Introduction and Acknowledgments:

The following document is one section of a report which is currently in preparation by VTDEC. It is provided for readers who have an active interest in the development of biological criteria for Vermont and New Hampshire lakes. A brief project overview is presented, followed by a detailed description of bioassessment methods employed. The assessment data collec ted in conjunction with this projectare to be used within a multimetric bioassessment framework (USEPA 1997, Reynoldson et. al., 1997) to generate draft criteria for assessing a lakes' overall compliance with standards for aquatic life use support. Our goal is to employ independently applied multivariate statistical techniques to evaluate the strength of the biological changes identified using the multimetric methods.

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Readers of this partial report are encouraged to contact Neil Kamman at VTDEC for additional information regarding the project, clarification, or for the full citations of references noted in text.

Overview:

The objective of this project is to determine the range of biological characteristics which constitute reference conditions for lakes of differing types across Vermont and New Hampshire. Our ultimate aim is the development of lake-specific biological criteria for inclusion in Vermont an New Hampshire's respective 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 this project form one of the case studies in that document (see Appendix F of USEPA 1998).

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 physico-chemical attributes of lakes which are not typically affected by anthropomorphic factors. These were subsequently corroborated using the biological measurements. The biological reference condition of three lake classes was defined, and was used as a measure by which known-impaired and lakes of unknown biological condition were assessed.

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.

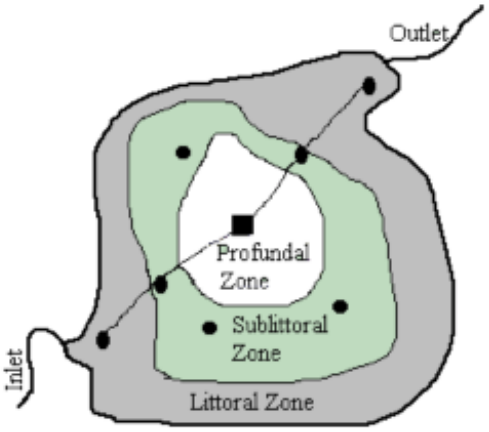
Table 2. Tier two chemical parameters and biological metrics evaluated for 1996-1997 Bioassessment and Paleolimnology of Vermont and New Hampshire Lakes Project study lakes.

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. 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.

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 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. 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 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.