth of twice the secchi depth (euphotic zone), or to within one meter of the bottom sediments. In locations where obtaining a minimum one meter hose sample was impractical (e.g. the bottom depth was < 1 meter in depth), a grab sample was obtained at 0.2 meters. A 100ml subsample was be obtained at each of the five individual lake sites, and these subsamples were composited to form a whole-lake sample representing algae present in the lake for that visit. Samples were preserved in Lugols' solution at 2.5ml per 100ml sample (preserved to a 'weak tea' color). Because of the highly variable nature of phytoplankton communities, sampling was conducted throughout the warmwater season (June through mid-September) on a bi-weekly basis. All bi-weekly lake-composite samples were further composited to form a whole-season, whole-lake sample. Individual single visit composites were archived for potential future analysis.

Individual whole-season phytoplankton composite samples were counted and identified by Aquatic Analysts Inc. of Portland, OR, using a stereo microscope. Individual algal species were enumerated from a subsample of the

composite using the Utermohl method, using a minimum 100 algal-unit count. Results were reported as density and relative percent density, and biovolume and relative percent biovolume for each species.

Zooplankton

Zooplankton samples consisting of three composited vertical net tows from the central deepwater station were collected using an 80 micron mesh Wisconsin net were archived from the deepwater station. These samples were preserved in 75% ETOH to achieve 30% residual ETOH.

Table 4. Field and analytical methods for physico-chemical water quality parameters collected in conjunction with the Bioassessment and Paleolimnology of Vermont and New Hampshire Lakes Project.

Analytical Parameter	Field collection method	VT method number[2]	NHDES analytical method number	VTDEC analytical method number[1]	EPA method[3]
Alkalinity	Kemmerer, 1 meter below surface, and 1 meter above the bottom if thermally stratified conditions exist.	2.2.3	5.5.1[5]	5.1.2	2320B[4]
Conductivity			2510B[4]	1.6.2[2]	120.1
Dissolved oxygen	Kemmerer, profile - VT YSI54A/Hydrolab- NH		Hydrolab/ YSI	5.7.2	360.2
Transparency	Secchi	1.2.1	n/a	n/a	n/a
Temperature	Thermistor, profile - VT YSI54A/Hydrolab- NH	1.1.2	Hydrolab/ YSI	n/a	n/a
Chlorophyll-a	Depth-integrated composite	2.2.2	n/a	5.4.2	10200[4]

[1] VTDEC, 1992. Laboratory Quality Assurance Plan.
[2] VTDEC, 1990.
[3] EPA, 1979 *rev*.1983 Analysis of Water and Wastes.
[4] APHA, 1992. Standard Methods Ed.18.
[5] EPA, 1987. Handbook of Methods for Acid Deposition Studies.

Results and Criteria Development

Lake Physical Attributes and Water Chemistry

Lakes sampled during the three-year project period varied widely in their physico-chemical makeupLake sizes ranged from 20 to 1,402 acres, basin-lake area ratios ranged from 4.2:1 to 63:1, and lake depth ranged from 3.3 to 43 meters. Flushing rates varied from a low of 0.4 to a maximum 52 refills per year for the very small and low-volume Little Elmore Pond.

Alkalinity measured in the study lakes ranged from –0.33 mg/l in the acidic Branch Pond to nearly 100 mg/l in Ewell Pond, and conductivity varied between 9.2 and 217 *u*mhos. Seasonwide mean Secchi transparencies varied from 1.7 to 11.5 meters in the oligotrophic Russell Pond, and mean chlorophyll-a concentrations ranged from a low of 0.98 *u*g/l to a high of 22.5 in the eutrophic French Pond.

Table 5. Physico-chemical attributes of 43 lakes sampled in conjunction with the Bioassessment of VT and NH Lakes Project.

LakeId	Reference Status	Lake Area (ac)*	Basin Area (ac)*	Basin to Lake Area ratio*	Max. Depth (m)*	Alk. mg/l as $CaCO_3$*	Cond. us/cm³*	Flushing Rate #/yr	Mean Secchi (m)	Mean Chl-a (ug/l)
BALD HILL	Ref.	108	2588	24.0	12.8	40.30	89.9	7.4	4.2	2.9
BEAVER (NH)	Test	133	6899	51.9	13.7	16.78	162.2	5.1	4.0	9.4
BRANCH	Test	34	330	9.7	10.7	-0.33	10.1	7.9	2.3	1.7
BUTTERNUT (NH)	Ref.	37	991	26.8	3.3	8.50	31.3	10.4	2.8	4.0
CARMI	Test	1402	7710	5.5	10	39.20	83.0	1.6	2.5	8.5
CASPIAN	Ref.	789	4510	5.7	43	63.50	149.0	0.5	7.6	1.3
COLE	Test	41	282	6.9	4	6.65	31.5	6.5	3.2	1.7
CRYSTAL (BARTON)	Ref.	763	14453	18.9	22	31.85	101.2	1.3	6.6	1.0
CURTIS	Test	72	917	12.7	9.5	74.30	168.0	2.1	3.6	10.3
DUDLEY (NH)	Ref.	30	1664	55.5	6.1	6.05	34.5	7.8	5.0	4.3
DUNMORE	Test	985	13068	13.3	32	26.60	65.0	0.9	4.2	3.2
EDEN	Test	194	2347	12.1	12	22.50	78.0	4.0	3.7	2.6
EWELL	Test	51	1981	38.8	18	99.85	217.0	8.0	4.5	1.4
FAIRFIELD	Test	446	3758	8.4	12.8	31.70	85.0	1.4	5.0	6.7
FRENCH (NH)	Test	42	486	11.6	12.5	9.38	82.9	1.3	3.3	22.5
GILMAN (NH)	Ref.	32	631	19.7	5.2	7.30	27.8	5.0	3.8	6.3
GREAT HOSMER	Test	140	860	6.1	17	86.80	179.0	0.6	5.7	7.0
HATCH (NH)	Ref.	25	475	19.0	17.4	9.62	46.6	1.6	5.4	5.8
HIGH (SUDBRY)	Ref.	20	173	8.7	16	59.75	131.1	0.5	5.9	4.1
HINKUM	Ref.	60	353	5.9	24	36.65	93.7	32.3	7.8	2.0
HOLLAND	Test	325	4431	13.6	11.9	4.80	20.0	4.0	2.8	2.2
INTERVALE (NH)	Test	43	1152	26.8	14.9	4.70	34.3	1.9	5.8	6.6
LITTLE ELMORE	Test	20	316	15.8	4	7.60	30.8	52.1	1.7	7.5
LONG (GRNSBO)	Ref.	100	1910	19.1	7	47.50	104.4	6.8	3.9	4.0
LONG (SHEFLD)	Ref.	38	204	5.4	9	20.40	49.8	14.4	4.1	2.2
MAIDSTONE	Ref.	745	3103	4.2	37	8.20	13.0	0.4	7.5	1.4
MCCONNELL	Ref.	87	3621	41.6	7	8.00	29.1	17.3	2.0	6.2
NATHAN (NH)	Ref.	42	2112	50.3	6.4	13.90	40.4	19.5	2.2	6.3
PARKER	Test	250	5418	21.7	13.7	63.00	114.9	1.7	--	--
RUSSELL (NH)	Ref.	39	365	9.4	23.7	2.31	24.4	0.8	11.5	2.6
SESSIONS (NH)	Ref.	35	512	14.6	10.3	6.20	26.3	1.5	3.6	12.2
SHADOW (GLOVER)	Ref.	210	3575	17.0	42.4	48.00	90.0	1.7	6.7	1.4
SMITH (NH)	Ref.	27	492	18.2	10.76	3.98	29.6	2.3	5.2	5.0
SPRING (SHRWBY)	Test	66	275	4.2	24	36.85	79.9	0.7	6.7	7.8
ST. CATHERINE	Test	883	7447	8.4	19.5	33.40	100.0	0.9	4.3	4.2
STRATTON	Test	46	266	5.8	5.5	1.41	9.2	5.1	2.2	4.8
TICKLENAKED	Test	54	1444	26.7	14.5	63.30	162.2	3.5	--	--
TURTLEHEAD	Ref.	69	3707	53.7	7	7.85	36.9	31.6	1.7	4.5
WALLINGFORD	Ref.	87	1470	16.9	7	5.70	22.8	20.2	1.9	4.1
WHEELER (BRUNWK)	Ref.	66	4159	63.0	10	7.52	27.8	42.3	2.0	4.3
WILLARD (NH)	Ref.	96	1024	10.7	17.7	1.58	22.5	6.1	9.1	3.6
WOLCOTT	Ref.	74	920	12.4	7	8.95	30.3	2.7	2.6	10.4
WOODWARD	Test	106	1878	17.7	14.6	28.30	108.0	6.4	--	--

*) Denotes environmental variables used in classification analyses

Development of Phytoplankton-Based Criteria

Overview

An overview of the lake classification procedure for plankton criteria development is as follows. To classify lakes, an initial grouping was inferred using canonical correspondence analysis (terBraak, 1988) using PC-ORD software (McCune and Medford, 1997). The purpose of this first analyses was to identify a potential classification scheme based on the multivariate relationships between phytoplankton metrics and environmental variables across the 40 lakes. The inferred classification was subsequently tested and formalized with multivariate linear discriminant analysis (Rencher, 1997) using only environmental variables. This and subsequent multivariate linear modeling analyses were performed using SAS (proc GLM with MANOVA, SAS Institute, 2000). The purpose of the discriminant analysis was to refine the classification scheme, and to generate a set of mathematical equations by which lakes could be classified with known confidence. Once the lakes were so-classified, differences in the phytoplankton metrics across the classes were tested using multivariate ANOVA (Rencher, 1997). This third procedure ensured that there existed a statistically significant difference in the phytoplankton community which could be explained by a lakes' membership to its' physico-chemical class. Metric distributions were then examined within classes to assess candidate metrics for inclusion into a multimetric phytoplankton community index, and statistical independence and variability of the metrics was assessed. Selected metrics were then analyzed within a second multivariate ANOVA to insure that the metrics were able to distinguish reference from test conditions, while accounting for variation attributable to class. This final set of metrics was then scored by 'bi-section' (USEPA 1998), and the metrics combined to comprise a phytoplankton community index. The replicability of this index was then assessed.

Inferential Classification by Canonical Correspondence Analysis:
A total of 134 distinct epipelagic species comprise the dataset used to develop phytoplankton metrics. These are shown in Appendix B. An average of 20 species were present within any given composite lake sample. The canonical correspondence analysis was performed in such a manner that it ordinated lakes as weighted averages of the phytoplankton metrics, and superimposed these weighted-averages over lake scores which were linear combinations of 6 environmental variables (Table 5). The analysis explained 25% of the total dataset variation (23.8% on the first two axes), and is graphically displayed in Figure 1. From this analysis, it was evident that lake and basin size explained the greatest separation in the phytoplankton community composition of individual lakes, since these variables have high
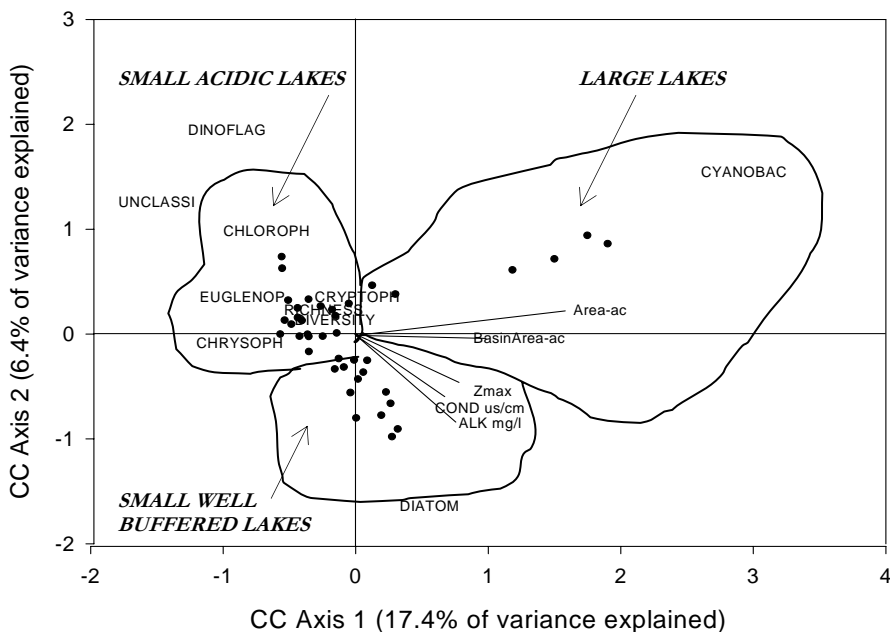

Figure 3. Canonical correspondence triplot of 40 lakes (•) as weighted averages of 8 phytoplankton compositional metrics (uppercase) in relation to 5 environmental variables (vectors). This ordination explains 25% of the total dataset variance. Inferred lake classes are identified.

correlations to the first ordination axis. Alkalinity, conductivity, and depth correlated well to the second axis. Since alkalinity and conductivity are good predictors of a lakes' acidity, the second axis is thus likened to an acidity gradient. Inspection of the individual environmental variable observation vectors (sets) for each individual lake suggested the existence of three lake classes: smaller acidic lakes; smaller well-buffered lakes; and large lakes. These inferred classes are shown on Figure 3.

Classification Verification by Multivariate Linear Discriminant Analysis

The classification was verified using multivariate linear discriminant analysis of the environmental variables, with individual lakes pre-assigned into one of the three inferred classes. For this analysis, environmental variables were log-transformed to satisfy requirements of normality. This analyses was performed using SAS proc CANDISC, requesting canonical scores, classification coefficients, and error analyses by crossvalidation (jackknifing).
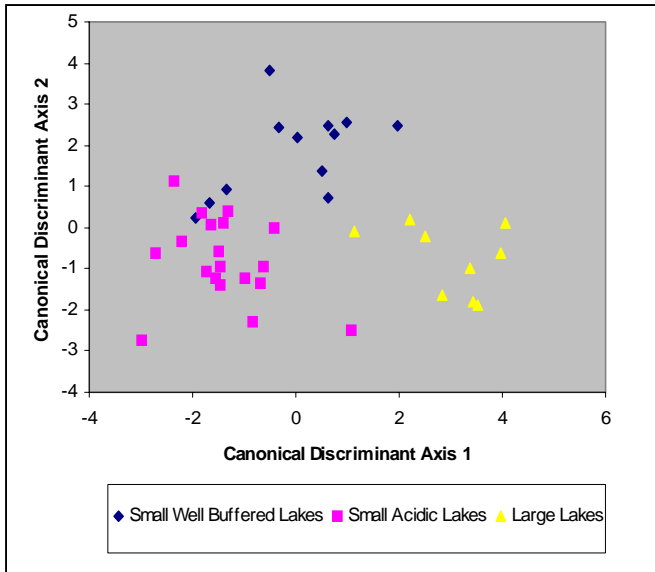
This analysis produced highly significant class separation (Wilks' 7 = 0.09, F= 12.21, p=0.001). Accounting for prior class size, the classification error rate was determined to be 85% correct allocation to a class overall. The separation between the three classes can be visualized by a scatterplot of all lakes within the first two canonical discriminant axes (Figure 4). The classification functions generated by the analysis are presented in Table 6, and the error analysis is provided in Table 7.

Figure 4. Plot of 40 lakes grouped by 6 environmental variables into three classes using multivariate linear discriminant analysis.

Table 6. 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 →	Small Well Buffered	Large	Small Acidic
Coefficient ↓			
CONSTANT	-214.12	-240.40	-217.32
Log-Lake Area (ac)	889.09	879.35	884.97
Log-Basin area (ac)	-871.96	-856.23	-866.60
Log-Basin/Lake Area Ratio	938.44	922.31	935.72
Log-Maximum depth (m)	-0.01	2.08	0.18
Log-Alkalinity (mg/l)	-22.51	-23.38	-27.55
Log-Conductivity (us/cm^3)	36.03	34.82	37.22

Table 7. Linear discriminant function classification error rates determined by crossvalidation for 40 lakes

LAKETYPE		Small Well Buffered	Large	Small Acidic	Total
Small Well Buffered	N classified to:	9	0	3	12
Large	N classified to:	2	7	0	9
Small Low Alkalinity	N classified to:	0	1	18	19
Classification Error Rate		25%	22.2%	5.3%	15%

10

The linear discriminant functions developed by this procedure are extremely useful in that they can be used to classify any lake into one of the three classes with a known probability of correct classification. The reader is referred to Appendix C for a complete description of how lakes can be classified using these discriminant functions. A description of lakes within each class is provided in Table 8. It should be noted that the term 'acidic' applied to the small acidic lakes class is not intended to suggest that these lakes are impaired by acidification. Rather, it is used as a descriptive term.

Table 8. Geometric mean observations of 6 environmental variables which were used to classify lakes for application of phytoplankton criteria.

Lake Class	Environmental Variables					
	Lake Area (ac)	Basin Area (ac)	Basin to Lake Area ratio	Max. Depth (m)	Alk. mg/l as $CaCO_3$	Cond. us/cm^3
Small Well Buffered	53	585	11	11	25	71
Small Acidic	55	1204	22	9	6	32
Large	599	5523	9	22	30	74
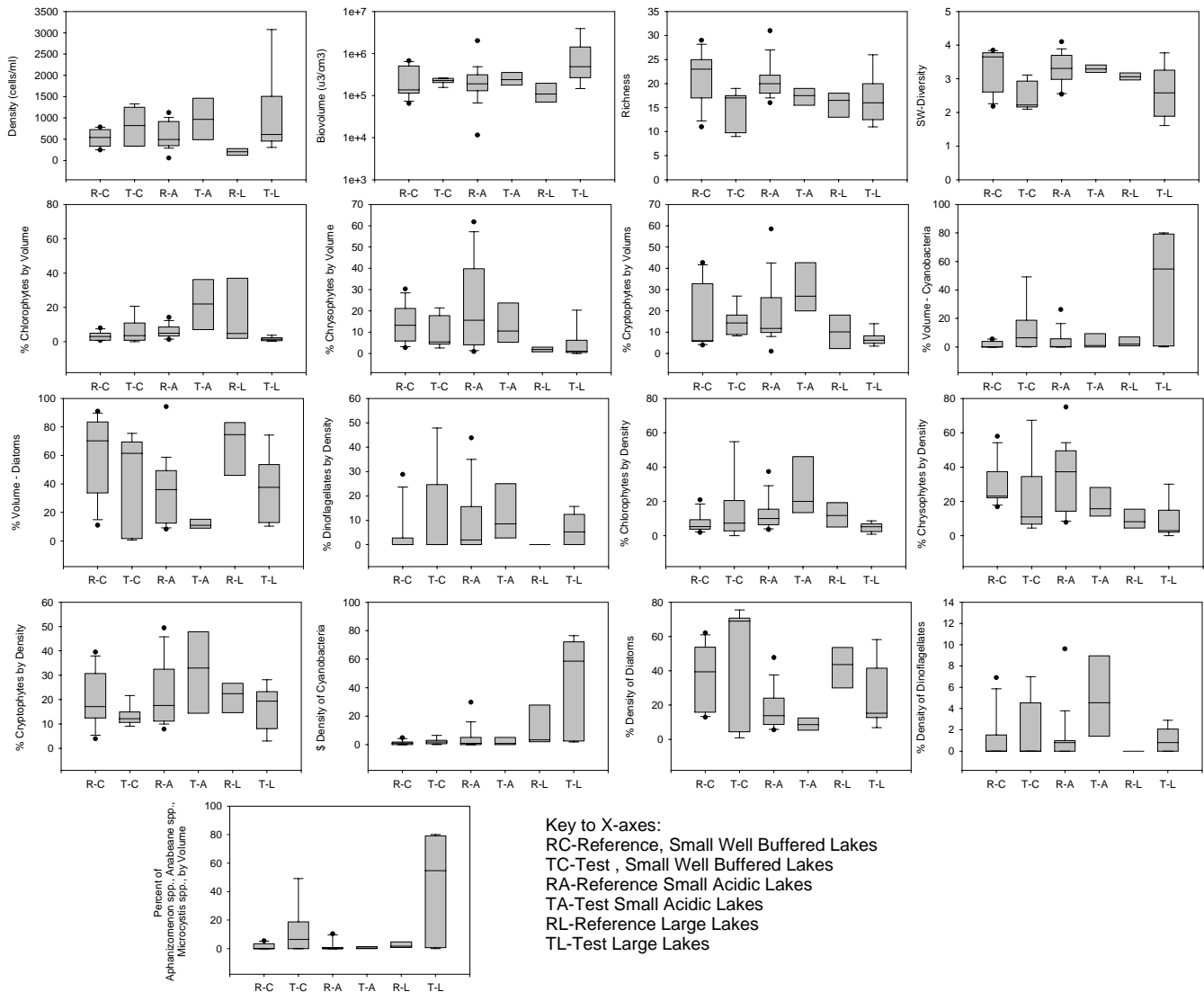
Figure 5. Box-and-whisker plots for 16 phytoplankton metrics, measured from 40 VT and NH lakes.

Verification That the Phytoplankton Community Varies Across the Three Classes

Multivariate ANOVA was then used to verify that the phytoplankton metrics themselves vary between the three classes for all sampled lakes. This analysis was first performed on all 38 of the 40 lakes in the set, with classes being defined as above, and with the phytoplankton community metrics identified in Table 2 as response variables. Variables were log-transformed to meet assumptions of normality. In addition, two lakes were omitted from the analysis. These were Lake Carmi and Fairfield Pond, both of which had extraordinarily strong cyanobacteria components within their phytoplankton communities, and both of which are classified as large lakes. Retaining these two outliers would have resulted in artificially enhanced *p*-values, and thus rejecting these two outliers yielded a more conservative statistical evaluation. The three lake classes were found to differ significantly from each other in their phytoplankton community composition (Wilks' 7=0.168, F=2.26, p=0.0076). Follow-up univariate ANOVA identified percent diatom by density (p=0.05), % chrysophyte by density (p=0.0035), and % cyanobacteria by density (p=0.023) as varying significantly between one or more

12

classes. Percent cryptophytes by volume also varied across the classes, but at a reduced significance level (p=0.08).

Metric Review and Selection

In order to select metrics for inclusion within a multimetric index, the distribution of reference and test lake metrics were plotted and examined, for all three identified classes (Figure 5). To assess statistical independence, a Spearman correlation matrix was generated using SPSS's SigmaStat package (1999). (Table 9). The goal of this evaluation was to select metrics which simultaneously vary between classes, appear to discriminate reference from test conditions, and are statistically independent.

Table 9. Spearman rank-order correlation matrix for 16 phytoplankton community metrics, calculated using data from 38 VT and NH lakes.

	Key:	Total BioVol	Richness	SW-Diversity	% CHLORO VOL	% CHRYSO VOL	% CRYPTO VOL	% CYANO VOL	% DIAT VOL	% DINO VOL	% CHLORO DEN	% CHRYSO DEN	% CRYPTO DEN	% CYANO DEN	% DIATOM DEN	% DINO DEN	% AphaAna. Mic.
Total density	**R→**	**0.763**	0.009	-0.182	-0.232	-0.145	-0.082	-0.022	0.105	0.104	-0.069	-0.109	-0.177	-0.210	0.133	0.059	0.077
	p-value→	**0.000**	0.958	0.272	0.159	0.382	0.621	0.896	0.527	0.532	0.680	0.511	0.285	0.204	0.423	0.725	0.643
Total BioVol			0.306	0.100	**-0.385**	**-0.348**	**-0.448**	0.041	0.291	0.149	0.012	-0.191	-0.131	-0.045	0.233	0.116	0.111
			0.061	0.548	**0.017**	**0.032**	**0.005**	0.804	0.076	0.371	0.943	0.250	0.432	0.785	0.158	0.485	0.504
Richness				**0.714**	-0.070	0.145	-0.122	-0.185	0.255	0.136	0.204	0.172	0.152	-0.169	0.161	0.131	-0.167
				0.000	0.674	0.381	0.463	0.263	0.122	0.412	0.217	0.300	0.359	0.309	0.333	0.431	0.315
SW-Diversity					0.196	-0.025	-0.044	-0.274	0.215	0.175	**0.555**	0.003	0.215	-0.158	0.029	0.267	**-0.383**
					0.236	0.878	0.792	0.096	0.193	0.292	**0.000**	0.983	0.194	0.342	0.864	0.104	**0.018**
% CHLORO VOL						0.129	**0.359**	-0.185	**-0.439**	0.086	**0.722**	0.039	0.058	-0.131	**-0.503**	0.085	**-0.320**
						0.436	**0.027**	0.263	**0.006**	0.608	**0.000**	0.813	0.730	0.432	**0.001**	0.611	**0.050**
% CHRYSO VOL							0.079	-0.045	-0.301	0.049	-0.152	**0.823**	-0.036	-0.087	**-0.392**	0.023	-0.140
							0.636	0.789	0.066	0.769	0.360	**0.000**	0.829	0.600	**0.015**	0.889	0.399
% CRYPTO VOL								-0.001	**-0.379**	0.025	0.109	-0.101	**0.611**	-0.066	-0.260	0.049	-0.149
								0.994	**0.019**	0.879	0.511	0.544	**0.000**	0.691	0.114	0.771	0.369
%CYANO VOL									-0.220	-0.014	-0.206	-0.170	-0.200	**0.932**	-0.053	-0.023	**0.814**
									0.183	0.931	0.213	0.306	0.227	**0.000**	0.753	0.892	**0.000**
%DIAT VOL										**-0.541**	-0.125	-0.218	0.003	-0.147	**0.842**	**-0.507**	-0.012
										0.000	0.453	0.187	0.986	0.376	**0.000**	**0.001**	0.940
%DINO VOL											0.192	0.082	-0.002	-0.066	**-0.441**	**0.953**	-0.113
											0.247	0.623	0.992	0.692	**0.006**	**0.000**	0.498
%CHLORO DEN												-0.115	0.106	-0.147	-0.292	0.245	-0.300
												0.489	0.525	0.375	0.075	0.138	0.067

Key:	Total BioVol	Richness	SW-Diversity	% CHLORO VOL	% CHRYSO VOL	% CRYPTO VOL	% CYANO VOL	% DIAT VOL	% DINO VOL	% CHLORO DEN	% CHRYSO DEN	% CRYPTO DEN	% CYANO DEN	% DIATOM DEN	% DINO DEN	% AphaAna. Mic.
%CHRYSODEN												-0.168	-0.221	-0.480	0.031	-0.185
												0.310	0.181	0.002	0.854	0.265
%CRYPTO DEN													-0.144	-0.024	0.049	-0.296
													0.385	0.886	0.768	0.071
%CYANO DEN														-0.029	-0.044	**0.699**
														0.864	0.792	**0.000**
% DIAT DEN															-0.408	0.175
															0.011	0.290
% DINO DEN																-0.152
																0.360

Among the 16 metrics evaluated, metrics were retained for further evaluation if they discriminated reference from test conditions within one or more classes, and displayed statistical independence. Metrics were considered to discriminate if either median observation (test or reference) fell outside the interquartile range (25th – 75th percentile of the distribution) of the other. In no cases were two metrics representing the same taxonomic group retained (e.g. % *Aphanizomenon spp., Anabaena spp., Microcystis spp.* And % cyanobacteria by volume). The following metrics were selected for further evaluation.

Total density: Discriminates reference from test lakes within all three classes, varies between classes; correlates only to total biovolume.

% Cryptophytes by volume: Discriminates reference from test lakes within the small well buffered and small acidic lake classes; correlated to % diatoms by volume and to total biovolume.

% Chrysophytes by density: Discriminates reference from test lakes within small well-buffered and large lakes; correlated to % diatoms by density.

% Diatoms by density: Discriminates reference from test lakes within small acidic and large lakes. This metric may also have discriminatory ability within small, well buffered lakes, however test lakes respond in the opposite direction than they do in the other classes; correlated to % chrysophytes and to % dinoflagellates (by both density and volume).

% *Aphanizomenon spp., Anabaena spp., Microcystis spp.* : Strongly discriminates reference from test lakes within small well-buffered and large lakes; correlated to % cyanobacteria by volume and density, to the Shannon-Weiner index of diversity, and to % chlorophytes by volume. These correlations relate to this noxious algal guild's competitive advantage conferred by it's ability to fix atmospheric nitrogen, the effect of which is to reduce the proportion of the more common green algae, thereby reducing diversity.

Evaluation of Metric Sensitivity and Discrimination of Conditions Not Meeting Reference

The interquartile coefficient (USEPA 1998) was calculated as a measure of each metrics sensitivity to detecting a deviation from reference conditions. The interquartile coefficient is defined as the interquartile range of the candidate reference metric, divided by the scope for detection of the same metric, and is expressed as follows.

$$IC = IQ/ScD \qquad \qquad \text{Eq. 1}$$

     where: IC= Interquartile coefficient
       IQ=   75th percentile - 25th percentile of the metrics distribution within reference lakes;
ScD= The absolute value of the difference between the upper (or lower) quartile of the reference range, and the maximum (or minimum) value for the test distribution.

USEPA (1998) indicates that metrics with interquartile coefficients of > 1 are overly variable to detect deviation from reference conditions. Interquartile coefficients for the five selected metrics are presented in Table 10.

Finally, in order to verify that the selected phytoplankton metrics indeed discriminate reference from test conditions within individual lake classes, a two-way multivariate ANOVA was performed. This analysis specifically evaluated the hypothesis that the average phytoplankton community within test lakes was significantly different from that found on reference lakes, when accounting for variation in the metrics which could be attributed to lake class. The analysis was performed on all 40 lakes, and showed no significant lake class x lake disturbance interaction (Wilks' 7=0.826, F=0.60, p=0.806), meaning that the ability of the metrics to discriminate reference from test conditions did not depend on lake class. The phytoplankton community metrics varied significantly with lake class when the effect of disturbance was accounted for (Wilks' 7=0.314, F=4.70, p<0.001), which was expected given the results of the multivariate ANOVA previously used to independently

verify the physico-chemical classification system (see above). Finally, when accounting for variation attributable to lake class, the average phytoplankton community varied significantly with disturbance (Wilks' 7=0.657, F=3.13, p=0.022), meaning that the five selected metrics were capable of identifying conditions which depart from reference within each lake types.

Table 10. Interquartile coefficients for five phytoplankton community metrics across three lake classes.

Lake class	Total Density	% Cryptophytes by volume	% Chrysophytes by density	% Diatoms by density	% Aphanizomenon spp., Anabaena spp., Microcystis spp. by volume
Small Well Buffered Lakes	0.42	>1	0.74	>1	0.05
Small Acidic Lakes	0.74	0.42	>1	>1	0.56
Large Lakes	0.05	>1	>1	0.47	0.04

Given the information provided in Table 10, it is clear that two metrics, total density and % *Aphanizomenon spp., Anabaena spp., Microcystis spp.* by volume, discriminate reference from test conditions within each lake class, and therefore both metrics were retained for application to each lake class. The other three metrics vary with disturbance only within one lake class each, as follows. First, under non-reference conditions, % cryptophytes by volume becomes elevated in small, acidic lakes. On small, well buffered lakes, the proportion of chrysopytes by density is relative to reference. Finally,the portion of the community occupied by diatoms is reduced in large lakes under conditions which depart from reference. The set of metrics used to build a plankton index for each lake class is therefore as follows:

Small, Well Buffered Lakes:
Total density, % *Aphanizomenon spp., Anabaena spp., Microcystis spp.* by volume, % chrysophytes by density

Small, Acidic Lakes:
Total density, % *Aphanizomenon spp., Anabaena spp., Microcystis spp.* by volume, % cryptophytes by volume

Large Lakes:
Total density, % *Aphanizomenon spp., Anabaena spp., Microcystis spp.* by volume, % diatoms by density

Bi-Section Scoring and Phytoplankton Index Construction

To develop a phytoplankton index, the 'bisection' scoring algorithm (USEPA, 1998) was used to identify numeric scores corresponding to the metrics position in relation to the reference range. This algorithm allocates a score of five for all metric values which fall 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 is then bisected, with a score of three and one being allocated to the ranges of values corresponding to 'better' and 'worse' values, respectively. Table 11 identifies the scoring ranges for the five selected metrics.

To calculate a given lakes' overall phytoplankton index score, the scores corresponding to the actual metric value measured for the test lake are summed. For this index, actual scores for the 40 lakes ranged from a maximum of 15 for lakes fully meeting reference conditions for all three measures, to three for lakes not meeting reference expectations for any metric. Figure 6 displays the range of plankton index scores corresponding to reference and test lakes across the three classes.

Table 11. Scoring algorithms for five phytoplankton community metrics measured from 40 VT and NH lakes. Metrics which do not discriminate between reference and test lakes are not included in the overall index calculation.

Small, Well Buffered Lakes:			
score attributed→ Metric under evaluation↓	**1**	**3**	**5**
Total Density	**>1001**	**675-1001**	**<675**
%Cryptophytes by Volume	Non-discriminating metric		
% Chrysophytes by Density	**<11%**	**11-22%**	**>22%**
% Diatoms by Density	Non-discriminating metric		
% of *Aphanizomenon spp., Anabaena spp., and Microcystis spp.* by Volume	**>28%**	**2.3% - 28%**	**<2.3%**
Small Acidic Lakes:			
score attributed→	**1**	**3**	**5**
Total Density	**>1275**	**903-1275**	**<903**
% Cryptophytes by Volume	**>39%**	**23% - 39%**	**<23%**
% Chrysophytes by Density	Non-discriminating metric		
% Diatoms by Density	Non-discriminating metric		
% of *Aphanizomenon spp., Anabaena spp., and Microcystis spp.* by Volume	**>5%**	**1-5%**	**<1%**
Large Lakes:			
score attributed→	**1**	**3**	**5**
Total Density	**>1404**	**269 - 1404**	**<269**
% Cryptophytes by Volume	Non-discriminating metric		
% Chrysophytes by Density	Non-discriminating metric		
% Diatoms by Density	**<21.4%**	**21.4% - 34.9%**	**>34.9%**
% of *Aphanizomenon spp., Anabaena spp., and Microcystis spp.* by Volume	**>41.8%**	**3.6% - 41.8%**	**<3.6%**

The phytoplankton index scores and subsequent assessments for all 40 lakes used to generate this index are provided in Table 12, as are replicate scores from four additional lakes. Two lakes subject to special studies by VTDEC were also independently evaluated. These were Ticklenaked Pond (Ryegate, VT), and Lake Parker (Glover, VT). In all cases, assessments conducted from duplicate plankton samples yielded excellent replication (mean relative difference in scores = 5%), and identical assessments.

Table 12. Calculation of the phytoplankton index and assessment of conditions on 42 VT and NH lakes.

Small Acidic Lakes	Ref/ Test	Total Density	Crypto. Vol.	Apha Ana Mic Vol	Density Score	Crypto Score	Apha Ana Mic Score	Total Index Score	Assessment
DUDLEY (NH)	Ref.	491.2	29.3	0.0	5	3	5	13	Meets reference
GILMAN (NH)	Ref.	783.1	16.9	3.6	5	5	3	13	Meets reference
HOLLAND	Ref.	292.4	31.9	0.0	5	3	5	13	Meets reference
INTERVALE (NH)	Ref.	354.4	8.0	0.8	5	5	5	15	Meets reference
LITTLE ELMORE	Ref.	1122.3	10.2	10.3	3	5	1	9	May deviate
MARSHFIELD	Ref.	713.2	10.0	0.0	5	5	5	15	Meets reference
MCCONNELL	Ref.	930.5	8.9	0.0	3	5	5	13	Meets reference
NATHAN (NH)	Ref.	344.7	15.9	0.5	5	5	5	15	Meets reference
RUSSELL (NH)	Ref.	55.6	14.6	0.0	5	5	5	15	Meets reference
SESSIONS (NH)	Ref.	453.9	58.5	0.0	5	1	5	11	Meets reference
SMITH (NH)	Ref.	469.6	10.1	0.0	5	5	5	15	Meets reference
WALLINGFORD	Ref.	875.6	42.5	0.0	5	1	5	11	Meets reference
WHEELER (BRUNWK)	Ref.	1008.7	9.8	0.0	3	5	5	13	Meets reference
WILLARD (NH)	Ref.	301.7	11.8	9.7	5	5	1	11	Meets reference
WOLCOTT	Ref.	1002.2	1.0	0.0	3	5	5	13	Meets reference
BEAVER (NH)	Test	653.8	23.2	0.8	5	3	5	13	Meets reference
BRANCH	Test	324.0	30.7	0.0	5	3	5	13	Meets reference
FRENCH (NH)	Test	1646.5	54.7	1.9	1	1	3	5	Fails to meet
STRATTON	Test	1278.8	16.8	0.0	1	5	5	11	Meets reference
FRENCH (NH) replicate	Test	1403.4	34.1	10.1	1	3	1	5	Fails to meet
MCCONNELL replicate	Ref	951.2	19.8	0	3	5	5	13	Meets reference
Small Well Buffered Lakes		Total Density	Chryso. Den.	Apha Ana Mic Vol	Density Score	Chryso Score	Apha Ana Mic Score	Total Index Score	Assessment
BALD HILL	Ref.	539.1	23.1	0.0	5	5	5	15	Meets reference
BUTTERNUT (NH)	Ref.	266.4	16.8	0.0	5	3	5	13	Meets reference
HATCH (NH)	Ref.	531.9	22.6	0.0	5	5	5	15	Meets reference
HIGH (SUDBRY)	Ref.	567.5	57.9	4.6	5	5	3	13	Meets reference
HINKUM	Ref.	245.2	39.5	0.0	5	5	5	15	Meets reference
LONG (GRNSBO)	Ref.	782.7	21.9	5.4	3	3	3	9	May Deviate
LONG (SHEFLD)	Ref.	780.7	31.1	0.0	3	5	5	13	Meets reference
COLE	Test	340.1	23.5	0.0	5	5	5	15	Meets reference
CURTIS	Test	337.4	67.3	49.1	5	5	1	11	Meets reference
EWELL	Test	1327.9	7.5	6.5	1	1	3	5	Fails to
GREAT HOSMER	Test	820.0	10.9	8.7	3	1	3	7	Fails to Meet
SPRING (SHRWBY)	Test	1224.9	4.5	0.0	1	1	5	7	Fails to Meet
TICKLENAKED (independent)	Test	1449.6	10.26	75.75	1	1	1	3	Fails to Meet
CURTIS replicate	Test	383.7	59.6	20.2	5	5	3	13	Meets reference
Large Lakes		Total Density	Diatom Den.	Apha Ana Mic Vol	Density Score	Diatom Score	Apha Ana Mic Score	Total Index Score	Assessment
CASPIAN	Ref.	87	41.9	1.0	5	5	5	15	Meets reference
CRYSTAL (BARTON)	Ref.	258	61.7	2.5	5	5	5	15	Meets reference
MAIDSTONE	Ref.	300	45.4	0.7	3	5	5	13	Meets reference
SHADOW (GLOVER)	Ref.	158	18.1	6.8	5	1	1	7	Fails to meet
CARMI	Test	3077	14.7	54.7	1	1	1	3	Fails to meet
DUNMORE	Test	504	58.2	0.9	3	5	5	13	Meets reference
EDEN	Test	309	35.9	0.0	3	5	5	13	Meets reference

FAIRFIELD	Test	613	6.9	78.8	3	1	1	5	Fails to meet
ST. CATHERINE	Test	984	15.2	80.0	3	1	1	5	Fails to meet
CASPIAN replicate	Ref.	144.4	68.8	1.2	5	5	5	15	Meets refrence
PARKER (independent)	Test	416	47.2	1.7	3	5	5	13	Meets reference
PARKER (independent replicate)	Test	594	52.9	5.7	3	5	3	11	Meets reference
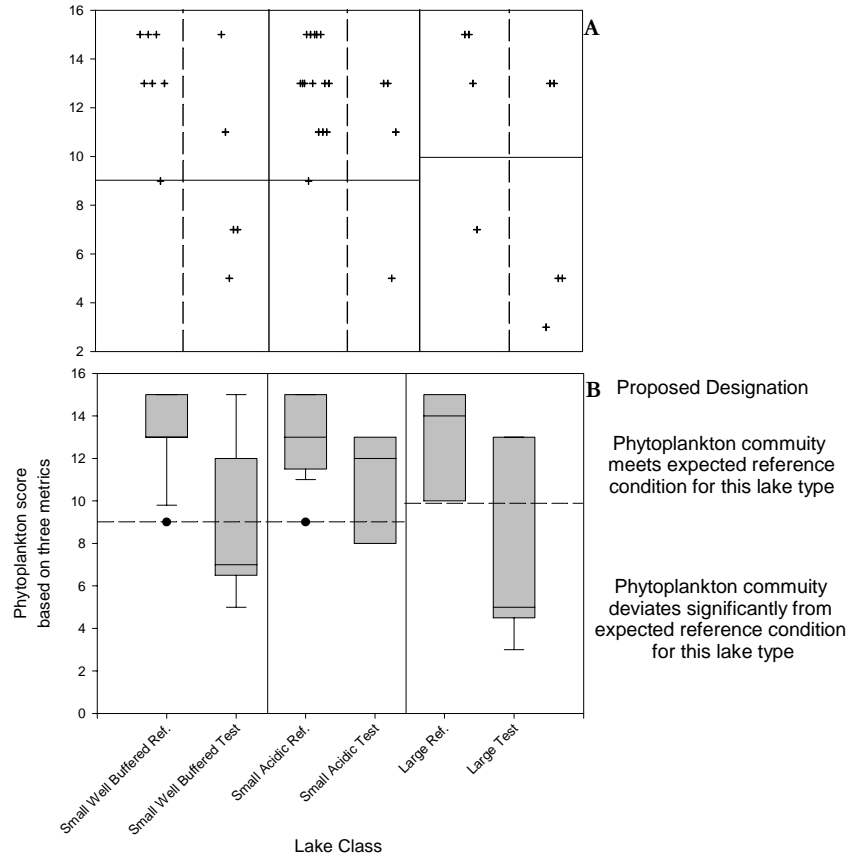
Figure 6. Phytoplankton index scores for 40 VT and NH lakes. Individual lake scores (A), and Tukey box-plots (B) are shown, as are proposed cut-points for determining deviation from reference conditions.

Development of Macroinvertebrate-Based Criteria

Overview

The analytical approach for macroinvertebrate criteria development was similar to that employed for the phytoplankton assemblage. The analysis was, however, more complex owing to the evaluation of five separate community types within each lake. These community types are named based on the habitats samples, as follows: rocky-littoral epibenthos; macrophytic epibenthos; muddy littoral epi- and infaunal benthos; subblitoral epi- and infaunal benthos; and, profundal benthos. The process of metric evaluation, followed by independent statistical evaluation, was structured to iteratively 'weed out' metrics which did not contribute towards determining whether the biota occupying one habitat of an individual lake might deviate from the reference expectation for that lake class. 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. This general classification of well buffered lakes, low alkalinity lakes, and large lakes was therefore the starting point for the macroinvertebrate analysis.

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 plotted using Tukey box-plots. In these two preliminary graphical examinations, it was apparent that metrics from the profundal zone held little promise in contributing to a meaningful biological assessment, and metrics from this community were thus rejected from further consideration. The retained metrics were then subjected to a Spearman non-parametric correlation analysis, to identify metrics which were highly autocorrelated. Metrics which were identified as redundant (e.g. Spearman R≥0.75), and which contained a lower quantity of original information, were further rejected from the dataset.

The next step in the analysis was to perform MANOVA on the trimmed metric sets, to evaluate the statistical significance of observed differences in the joint distributions of metrics across classes and between reference and test lakes. These tests were performed within community type, and the interaction between lake class and reference status was assessed under the hypothesis that the direction of biological departure indicated by test lake metric set depended on the lake class. These statistical evaluations necessitated that some of the metrics be rescaled to satisfy assumptions of multivariate normality.

Scoring algorithms were developed for this final metric set largely using the bi-section scoring method, but with modifications for select metrics. Interquartile coefficients were calculated for each metric, and metrics with low interquartile coefficients were not retained in the final metric set. Assessments were conducted for each lake, using scores for metrics representing each community type. For the well buffered lakes, there were eight final metrics retained, with at least one metric from each community type. For the low alkalinity lakes, six metrics were retained, with two metrics each from the rocky-littoral, macrophyte, and sublittoral habitats. For large lakes, eleven metrics were retained, with two or more metrics representing each community type. Recommendations for additional validations of the final trial criteria system are provided.

Classification, Candidate Metrics, and Initial Metric Evaluation

As stated above, development of a robust criteria system initially depends on development of a classification scheme which explains variation in biological communities as a function of natural waterbody attributes. Since three biological assemblage types are under evaluation in this project (phytoplankton, macroinvertebrates,

macrophytes), it is possible to derive three separate classification systems; one for each assemblage. The ultimate goal of this project is to develop a set of biological criteria by which a lake system can be evaluated for aquatic life use support. As a practical application, maintaining a single physico-chemical classification scheme is preferable to deriving classifications for each biological assemblage. While assemblage-specific classification may be useful in explaining the maximum amount of biological variation within reference waters for each assemblage, there are several advantages to retaining a single classification which is applied to all assemblages. First, a single-class system is more easily understood by multiple audiences. Second, implementation of the criteria is simpler from a programmatic standpoint. Finally, a single classification which captures biological variation in multiple assemblages across reference waters is far simpler analytically. Essentially, a lake can be classified a-priori, then evaluated for biological integrity within any or all biological assemblages.

For these reasons, the physico-chemical lake classification inferred by CCA using phytoplankton data was retained for development of macroinvertebrate criteria. The validity of this approach was assessed by performing several CCA analyses, with the two criteria for accepting the phytoplankton-inferred classification being a similar clustering of sites within classes as was noted during the phytoplankton analyses, and a reasonably high percent variance explained within the ordination. Figure 7 shows a CCA ordination diagram where reference lakes, biometrics, and physico-chemical variables are arranged by their relative positions in ordination space, with 40.1 percent of the total dataset variance explained. A similar analysis performed using combined reference and test lakes yielded similar site, and explained 23.1 percent of the
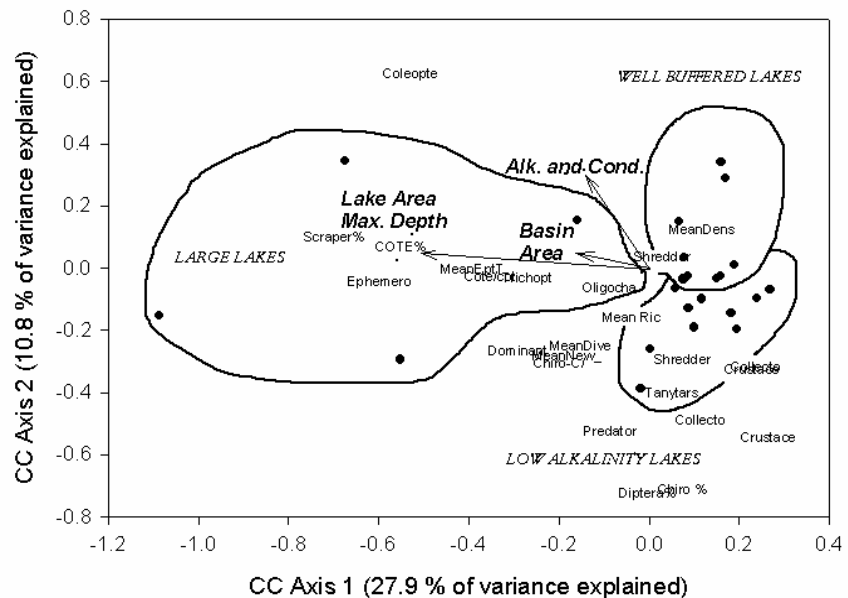

Figure 7. Canonical correspondence triplot of 26 reference lakes (•) as weighted averages of 23 macroinvertebrate biometrics collected from the rocky-littoral community, in relation to 5 environmental variables (vectors). Sites are plotted as linear combinations of environmental variables. This ordination explains 40.1% of the total dataset variance on the first three axes. Boundaries are inscribed around sites which were identified by discriminant function analysis as belonging to one of three lake classes (identified in uppercase).

total dataset variance on the first three axes. These analyses suggest that the phytoplankton-inferred, physicochemical classification is valid for macroinvertebrates.

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 (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, VTDEC2001), and is presented in Table 13.

Table 13. 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
MeanEpt/EptChiro	Structural	Proportion of Ephemoptera, Plecoptera, Tricoptera to EPT+Chironomidae
MeanEptRichness	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&ch&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
Crustacea/Mollusca % and R	Compositional	Expressed as percent of community and as richness
COTE% and R	Compositional	Expressed as percent of community and as richness
Tanytarsus sp. %	Compositional	self explanatory
Chiro % and R	Compositional	Expressed as percent of community and as richness
Chaoboridae%	Compositional	self explanatory
CollectorGatherer%	Functional	self explanatory
CollectorFilterer%	Functional	self explanatory
Predator%	Functional	self explanatory
ShredderDetritivore%	Functional	self explanatory
ShredderHerbivore%	Functional	self explanatory
Scraper%	Functional	self explanatory

As noted above, spread-location plots of all 34 candidate metrics were generated by habitat type, showing the relative distributions of reference and test lake metric values within lake classes. One set of spread-location plot for eight metrics is shown in Figure 8.
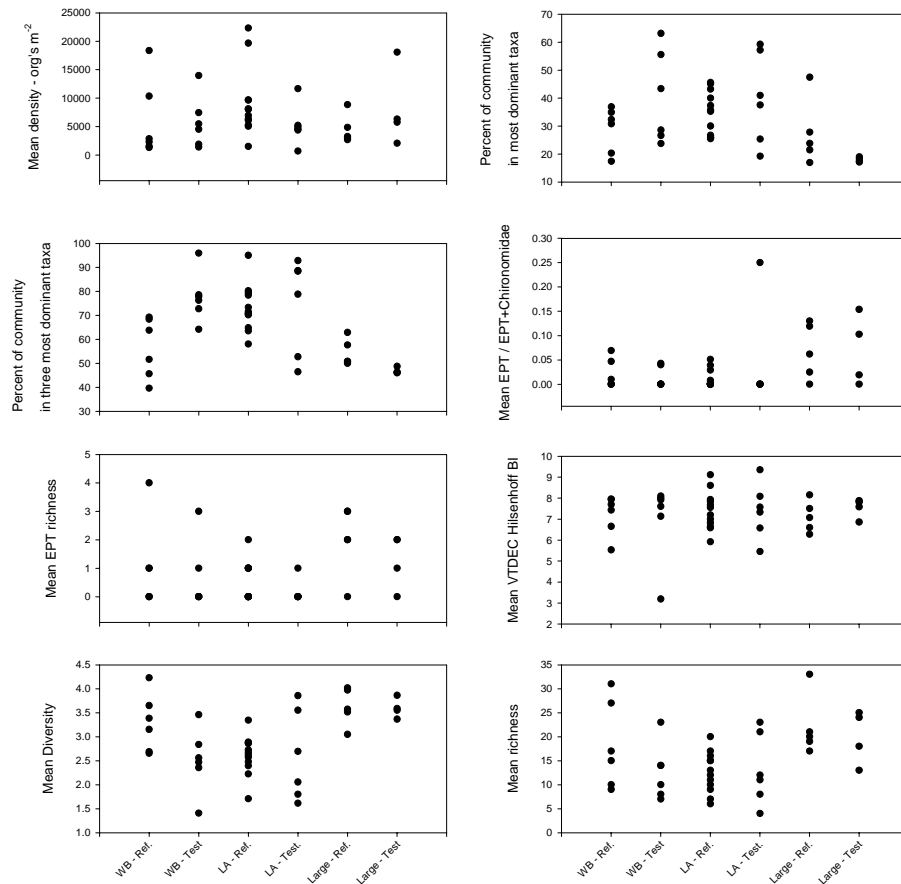

Figure 8. Spread-location plot of eight macroinvertebrate metrics separated by lake class and reference status, for samples collected from the sublittoral zone. WB: well buffered reference lakes. LA: low alkalinity lakes. Large: large lakes. Ref.: reference status lakes. Test: lakes of impaired or unknown status.

Plots such as that shown in Figure 8 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. For example, these plots show an apparent difference in the distribution of *percent of community in most dominant* and in the *percent of community in the three most dominant taxa*, between reference and test lakes, for well-buffered and large lakes. Similarly, there are likely detectable differences in the distributions of several metrics across reference lakes of all three classes, as is well shown by the *mean diversity* and *mean richness* metrics. These comparisons are not statistically-based, but do serve as a guide for retaining or rejecting candidate metrics. Similar
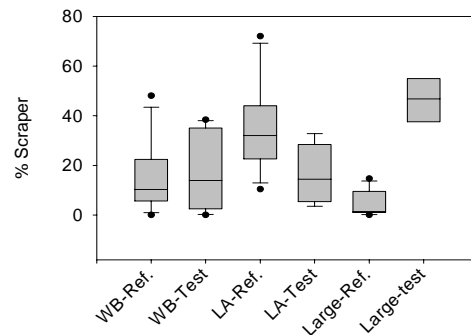

Figure 9. Tukey box-plot for the metric percent scraper, for rocky-littoral habitats. X-axis key is shown in Figure 8.

plots were prepared for all metrics within all community types.

Following this, Tukey box-plots (SigmaPlot 5.0, SPSS, 2001) were prepared to more effectively visualize metric distributions, quartiles, and extreme values. One such example is provided in Figure 9. In this example, it appears clear that the percent of community as functional scrapers differs significantly between reference and test lakes, for low alkalinity and large lakes. From the collective set of box-plots, a further trimmed set of metrics was selected for a statistical 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 value of 0.75. Table 13 provides a trimmed roster of candidate metrics used to perform multivariate analysis, and to generate scoring algorithms for index development. Spearman correlations are provided in Table 14.

Table 13. 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.

		Community type			
		Rocky littoral epibenthos	Macrophytic epibenthos	Muddy littoral epi- and infaunal benthos	Sublittoral epi- and infaunal benthos
Lake class	Well buffered	*MeanEpt/EptChiro Cote/cote&ch&oli Coleoptera%	Chiro R ShredderDetritivore% MeanRichness Tanytarsus sp. %	MeanNew_BI MeanRichness	*MeanDiversity Dominant3Taxa % CollectorFilterer% *Chiro R %Dips as intol. chiros
	Low alkalinity	CrustacaeaMollusca R %Dips as intol. chiros Coleoptera% *OtherOrders%	CrutacaeaMollusca R Tanytarsus sp. % Mean Richness COTE%		%Dips as intol. chiros Tanytarsus sp. %
	Large	DominantTaxa% *MeanEPT/EPTChiro MeanDiversity CrustacaeaMollusca% Scraper% Ephemoptera% *OtherOrders% CollectorGatherer%	ShredderDetritivore% MeanRichness *CrustacaeaMollusca R Chiro R	MeanNew_BI Chiro R *MeanRichness	Dominant3Taxa % CollectorFilterer% Chiro R %Dips as intol. chiros

Table 14. Redundant metrics rejected from further analysis due to high Spearman correlations.

Community	Lake Class	Metric rejected	Autocorrelated with	Spearman R	p
Rocky littoral epibenthos	Well buffered	MeanEpt/EptChiro	Cote/cote&ch&oli	0.94	<0.001
	Low alkalinity	OtherOrders%	Crustacea/Mollusca%	0.929	<0.001
	Large	MeanEpt/EptChiro	Ephemeroptera%	0.901	<0.001
			CollectorGatherer%	-0.819	<0.001
		% Other orders	Crustacea/Mollusca%	0.916	<0.001
Sublittoral epi- and infaunal benthos	Well buffered	Mean diversity	Dominant3taxa%	-0.923	<0.001
		ChiroR	Dominant3taxa%	-0.889	<0.001
			MeanDiversiry	0.765	0.003

Macrophytic epibenthos	Large	Crustacea/Mollusca%	MeanRichness	0.833	0.002

Multivariate Analysis of Variance, Lake Class and Reference Status Within Community Type

In order to ascertain whether the shortened list of metrics could be used to statistically detect differences in community structure and function across classes, and between reference and test lakes, MANOVA was performed. MANOVA requires multivariate normality of the underlying data, and thus metrics were rescaled to approximate the normal statistical distribution where necessary and possible. Normality was assessed using the Kolmogorov-Smirnov test (SPSS, 1999), with follow-up diagnostic evaluation using normal probability plots. A variety of transforms were applied to the data to rescale them to normality. In most cases, the common $\log_e(1+x)$ transform was useful. In two instances, a square-root transform was applied to achieve normality. In a few instances, distributions were characterized by several like-values at the tail of the distribution, and transforms were unattainable. This is because regardless of the transform applied, a density of points at the end-member of a distribution could not be 'spread' across the lowest portions of that distribution without artificially altering the data. Figure 10 shows one such example. Where these distributions were apparent, the data were retained untransformed, and multivariate modeling was performed both with and without the difficult metrics, to assess the importance of those metrics in altering statistical findings of the models. Transforms applied to the data are shown in Table 15.
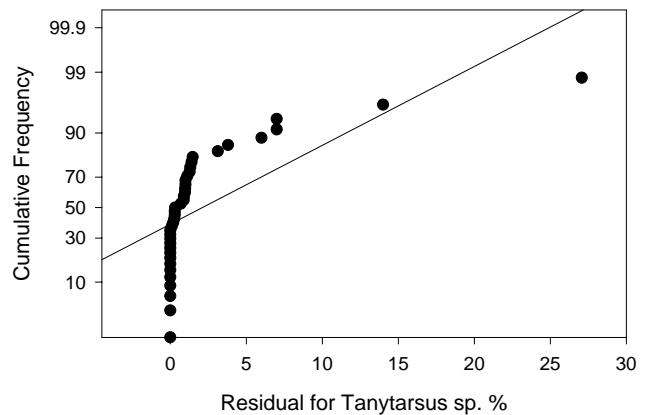

Figure 10. Normal probability plot of the distribution of % Tanytarsus sp. value for the muddy littoral habitat for all lake classes. A density of observations at the low end of the distribution illustrates the difficulty in deriving an acceptable transformation to achieve statistical normality for this metric.

MANOVA was used to assess the degree to which the multivariate distributions of the metrics varied due to lake class and to reference status. These analyses were performed within community types, 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. The purpose of these analyses was to ascertain whether the variation across classes or reference status observed within the spread-location and Tukey box-plots was statistically valid. Multivariate ANOVA was preferred to sequential univariate ANOVA to account for the residual co-variance among many of the metrics, even given the trimming of the metric set following the Spearman correlation analysis. Results of the MANOVA analyses were satisfactory, as the multivariate distributions of metrics varyied significantly between refrence and test lakes in most cases (Table 16).

Table 15. Transformations used to rescale biological metrics to achieve statistical normality. Only metrics where transforms were necessary (or uinattainable) are shown in this table.

Community	Metric	Transform applied	Community	Metric	Transform applied
Rocky littoral epibenthos	DominantTaxa% MeanEpt/EptChiro Coleoptera% Ephemoptera% OtherOrders% Scraper% CrustacaeaMollusca% %Dips as intol. chiros Cote/cote&ch&oli	$\log_e (1+x)$ $\log_e (1+x)$ $\log_e (1+x)$ $\log_e (1+x)$ $\log_e (1+x)$ $\log_e (1+x)$ $\log_e (1+x)$ Unattainable $\log_e (1+x)$	Muddy littoral epi- and infaunal benthos	Mean Richness	$\log_e (1+x)$
Macrophytic epibenthos	Tricoptera% ShredderDetritivore% CrustacaeaMolluscaR Tanytarsus sp. % ChiroR	$\log_e (1+x)$ unattainable $\log_e (1+x)$ unattainable square root	Sublittoral epi- and infaunal benthos	Tanytarsus sp. %	unattainable

These MANOVA highlighted several noteworthy findings. First, a significant interaction effect exists between lake type and reference status, for the rocky-littoral epibenthos and sublittoral epi and infaunal benthos, and a marginally significant interaction exists for the macrophytic epibenthos (p=0.055). These interactions indicate that the direction of change in the mean set of metric observations for conditions deviating from reference will vary depending on lake class. This is well illustrated in a univariate sense by Figure 9, where % scrapers are enhanced under disturbance for large lakes, and reduced for low alkalinity lakes. Second, the mean observation varied significantly between lake classes for rocky-littoral epibenthos, sublittoral epi- and infaunal benthos, and macrophytic epibenthos. Finally, in the macrophytic epibenthos, the preliminary metric set yields a highly significant spearation between reference and test lakes.   A weak separation between reference and test lakes is also apparent in the sublittoral epi- and infaunal benthos (p=0.093). That the strength of separation between reference and test lakes is not greater is unsurprising, as the entire metric set for each lake class being used to assess discrimination of reference status. As was shown in Table 13, not every metric is useful at separating reference from test conditions within each community.   Therefore, the statistical strength of reference discrimination is diluted in this two-factor MANOVA by non-discriminating metrics. A final series of MANOVA analyses, presented below, accounts for this problem.

Table 16. Results of MANOVA analyses to assess separation between lake class x reference status interaction, lake class, and reference status, for preliminary metric sets on four community types.

Habitat	Wilks' ⋎, F-statistic, and p-value for **interaction** effect			Wilks' ⋎, F-statistic, and p-value for **lake class** effect			Wilks' ⋎, F-statistic, and p-value for **reference status** effect			Difference in analysis when retaining normality-unattainable metrics
	⋎	F	p				⋎	F	p	
Rocky littoral epibenthos	0.350	1.79	0.048	0.332	1.91	0.032	0.705	1.09	0.405	loss of interaction significance at 95% (p = 0.060), no difference in main effects
Macrophytic epibenthos	0.558	1.96	0.055	0.065	16.97	0.0001	0.372	9.78	0.0001	no difference in resulting model

Muddy littoral epi- and infaunal benthos	0.731	1.53	0.187	0.835	1.77	0.176	0.731	2.78	0.187	N/A
Sublittoral epi- and infaunal benthos	0.457	2.77	0.007	0.409	3.26	0.002	0.733	2.11	0.093	no difference in resulting model

Evaluation of Metric Sensitivity and Index Development

Interquartile coefficients were calculated for all remaining metrics following Eq.1. Individual metric distribution quartiles were calculated using SAS Proc Univariate (SAS Institute 2000). With the exception of Tanytarsus sp.%, metrics were rejected if their interquartile coefficient exceeded a value of one. Due to the highly skewed distribution of Tanytarsus sp.% (Figure 11), it was impractical to calculate an interquartile coefficient, since the interquartile coefficient was always greater than one. However, the preponderance of test-lake values at or near zero, and below the 25th percentile of reference lakes, indicates that this metric should be retained. Table 17 provides interquartile coefficients for each community by metric combination, within lake class.
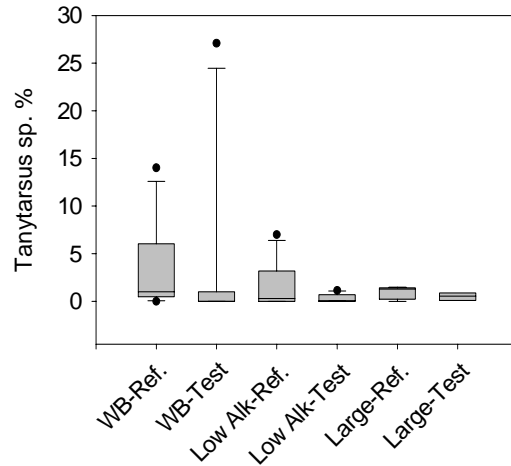

Figure 11. Tanytarsus sp.% from the macrophytic epibenthos community.

Table 17. Interquartile coefficients for candidate metrics. Shaded values exceed one, indicating that the metric was not included in the index developed for that community by lake class combination.

Community	Metric	Lake Type		
		Well buffered	Low alkalinity	Large
Rocky-littoral epibenthos	DominantTaxa%	--	--	0.375
	MeanDiversity	--	--	2.34
	Coleoptera%	3.70	--	--
	Ephemeroptera%	--	--	0.94
	CollecterGatherer%	--	--	0.99
	Crustacaea/Mollusca%	--	0.48	0.34
	%Dips as intol. chiros	--	0.09	--
	Cote/cote&ch&oli	0.40	--	--
Macrophytic epibenthos	ShredderDetritivore%	4.67	--	--
	MeanRichness	3.17	1.33	0.29
	Crustacaea/MolluscaR		3.63	--
	Tanytarsus sp.%	BPJ	BPJ	--
	ChiroR	1.4	0.23	0.43
Muddy littoral epi- and infaunal benthos	Mean_NewBI	0.45	--	1.00
	MeanRichness	1	--	--
	ChiroR	--	--	1.35
Sublittoral epi- and infaunal benthos	Dominant3Taxa%	0.83	--	1.42
	CollecterFilterer%	1.36	--	0.61

Tanytarsus sp.%	--	BPJ	--
ChiroR	--	--	0.25
%Dips as intol. chiros	0.51	0.76	0.79

Index Development, Scoring Algorithms, and Evaluation of the Final Metric Set

Individual metric scoring ranges were calculated using the bisection method, as described above for the phytoplankton metrics. These scores were summed to arrive at a final macroinvertebrate community index value, which is expressed in proportion to the maximum attainable score for each lake type. For the well buffered and low alkalinity lakes, the index is comprised of six metrics. For the large lakes, the index is comprised of 10 metrics. For the well buffered and large lake types, the index is comprised of metrics from each of the four habitat types. For the low alkalinity lakes, the index summarizes metrics from all but the muddy littoral habitat.

The final metric score values were evaluated using MANOVA to determine the likelihood that reference and test lakes differ significantly in their biometric ranges. These analyses were performed by lake class, and in each case, a significant difference was evident. The distributions of index score values for reference and test lakes, by lake class, is shown in Figure 12. P-values from the MANOVA analyses are also presented in that figure. Table 18 provides scoring algorithms, for individual metrics, within the each lake class by habitat type combination.
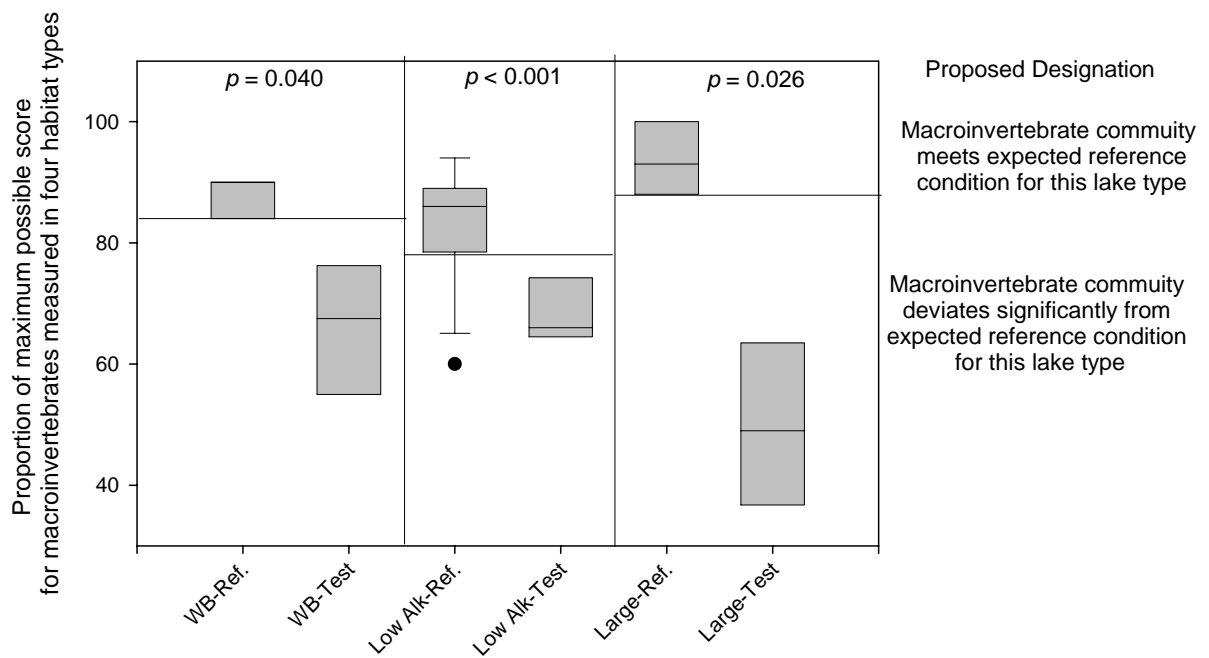

Figure 12. Overall macroinvertebrate index scores based on metrics from four habitats of three lake types, relative to the maximum available score. Proposed cutpoints indicating significant deviation from reference are shown. Significance of reference vs. test differences (p-values) from MANOVA are shown.

Table 18. *Scoring algorithms for macroinvertebrate metrics measured from four habitat types for three lake classes.*

Habitat type ↓	Metric ↓ Lake type → Score attributed →	Small, well buffered lakes			Small acidic lakes			Large lakes		
		1	3	5	1	3	5	1	3	5
Rocky littoral	Cote/Cote+Chiro.+Oligo.	<0.31	0.31 - 0.63	>0.63	-	-	-	-	-	-
	% Dipteran infauna as intolerant chironmids	-	-	-	<0.81	0.81 - ≤ 0.97	> 0.97	-	-	-
	Collector gatherer %	-	-	-	-	-	-	>33.8	≥24.1 - 33.8	<24.1
	Crustacaea Mollusca %	-	-	-	<18.9	18.9 – 37.9	>37.9	>23	>10 - 23	≤ 10
	% in most dominant taxa %	-	-	-	-	-	-	<20.0	20.0 - <30.0	≥ 30.0
	Ephemoptera %	-	-	-	-	-	-	<27.1	≥27.1 - <46.0	≥46.0
Macro-phytes	% Tanytarsus spp.	-	<1	≥ 1	-	<1	≥ 1	-	-	-
	Chironomidae richness	-	-	-	<4.7	4.7 - <9.5	≥9.5	>11.5	>8 - 11.5	≤ 8
	Mean richness	-	-	-	-	-	-	>36.5	>28 - 36.5	≤ 28
Muddy littoral	Mean VTDEC "NewBI"	>8.5	7 - 8.5	<7	-	-	-	>8.25	>6.5 - 8.25	≤6.5
	Mean richness	<20	20 - <27	≥ 27	-	-	-	-	-	-
Sublittoral	% in dominant 3 taxa	>82.2	68.4 - 82.2	<68.4	-	-	-	-	-	-
	% Tanytarsus spp.	-	-	-	-	<2.3	≥ 2.3	-	-	-
	% Dipteran infauna as intolerant chironmids	<0.53	0.53 - <0.7	≥7	<0.2	0.2 - <0.34	≥0.34	<0.39	0.39 - <0.59	≥ 0.59
	Collector filterer %	-	-	-	-			>31.4	23.2 - ≤ 31.4	<23.2
	Chironomidae richness	-	-	-	-	-	-	<8	8 - ≤12	>12

References

American Public Health Association. 1992. Standard Methods for the Analysis of Water and Wastewater, Ed. 18. Washington, D.C.

Carlson, R. 1995. Personal communication.

Carlson, R. and J. Simpson, 1996. A Coordinators Guide to Volunteer Lake Monitoring Methods. N. Amer. Lake Manage. Soc. Madison, WI.

Collins, C.D., Sheldon, R.B., and C.W. Boylen. 1987. Littoral Zone Macrophyte Community Structure: Distribution and Association of Species along Physical Gradients in Lake George, New York, U.S.A. Aquat. Bot., 29:177-194

Chapra, S.C. and K.H. Reckhow. 1983. Engineering Approaches for Lake Management Vol 1: Data Analysis and Empirical Modeling. Butterworth Pub. Boston.

Dibble, E.D., K.J. Kilgore and G.O. Dick. 1996. Measurement of Plant Architecture in Seven Aquatic Plants. J. Freshwat. Ecol. 11:3.

Gerritsen, J., B. Jessup, E. Leppo, and J., White. Development of Lake Condition indexes for Florida. Florida Department of Environmental Protection and Tetra-Tech Inc. Tallahassee, FL and Owings Mills, MD.

Hilsenhoff, W.L. 1987. An Improved Biotic Index of Organic Stream Pollution. Great Lakes Entomol. 20:31-39.

Picotte, A. 1995. Lay Monitoring Program Quality Assurance Project Plan. Vermont Department of Environmental Conservation. Waterbury.

PCORD

SAS Institute, 2000. SAS Statistical Analysis Software, V 6.12.

SPSS Science Inc. 1999. SigmaStat Statistical Software. V. 2.03.

Rencher, A. 1997. Methods of Multivariate Analysis. J. Wiley and Sons.

Reynoldson, T.B., R.H. Norris, V.H. Resh, K.E. Day, and D.M. Rosenburg. The reference condition: a Comparison of Multimetric and Multivariate Approaches to Assess Water-Quality Impairment using Benthic Macroinvertebrates. J. N. Am. Benthol. Soc. 1997, 16(4):833-852.

Sweet, J. W. 1986. A Survey and Ecological Analysis of Oregon and Idaho Phytoplankton. Final report to USEPA Region X. Seattle, WA.

terBraak, C. J. F., 1988 rev. 1992. CANOCO - a FORTRAN program for Canonical Community Ordination. Microcomputer Power, Ithaca, N.Y.

United States Environmental Protection Agency, 1979 (revised 1983). Chemical Analysis of Water and Wastes. EPA/600/4-79/020.

United States Environmental Protection Agency, 1987. Handbook of Methods for Acid Deposition Studies. EPA 600/4-87/026.

United States Environmental Protection Agency, 1997. Revisions to Rapid Bioassessment Protocols For Use in Streams and Rivers: Periphyton, Benthic, Macroinvertebrates, and Fish. Internet URL: http://www.epa.gov/owowwtr1/monitoring/AWPD/RBP/bioasses.html last revised 08/1997.

United States Environmental Protection Agency, 1998. Lake and Reservoir Bioassessment and Biocriteria Technical Guidance Document. Washington.

Vermont Department of Environmental Conservation. 1990. Field Methods Manual. Waterbury.

Vermont Department of Environmental Conservation and New York State Department of Environmental Conservation. 1997. A Phosphorus Budget, Model, and Load Reduction Strategy for Lake Champlain, Lake Champlain Diagnostic-Feasibility Study Final Report. Waterbury.

Vermont Department of Environmental Conservation. 2001. Wadeable Stream Biocriteria Development and Implementation Procedures for Fish and Macroinvertebrate Assemblages in Vermont Streams and Rivers. Waterbury, VT, U.S.A.

Warren, S. 1995. Aquatic Plant Surveys Quality Assurance Project Plan. Vermont Department of Environmental Conservation. Waterbury.