

Title & Approvals

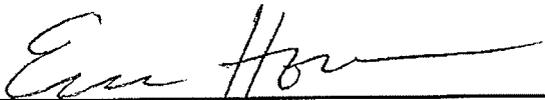
MISSISQUOI BAY BASIN PROJECT:  
SHORT-TERM MONITORING PROGRAM

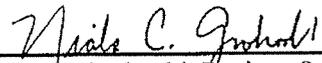
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Version 4

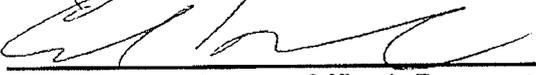
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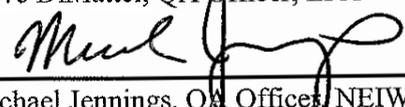
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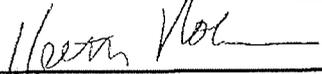
  
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### **Problem Definition/Background**

Missisquoi Bay has one of the highest in-lake phosphorus concentrations of any segment of Lake Champlain. Phosphorus loads to and concentrations in the Bay greatly exceed target levels designated by water quality criteria for phosphorus endorsed by the governments of New York, Quebec, and Vermont. Furthermore, this phosphorus contributes significantly to blue-green algae blooms (cyanobacteria) in Missisquoi Bay during the summer months. These blooms are frequently dense enough to preclude recreational water contact for many weeks at a time. Loads of sediment and nitrogen to the Bay are also a concern.

While the governments of Vermont and Quebec have made significant progress in reducing the loads of phosphorus to the Bay, more needs to be done in order to meet the target loads. A 2002 agreement between the governments of Vermont and Quebec established that their relative target contributions of phosphorus in the watershed are 60% and 40%, respectively. The Province of Quebec has undertaken several programs to reduce its share of phosphorus loads. Similarly, Vermont has initiated phosphorus reduction programs, but has found reducing phosphorus to be more problematic in its sector of the Basin. On August 1, 2008, the Governments, pursuant to Article IX of the Boundary Waters Treaty of 1909, requested the International Joint Commission (IJC) to assist in the implementation of the transboundary initiative to reduce phosphorus loadings. Recognizing the recent advances made by the Province of Quebec within its areas of jurisdiction, the Commission was requested to coordinate a number of tasks on the U.S. side of the border, in close partnership with the Lake Champlain Basin Program (LCBP).

As part of this coordination, the IJC has contracted with the LCBP to establish water quality monitoring locations on tributaries within the Missisquoi Bay Basin to be sampled over a two-year period. Increased sampling in the Basin will address the need for more detailed spatial data of tributary nutrient concentration and load as well as support improved calibration and validation of nutrient-load modeling planned as part of the IJC project. It is the aim of the monitoring and modeling to locate critical source areas of phosphorus pollution to the Bay in order to mitigate them. Ultimately, the goal of this work is to reduce external loads of phosphorus, nitrogen, and sediments into Missisquoi Bay so that water quality standards are consistently achieved.

### **Project Purpose/Task Description**

The primary purpose for the two-year Monitoring Program described in this plan is to increase knowledge of tributary nutrient loads in the Missisquoi Bay Basin and support modeling of pollution sources. The increased sampling in the Basin will address the need for more detailed spatial data of tributary nutrient loads and related meteorological (precipitation) data. Parameters to be sampled, sampling frequencies, and sampling locations were selected to complement the current long-term monitoring program in the Basin. The data collected in this monitoring program will enable a more accurate assessment of nutrient loads within the Basin.

### **Intended Uses of Data**

The water quality, stream flow and meteorological data generated by this monitoring program are intended to be used to identify and delineate critical source areas of nonpoint source pollution in the Basin as part of the IJC funded project on the Missisquoi Bay. In addition, the data may be utilized by a wide range of stakeholders for many purposes including providing general information to the public, supporting additional research projects and helping to direct management efforts.

Tributary monitoring data will be collected and analyzed similarly to sampling for the Long-Term Water Quality and Biological Monitoring Project for Lake Champlain (LTMP) so that it can be integrated with existing data to create a more spatially detailed assessment of water quality in the Missisquoi Bay Basin. This increased sampling will address the need for more detailed spatial data of tributary nutrient concentration and load as well as support improved calibration and validation of nutrient-load modeling planned as part of the IJC project. Multiple parameters monitored through this project, including total and dissolved phosphorus and nitrogen concentrations in the water column and suspended sediments, will be used to assess the health of individual tributaries and their contribution to nutrient loading in the Basin. The data should assist in locating critical source areas of nutrient pollution, which in turn will help to target resources for nutrient reductions.

In addition to collecting water quality data, the LCBP will establish new meteorological stations to create a more comprehensive network in the Basin. The current lack of consistent weather data restricts the understanding of nutrient runoff and stream discharge and diminishes the effectiveness of modeling. The LCBP will establish meteorological recording stations in accordance with standards outlined by the United States Geological Survey (USGS).

### **Protocols and Procedures**

#### **Water Quality Tributary Monitoring**

Water quality monitoring stations will be established near the mouths of the following five tributaries in the Missisquoi Bay Basin: Hungerford Brook, Tyler Branch, Trout River, Black Creek, and Mud Creek (see attached map). Water quality sampling will follow all pertinent procedures and protocols outlined in the approved Long-Term Water Quality and Biological Monitoring Project for Lake Champlain (see attached document).

Water quality will be sampled throughout the year as specified in the protocols for the long-term monitoring program conducted by the Vermont Department of Environmental Conservation (Lake Champlain LTM QAPP Section 10.4, page 17). Winter month sampling will be dependent on the freeze/thaw cycles. The yearly sampling timeline is shown in Table 1. Water quality samples will be taken following precipitation events with a target of 20 high-flow total phosphorus samples per year. In addition, 4 low-flow total phosphorus samples will be taken

each year. The low-flow samples will be used to define the concentrations vs. flow relationship over the full range of flow conditions.

*Table 1: Water Quality Sampling Timeline.*

<i>Task</i>	<i>Jan</i>	<i>Feb</i>	<i>Mar</i>	<i>Apr</i>	<i>May</i>	<i>Jun</i>	<i>Jul</i>	<i>Aug</i>	<i>Sep</i>	<i>Oct</i>	<i>Nov</i>	<i>Dec</i>
Tributary Monitoring												

Light Gray: Moderate Activity; Dark Gray: High Activity

Winter month sampling will be contingent on freeze/thaw cycles

Tributary samples will be collected according to the procedures outlined in section 11.2 (page 26) in the Lake Champlain LTM QAPP. The following parameters will be analyzed for each sampling event: temperature, pH, conductivity, alkalinity, total and dissolved phosphorus, total nitrogen, total suspended solids, chloride, and metals (Ca, Mg, Na, K, Fe). All samples will be handled according to section 12.0 (page 28) of the Lake Champlain LTM QAPP and analyzed according to section 13.1 (page 28).

The United States Geological Survey (USGS) will install an automated sampler (ISCO) on Hungerford Brook. The sampler will be co-located with the flow gage and will collect hydrologic and/or precipitation event-based samples. These samples will be analyzed for total phosphorus and suspended sediment concentrations. Samples will be processed and analyzed according to procedures outlined in the Lake Champlain LTM QAPP (section 12.0, page 28 and section 13.1, page 28). A total of 40 samples per year will be collected between the months of March and November. The automatic water sampler will be controlled by dataloggers, which will be triggered to sample at desired flow thresholds.

### **Streamflow Measurements**

Continuous-record, real time flow gages will be constructed and operated at the farthest feasible downstream location of the following five tributaries in the Missisquoi Bay Basin: Hungerford Brook, Tyler Branch, Trout River, Black Creek, and Mud Creek. In addition, a flow gage will be established on the Sutton River on the U.S. side of the border (see attached map).

Field reconnaissance by United States Geological Survey (USGS) personnel will determine the most suitable sites for flow gages using protocols described in Rantz and others (1982a). Station installations will follow the schedule outlined in Table 2 and be done by the USGS in accordance with their procedures and protocols. If necessary, permission and lease arrangements with land owners will be made.

*Table 2. Stream Gage and Weather Station Installation Timeline*

Installation Order	Station	FY09				
		May	June	July	Aug	Sept
	<b>Hydrologic Station</b>					
1	Hungerford Brook (includes meteorological)					
2	Tyler Branch					
3	Trout River (includes meteorological)					
4	Black Creek					
5	Mud Creek (includes meteorological)					
6	Sutton River					
	<b>Meteorological Station</b>					
7	East Fletcher					

The flow gages will be operated and maintained by the USGS for the duration of this project, according to their procedures and protocols. Data collection procedures will be followed as outlined within individual reports on Techniques of Water Resources Investigations (located at <http://pubs.usgs.gov/twri/>).

Standard USGS procedures for streamflow data collection and analysis are included within reports by Sauer (2001) and Rantz et al. (1982a, 1982b). Data collection platforms (DCPs) and stage sensors will measure and record stream stage at 15 minute intervals. The data will be transmitted using satellite telemetry equipment to the USGS National Water Information System (NWIS) website web at: <http://waterdata.usgs.gov/nh/nwis/current/?type=flow>.

In the case of any faulty or missing stage record, estimates will be made by hydrographic comparison, regression or other standard techniques for estimation of missing records as presented in Sauer (2001). Procedures for the correction of errors or discontinuities of the data will be followed as outlined in the USGS Water Supply Papers (Rantz et al., 1982a, 1982b).

### **Meteorological Condition Monitoring**

Meteorological stations will be established by the USGS at four locations and installed according to the schedule outlined in Table 2. Three of the weather stations will be co-located with stream-gaging stations; the fourth station will be located in East Fletcher, Vermont (see attached map). The meteorological stations will continuously record precipitation and air temperature data using DCPs at 15 minute intervals. The data will be transmitted by satellite telemetry at 1-hour intervals to the USGS website at <http://waterdata.usgs.gov/nh/nwis/current/?type=precip>.

The USGS will maintain the meteorological stations for the duration of this project. Precipitation data will be collected using standard USGS procedures (USGS Technical Memorandum 2006.01). No precipitation data will be collected during snow and freezing temperatures. Air temperature data will also be collected using standard USGS procedures (Wilde, 2006; Wagner et al., 2006). Thermistors will be mounted within a radiation shield and approximately 2 meters above ground level.

### **Data Management and Integration**

Data acquired from tributary monitoring will be processed and managed according to section 19.0 (page 38) of the Lake Champlain LTM QAPP and ultimately incorporated into the LTM electronic project database. The USGS will process, manage and publish daily mean streamflow values and air temperature and precipitation data according to their procedures and protocols. Real time data will be available on the USGS website and final data will be made available within approximately 6 months following data collection. Additionally, the USGS will provide streamflow and meteorological data to the LCBP.

In order to maximize the utility of the data gathered in the STMP, they must be suitable for integration with existing and future Vermont and Quebec data. Because water quality tributary monitoring will be done in accordance with the approved LTMP, it can be joined with Vermont data in the same fashion as LTMP data. Additionally, split sampling of water quality parameters will include a full set of samples for analyses to be conducted by the Ministère du Développement durable, de l'Environnement et des Parcs (MDDEP) laboratories in Quebec, to provide information on the comparability of analytic procedures on both sides of the international border. The results of split sampling will allow further refinements of the existing protocol for integrating data between Vermont and Quebec, as reported in: *Phosphorus Loading to the Missisquoi Bay from Sub-Basins in Vermont and Quebec, 2002-2005*, recently completed by Eric Smeltzer, VT DEC, and Marc Simoneau, MDDEP (available at <http://lcbp.org/techreportPDF/P-Load-Missisquoi-Bay-25Nov2008-en.pdf>). Stream flow measurements and meteorological data, calculated and reported by the USGS in accordance with their quality controls, will be easily comparable within the Basin.

### **Quality Control Requirements**

Field and laboratory quality control for tributary monitoring will be conducted according to section 14.0 (page 32) of the Lake Champlain LTM QAPP. Instrument and equipment maintenance and calibration will be subject to sections 15.0 and 16.0 (page 35), respectively. Streamflow and meteorological measurements will be subject to USGS quality assurance/quality control standards, as established at the Vermont/New Hampshire USGS office based on national protocols.

NEIWPCC may implement, at their discretion, various audits or reviews of this project to assess conformance and compliance to the quality assurance project plan in accordance with the NEIWPCC Quality Management Plan.

### References

- Rantz, S.E., 1982a, Measurement and computation of streamflow: volume 1. measurement of stage and discharge: U.S. Geological Survey Water-Supply Paper 2175, p. 1-284.  
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- U.S. Geological Survey, 2006, Office of Surface Water Technical Memorandum No. 2006.01, Collection, quality assurance, and presentation of precipitation data.
- Wagner, R.J., Boulger, R.W., Oblinger, C.J., and Smith, B.A., 2006, Guidelines and standard procedures for continuous water-quality monitors: station operation, record computation, and data reporting, U.S. Geological Survey Techniques and Methods 1-D3, 51 p.

### Appended Files

1. Map entitled *Missisquoi Bay Basin: Proposed Short-Term Monitoring Sites*
2. *Long-Term Water Quality and Biological Monitoring Project for Lake Champlain: 2009 Quality Assurance Project Plan*

# Missisquoi Bay Basin

## Proposed short-term monitoring sites



Points represent approximate locations

- Proposed short-term monitoring station with USGS flow gage
- ✕ Proposed USGS flow gage
- Proposed USGS weather station
- Existing NOAA weather station
- Major waters
- - - Sub-basin bounds (HUC12)

DRAFT: May 2009  
For Planning  
Purposes ONLY



Sources: Geobase, NOAA, USGS, VCGI

1.0 Title & Approvals

LONG-TERM WATER QUALITY AND BIOLOGICAL MONITORING  
PROJECT FOR LAKE CHAMPLAIN  
2009 Quality Assurance Project Plan

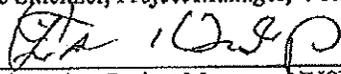
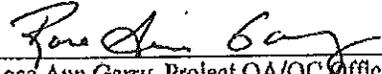
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PROJECT FOR LAKE CHAMPLAIN  
2009 Quality Assurance Project Plan

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Lake Champlain LTM QAPP/Workplan  
Revision 1.4 March 20, 2009  
Page 1 of 54

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2009 Quality Assurance Project Plan

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**2.0 Table of Contents**

1.0 Title & Approvals ..... 1

2.0 Table of Contents ..... 2

3.0 Distribution List ..... 4

4.0 Task/Organization ..... 5

5.0 Problem Definition/Background ..... 6

6.0 Project Purpose/Task Description ..... 7

7.0 Data Quality Objectives for Measurement Data ..... 11

8.0 Training Requirements/Certifications ..... 11

9.0 Documentation and Records ..... 11

10.0 Sampling Process Design ..... 12

11.0 Sampling Methods Requirements ..... 22

12.0 Sample Handling and Custody Requirements ..... 28

13.0 Analytical Methods Requirements ..... 28

14.0 Quality Control Requirements ..... 32

15.0 Instrument/Equipment Testing, Inspection, and Maintenance Requirements ..... 35

16.0 Instrument Calibration and Frequency ..... 35

17.0 Inspection and Acceptance Requirements for Supplies ..... 37

18.0 Non-direct measurements ..... 37

19.0 Data Management ..... 38

20.0 Assessments and Response Actions ..... 39

21.0 Reports ..... 40

22.0 Data Review, Validation, and Verification Requirements ..... 41

23.0 Validation and Verification Methods ..... 41

24.0 Reconciliation with Data Quality Objectives ..... 41

25.0 References ..... 42

Appendix A. Laboratory Methods for Biological Samples ..... 44

Appendix B: Data review procedures ..... 49

Appendix C. Project data forms ..... 51

**2.1 List of Tables**

Table 1. Phosphorus and pelagic food web indicators that will be developed from data collected by the LTMP ..... 7

Table 2. Project Schedule Timeline ..... 10

Table 3. Lake sampling locations and total station depths..... 14

Table 4. List of lake and tributary sampling station locations and total river drainage areas. .... 16

Table 5. Openwater and nearshore sampling site locations for zebra mussel and mysid monitoring in Lake Champlain..... 19

Table 6. Lake monitoring parameters ..... 23

Table 7. Summary of processing, preservation, and storage containers for water quality parameters..... 24

Table 8. Tributary monitoring parameters ..... 27

Table 9. Analytical procedures for parameters and field measurements..... 28

Table 10. Numbers of archived phytoplankton samples at priority stations remaining to be analyzed. .... 30

Table 11. Parameter table for biological monitoring ..... 31

Table 12. Quality assurance information for analytes..... 32

Table 13. Field data quality objectives for veliger, settled juvenile and adult density duplicate samples for zebra mussels ..... 33

Table 14. Laboratory data quality objectives for veliger, season settled juvenile and adult density duplicate samples for zebra mussels ..... 33

Table 15. Data quality objectives for phytoplankton analyses..... 34

Table 16. Water quality sampling equipment ..... 35

Table 17. Biological sampling equipment ..... 35

Table 18. Calibration frequency, procedures, standards and acceptance criteria for major measurement systems ..... 36

**2.2 List of Figures**

Figure 1. Lake Champlain Basin Location ..... 9

Figure 2. Location of lake and tributary sampling stations..... 13

Figure 3. Detecting change in total phosphorus concentration in Lake Champlain..... 15

Figure 4. Precision of mean total phosphorus loading estimates as a function of sample size for the Missisquoi River..... 17

Figure 5. Open-water and nearshore sampling site locations for Lake Champlain zebra mussel and mysid sampling ..... 20

Figure 6. Inland lake and tributary sampling site locations for zebra mussels..... 21

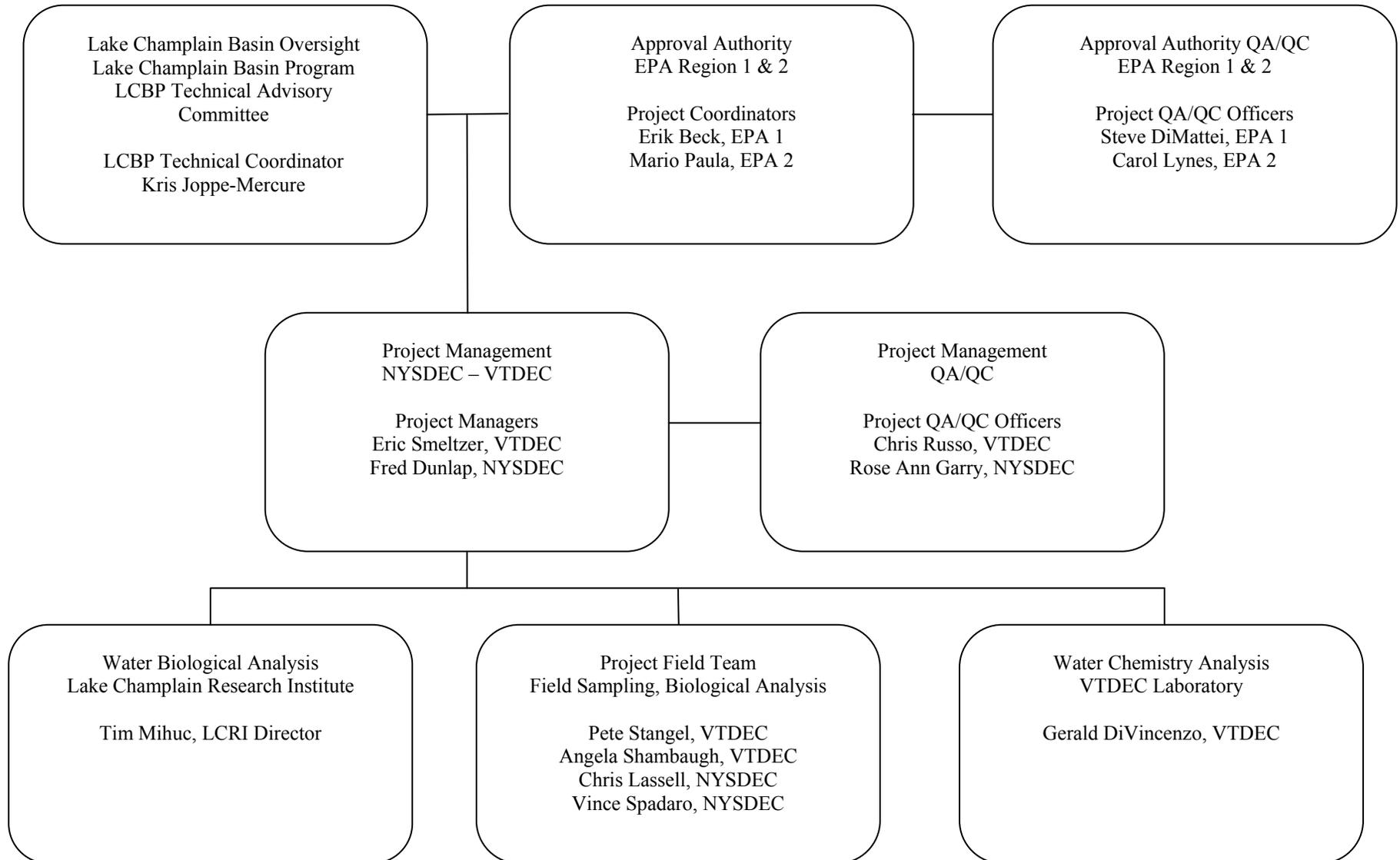
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## 4.0 Task/Organization

### Project Organization



## **5.0 Problem Definition/Background**

Lake Champlain is one of the largest lakes in the United States and is often called the "Sixth Great Lake". Stretching 120 miles, it forms the boundary between New York and Vermont (Figure 1). The contributing watershed area of approximately 8,200 square miles spans from the Adirondack Mountains of New York to the Green Mountains of Vermont and into the Province of Quebec, Canada. Although primarily a recreational lake, it also serves a multitude of uses ranging from a drinking water source to receiving treated wastewater from municipal and industrial facilities. It also receives non-point runoff from agricultural and urban areas. These activities, among others, have been identified as contributing to recognized or potential water quality problems within the lake system. Eutrophication, toxic substances, and the presence of nuisance plant species (Eurasian watermilfoil and water chestnut) have been identified as priority issues. The impact of zebra mussels on the Lake Champlain ecosystem and the threat of other exotic species entering from outside the Basin are of concern, as are algal blooms, the depletion of dissolved oxygen concentrations in some lake segments, low water transparency, contaminated fish flesh and declining fisheries stocks.

A considerable amount of chemical and biological data was collected at many locations in Lake Champlain during the 1970's and earlier, as reviewed in Myer and Gruendling (1979). These earlier studies provide some good historical baseline data, but are relatively limited in parameter coverage and seasonal and spatial extent. In many cases, measurements of all major nutrients were not made concurrently with the biological samples, as would be desirable for the purpose of analyzing ecological interrelationships. The Vermont Lay Monitoring Program has provided lake wide monitoring of eutrophication related parameters during the summer season using citizen volunteers and a consistent methodology every year since 1979. Further information on this program along with available data can be found at the VTDEC Volunteer monitoring website: [http://www.anr.state.vt.us/dec/waterq/lakes/htm/lp\\_volunteer.htm](http://www.anr.state.vt.us/dec/waterq/lakes/htm/lp_volunteer.htm) The most extensive recent monitoring programs on Lake Champlain are the Lake Champlain Diagnostic-Feasibility Study (Vermont DEC and New York State DEC, 1997), the Long-Term Water Quality and Biological Monitoring Project for Lake Champlain (Vermont DEC and New York State DEC, 1998), and the Lake Champlain Biomonitoring Program conducted by the Vermont Water Resources and Lake Studies Center (Brown *et al.*, 1992, 1993).

Water quality trend detection is often the first purpose many people have in mind when they consider the need for lake monitoring. It is important to be able to document environmental change in Lake Champlain. Biomonitoring greatly improves our knowledge of the response of aquatic ecosystems to changes in water quality conditions by providing a direct measure of aquatic community status. Aquatic communities integrate all aspects of seasonal and spatial variability in their environment and provide a more sensitive index of environmental change than water quality monitoring alone. Biomonitoring can serve as an "early warning" indicator by providing data and insights into incipient biological changes and long term indications of significant changes in system function or potential resource utilization. Evidence of water quality deterioration can provide a stimulus for initiating corrective actions. Water quality improvement or lack of improvement, in response to management programs is also important to demonstrate so that the effectiveness of pollution reduction efforts can be objectively evaluated.

The Lake Champlain Basin Program Technical Advisory Committee supports this effort and affirms that the monitoring program should continue to focus on developing information to track and evaluate management programs. Phosphorus loads and concentrations remain the highest priority, but other parameters that can serve as indicators of ecosystem health are also important.

## **6.0 Project Purpose/Task Description**

### **6.1. Objectives of Project**

The primary purpose for the on-going water quality and biological monitoring program described in this plan will be to detect environmental change in Lake Champlain. Environmental indicators, monitoring stations, monitoring frequencies, and sampling procedures have all been specifically selected for this purpose, and statistical considerations were applied to optimize the design of the monitoring program. The project will maintain a database that will serve as the basis for establishing the relationship between water quality, the biological community and lake environmental health.

The second main purpose of the Long Term Water Quality and Biological Monitoring Project for Lake Champlain (hereafter the LTMP) is to support the Lake Champlain Basin Program's Ecosystem Indicators Program. The Ecosystem Indicators Program is working to develop a suite of indicators in the pressure-state-response (PSR) framework that will describe the condition of the lake and track the effectiveness of management. The PSR framework is based on the premise that human activities exert pressures on the ecosystem that affect the state of the ecosystem. In response to a detrimental state or trend in the lake, humans develop management actions and policies in an effort to reduce the pressures. The indicators selected will be presented in the form of a scorecard that will be available to inform the public and lake managers (Table 1).

**Table 1. Phosphorus and pelagic food web indicators that will be developed from data collected by the LTMP**

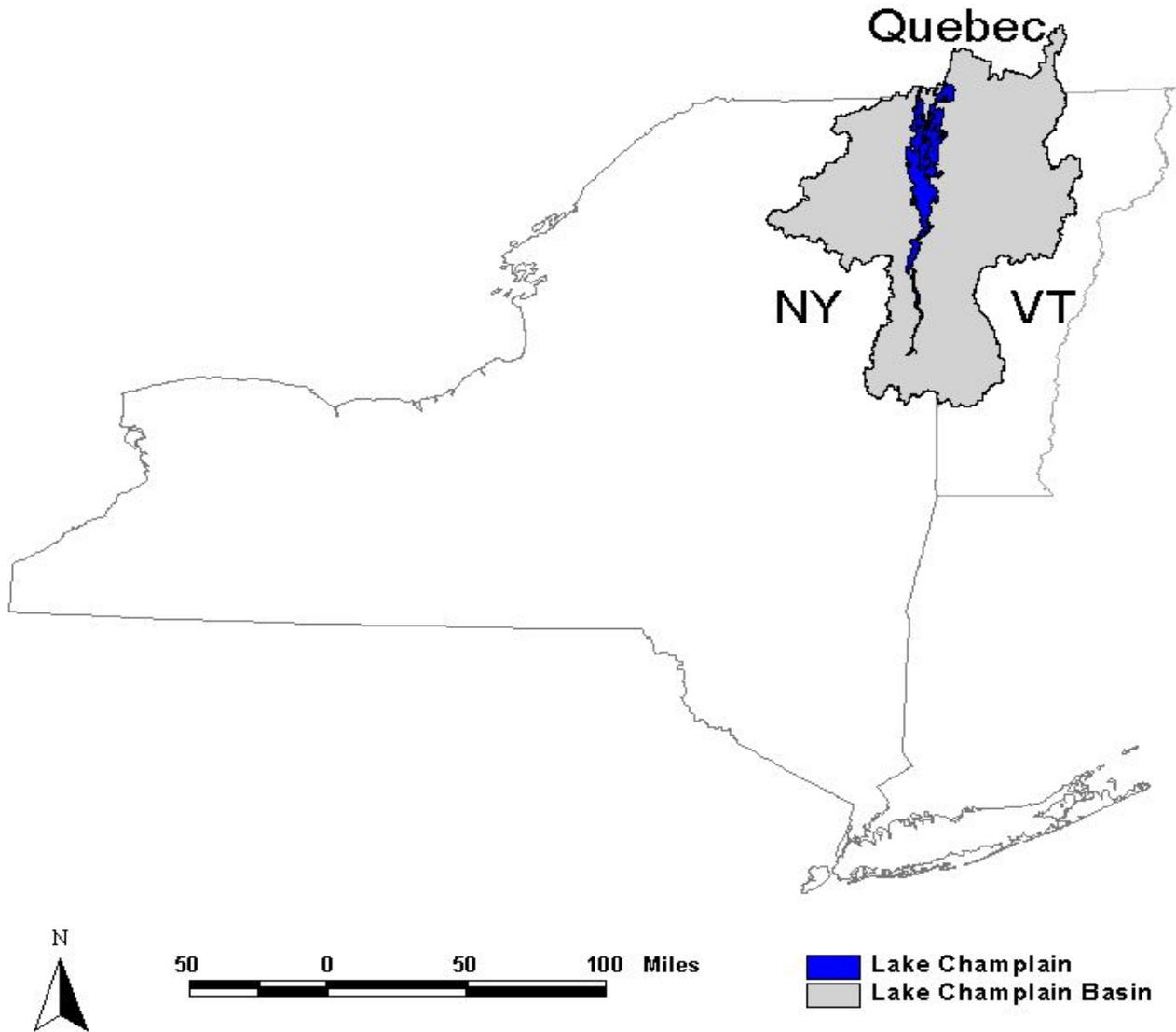
<b>Indicator</b>	<b>Supporting Measures</b>
Phosphorus in lake water	Annual mean total phosphorus concentration in each lake segment, and long-term trends.
Phosphorus in tributaries	Mean total phosphorus loads for each tributary (reported for two-year intervals) and long-term trends.
Chlorophyll-a in lake water	Annual mean chlorophyll-a concentration in each lake segment, and frequency of algae blooms.
Dissolved oxygen in lake water	Hypolimnetic dissolved oxygen concentrations in deep lake segments, and long-term trends.
Phosphorus in wastewater discharges	Annual phosphorus loads from each treatment facility, summarized by state/province and by lake segment subwatershed.
Nitrogen to phosphorus ratios	Annual mean total nitrogen to total phosphorus ratios in each lake segment.
New exotic species	Number of new invasive exotic species detected each year (phytoplankton, zooplankton, fish, vascular plants).
Phytoplankton community	Taxonomic composition and relative abundance of major groups. Percent potential toxin producing cyanobacteria.
Zooplankton community	Taxonomic composition and relative abundance. Average size of zooplankton. Ratio of phytoplankton biomass to zooplankton biomass.

## 6.2. Intended Uses of Data

The statistically reliable water quality trend information generated by the LTMP may be utilized by a wide range of audiences for many purposes from providing general lay information to supporting additional research projects to helping to direct management efforts. The principal investigators, the states of New York and Vermont, may use the data to assist in developing and supporting policy and management decisions, and to evaluate TMDL implementation. Additionally, the data may assist in narrowing down and identifying nutrient sources, which in turn, will help in targeting resources for nutrient reductions. The project may be deemed successful if the project objectives and data quality indicators, (precision, accuracy, representativeness, completeness, comparability, and sensitivity) are met.

The data generated by this project will be evaluated and presented in the form of an indicators scorecard to be developed by the LCBP to assess the condition and trends in the lake ecosystem. Multiple parameters monitored through this project, including phosphorus concentrations, chlorophyll-*a*, and nitrogen concentrations in the water column, and the composition and abundance of phytoplankton and zooplankton will be used as indicators to assess the pressures on the lake, the state of the lake, and the response of the lake to management actions and policies. The data analyses for the development of the actual indicator values and scorecard presentations will be the shared responsibility of the Lake Champlain Basin Program staff, Technical Advisory Committee, and the LTMP personnel, and is beyond the scope of this QAPP.

Figure 1. Lake Champlain Basin Location



### 6.3 Project Schedule

**Table 2. Project Schedule Timeline**

Task	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Lake monitoring												
Tributary monitoring <sup>1</sup>												
Water chemistry analysis												
Phytoplankton analysis												
Zooplankton analysis												
Zebra mussel analysis												
Mysids analysis												
Update work plan/QAPP												
Database management <sup>2</sup>												
Project website updates <sup>3</sup>												
Reporting Quarterly/Annual	Quarterly			Annual			Quarterly			Quarterly		

White = low activity, Light gray=moderate activity, Dark gray=high activity

<sup>1</sup>Event based sampling. Winter month sampling contingent on freeze/thaw cycles.

<sup>2</sup> Download of data from DEC Laboratory Information Management System, data review and checking, update of Access Database

<sup>3</sup> Annually updated data made available in statistical summary, graphical and full tabular form on the project website

## **7.0 Data Quality Objectives for Measurement Data**

Data collected by the monitoring program are used to assess progress towards basin-wide water quality goals. The quality assurance program established for the LTMP specifies the criteria used to assess precision and accuracy of the data collected each year. These are discussed in detail in Section 14 and noted throughout this QAPP. Quality objectives and criteria for chemical analyses are documented in the VTDEC Laboratory QAP (VTDEC, 2009; <http://www.anr.state.vt.us/dec/lab/htm/QualityControl.htm>) and noted throughout this QAPP.

## **8.0 Training Requirements/Certifications**

Many project team members have been involved in Lake Champlain basin monitoring on a continuous basis since the Diagnostic/Feasibility study was initiated in 1990. Additionally, the project team members are professional career employees of the State's of New York and Vermont Departments of Environmental Conservation working in the respective Water Quality Divisions.

Team members are fully trained and experienced in ambient sample collection for both water chemistry and biological parameters. Staff remains up-to-date with equipment use and field protocols. No additional specialized training is necessary for the field aspects of this project. All temporary and seasonal staff that may be associated with this project work under the supervision of project team members. VTDEC Laboratory personnel (chemistry) are supervised by the laboratory directors, and meet the training/certification requirements specified by the Laboratory.

Taxonomic expertise is required for the analysis of both phytoplankton and zooplankton. Phytoplankton analyses will be conducted at the Vermont DEC by an experienced taxonomist with more than 15 years experience with freshwater plankton from Lake Champlain. Zooplankton analyses will be conducted at the Lake Champlain Research Institute under the supervision of Dr. Timothy Mihuc. Mysid analyses will be overseen by NYSDEC staff having over 10 years of experience with Lake Champlain mysids identification and enumeration.

## **9.0 Documentation and Records**

Project field teams document field generated data on Field Log Sheets. NY field teams provide copies to the VT office and copies of all field sheets (VT and NY) reside in an archive in the Lakes and Ponds section of the Water Quality Division, VT DEC. Copies of all NY field sheets and in-situ generated data are also maintained at the NYDEC Division of Water office in Ray Brook, NY. All data generated by participating laboratories are collected by the project managers in an electronic format that can be incorporated into the project master database.

The project data is maintained by Vermont DEC and is stored in a Microsoft SQL Server 2005 database. Daily tape backup is provided, and copies of backup files are archived in separate locations. Database security features are employed to prevent editing or deletion of the original data by users other than the authorized database administrators. Copies of the current database are also available at the New York State DEC. The data are available to other government agencies, researchers, consultants, students, and the general public on request in either electronic, paper copy form or on the web at [www.anr.state.vt.us/dec/waterq/lakes/htm/lp\\_longterm.htm](http://www.anr.state.vt.us/dec/waterq/lakes/htm/lp_longterm.htm)

Graphical summaries of the data are made each year and posted on the website. Annual reports and the current QAPP plan are also available through this site. Past reports and plans are stored electronically and are available upon request.

## **10.0 Sampling Process Design**

### **10.1 Selection of Lake Station Locations**

The LTMP for Lake Champlain originally included lake monitoring at 12 lake stations (Nos. 2, 4, 7, 19, 21, 25, 33, 34, 36, 40, 46, 50) during the period 1992-2000 (Figure 2, Table 3). These stations were selected to represent major lake segments between which distinct water quality differences exist.

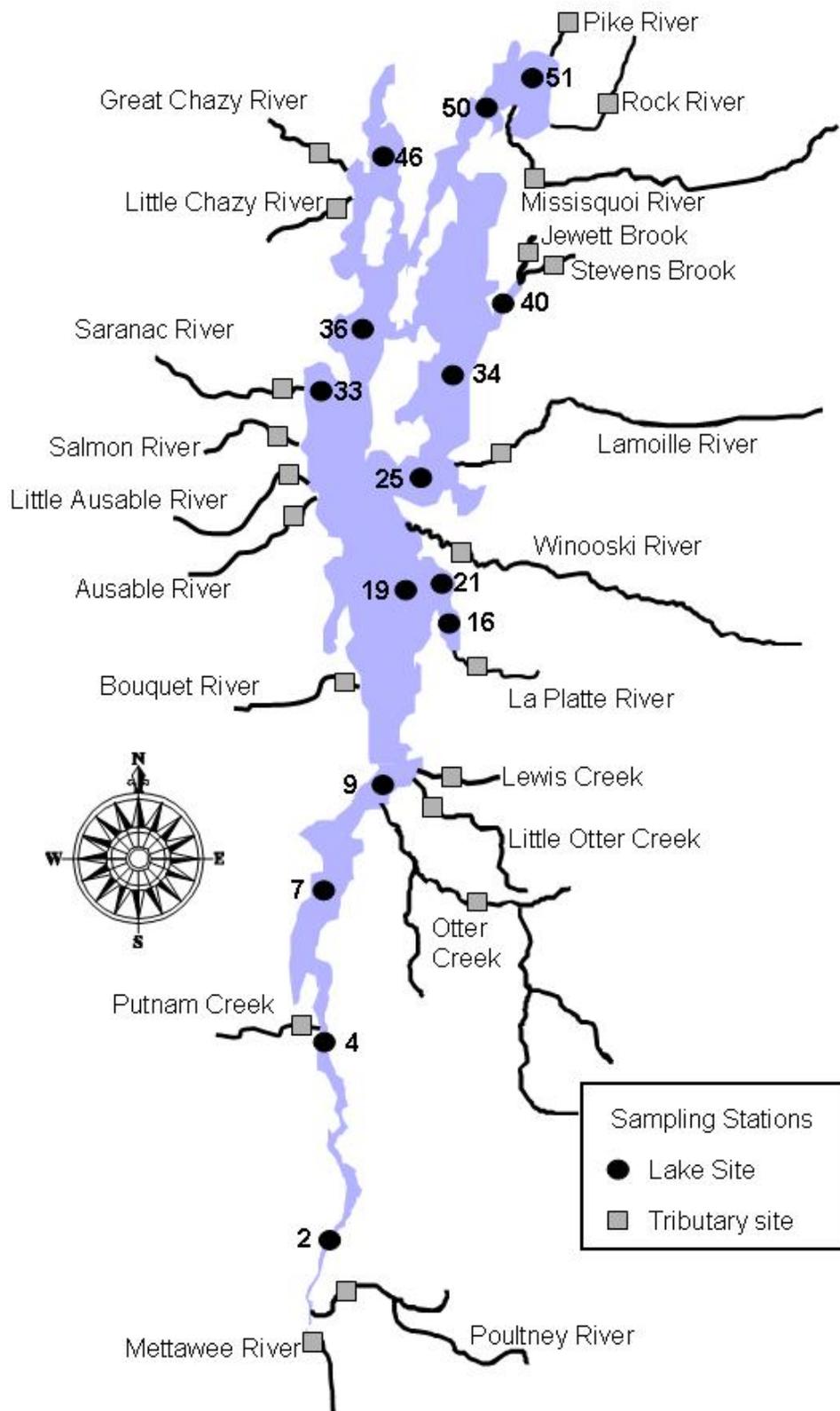
Beginning in 2001, two lake water quality and biological sampling stations (9 and 16) were added to the program. The Lake Monitoring Project Review Team of the Lake Champlain Basin Program determined that the program should include at least one sampling station in each of the 13 lake phosphorus management segments in order to track progress toward attainment of the in-lake total phosphorus concentration criteria established for each segment of the lake. Lake stations representing the Otter Creek and Shelburne Bay segments were added to provide sampling coverage for all lake segments for which phosphorus concentration criteria have been established (Lake Champlain Basin Program, 2003; Vermont DEC and New York State DEC, 2002).

An additional lake water quality and biological sampling station (51) was added to the program in 2006. The Lake Champlain Basin Program's Ecological Indicators Task Force recommended that an additional station be added in Missisquoi Bay to provide more complete spatial coverage and to better characterize water quality status and trends in this high priority lake segment. The in-lake phosphorus concentration criteria for Lake Champlain apply to central, open water locations in each lake segment. Station 51 was centrally located in Missisquoi Bay in order to provide data more consistent with the way the criteria are defined.

In summary, the locations of the lake sampling stations were selected based on the following considerations:

- Include a centrally located station in each phosphorus management segment.
- Avoid duplicating stations within lake areas where spatial water quality differences are small.
- Avoid sites with strong, spatially shifting concentration gradients such as locations near river mouths or in transition zones between adjoining segments.
- Co-locate sites with stations that have been monitored historically by other programs such as the Vermont Lay Monitoring Program and the Lake Champlain Diagnostic-Feasibility Study.

Figure 2. Location of lake and tributary sampling stations.



**Table 3. Lake sampling locations and total station depths**

Station #	Latitude N	Longitude W	Depth (meters)
02	43° 42.89'	73° 22.98'	5
04	43° 57.10'	73° 24.47'	10
07	44° 07.56'	73° 24.77'	50
09 <sup>1</sup>	44° 14.53'	73° 19.75'	97
16 <sup>1</sup>	44° 25.55'	73° 13.92'	25
19	44° 28.26'	73° 17.95'	100
21	44° 28.49'	73° 13.90'	15
25	44° 34.92'	73° 16.87'	32
33	44° 42.07'	73° 25.09'	11
34	44° 42.49'	73° 13.61'	50
36	44° 45.37'	73° 21.30'	50
40	44° 47.12'	73° 09.73'	7
46	44° 56.90'	73° 20.40'	7
50	45° 00.80'	73° 10.43'	4
51 <sup>2</sup>	45° 02.50'	73° 07.78'	5

<sup>1</sup> Added beginning in 2001

<sup>2</sup> Added beginning in 2006

## 10.2 Selection of Lake Sampling Frequency

Lake sampling frequencies were determined so that there is a reasonably high probability (power) of statistically detecting a meaningful environmental change over time, when such a change actually occurred (Green, 1989; Peterman, 1990). A power analysis was conducted for several lake chemical monitoring parameters in order to determine the proper sampling frequencies to achieve adequate power of detecting environmental change over time in Lake Champlain using the procedure provided by Walker (1988). Because total phosphorus was considered to be the highest priority lake monitoring parameter, the power analysis focused on total phosphorus for the purpose of determining optimum sampling frequencies.

The procedure assumed that environmental change would be analyzed using a t-test for the difference in the mean phosphorus value between two time periods (e.g., a baseline period *vs.* a post-treatment period) Walker's (1988) procedure allows for a consideration of both within-year and between-year components of variance in lake sampling data. Within-day variance (i.e., variance of replicate samples obtained at the same station on the same day) is generally small relative to the within-year (date to date) and between-year variance components for common lake monitoring parameters (Knowlton *et al.*, 1984), and was not included in the analysis.

Lake Champlain monitoring data from the Lake Champlain Diagnostic-Feasibility Study (1990-1991) and the LTMP for Lake Champlain (1992-1993) were used to estimate the variance components for total phosphorus according to methods given in Walker (1988) and Smeltzer *et al.* (1989). The power analysis was conducted using the median values of the variance components across all lake stations.

The power analysis requires specification of the magnitude of the environmental change to be detected. This is a somewhat arbitrary judgment, but it is an important specification because the required level of sampling effort and the program cost increase greatly as smaller change detection goals are considered. A minimum phosphorus change of 15% was specified for the power analysis, corresponding approximately to the phosphorus reduction needed for the Main Lake segment in order to comply with its water quality criterion value. Larger reductions are needed in other lake segments (e.g., Missisquoi Bay, South Lake), so using 15% should ensure adequate power for detecting the targeted changes in these other segments. However, the

variances for some lake segments were larger than the median values, which could reduce statistical power in those cases.

**Figure 3. Detecting change in total phosphorus concentration in Lake Champlain**

The results of the power analysis for lake total phosphorus are shown in Figure 3. The probability of detecting a 15% change in a multi-year mean phosphorus concentration is plotted vs. the number of years of sampling for several alternative within-year sampling frequency schedules. The “total number of years of monitoring” in Figure 3 refers to the number of years of sampling over two time periods (before and after) for which a phosphorus mean value is estimated. The number of years is assumed to be equal for each time period. For example, a value of 20 years of monitoring in Figure 3 indicates 10 years of baseline pre-monitoring followed by 10 years of post-monitoring. This analysis used a significance criterion of 0.05 for two-tailed t-tests.

Figure 3 shows how power increases with longer monitoring program duration and with increased sampling frequency within each sampling season. Sampling seasons are assumed to be six months (180 days) in length. The choice of a desired level of power to serve as a monitoring program design criterion is a somewhat arbitrary decision, but a relatively high power of about 80% is a commonly used criterion (Snedecor and Cochran, 1967; Green, 1989).

For total phosphorus, a sampling frequency of at least monthly would be required to detect a 15% change between two monitoring periods of 10 years each with an 80% power. A biweekly sampling frequency would detect such a change more quickly, with only four year monitoring periods. Sampling frequencies greater than biweekly give diminishing returns of power improvement. Based on this analysis, a biweekly sampling frequency (12 samples per year) was chosen for this monitoring program.

### 10.3 Selection of Tributary Monitoring Stations

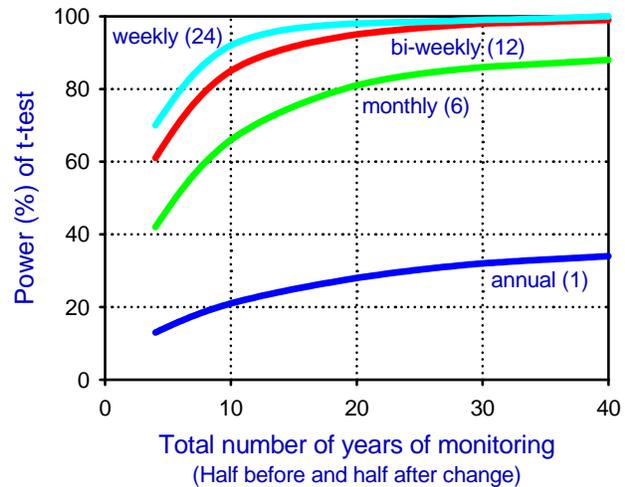
There are 21 Lake Champlain tributary rivers currently included in the monitoring program (Figure 2). The drainage areas of these rivers and the location of the sampling stations are given in Table 4.

The tributaries and sampling locations were determined based on the following considerations:

- Monitoring should include the largest tributaries (larger than 100 km<sup>2</sup> drainage area) or other sites (e.g., St. Albans Bay tributaries) where special management needs exist.
- Sampling locations should be as near to the river mouths as possible in order to capture loads from as much of the watershed as possible.
- All monitored rivers must have a continuous flow gage near the river mouth so that loads of phosphorus and other materials can be computed.

The 21 currently monitored rivers listed in Table 4 include all Lake Champlain tributaries larger than 100 km<sup>2</sup> in drainage area with the exception of the LaChute Creek (702 km<sup>2</sup>). All 21 monitored tributaries have flow

#### Power of detecting 15% change in total phosphorus



Statistical power for detecting a 15% change in total phosphorus concentration in Lake Champlain as a function of the number of years of monitoring and various within-year sampling frequencies.

gages operated by the U.S. Geological Survey or the Quebec Ministry of Sustainable Development, Environment, and Parks. The LaChute Creek (New York) does not have a flow gage station with publicly available data.

**Table 4. List of lake and tributary sampling station locations and total river drainage areas.**

<b>Tributary Station<sup>1</sup></b>	<b>Drainage Area at Mouth (km<sup>2</sup>)</b>	<b>Latitude N</b>	<b>Longitude W</b>
<b>Vermont/Quebec</b>			
Winooski (WINO01)	2,828	44° 31.52'	73° 15.41'
Otter (OTTE01)	2,462	44° 09.94'	73° 15.40'
Missisquoi (MISS01)	2,223	44° 55.23'	73° 07.63'
Lamoille (LAMO01)	1,909	44° 37.96'	73° 10.39'
Poultney (POUL01)	692	43° 34.24'	73° 23.53'
Pike (PIKE01)	517	45° 07.38'	73° 04.18'
Lewis (LEWI01)	209	44° 14.80'	73° 14.77'
Little Otter (LOTT01)	185	44° 12.24'	73° 15.11'
Rock River (ROCK02) <sup>2</sup>	152	44° 59.49'	73° 04.22'
LaPlatte (LAPL01)	137	44° 22.21'	73° 13.01'
Stevens (STEV01) <sup>3</sup>	39	44° 50.56'	73° 07.11'
Jewett (JEWE02) <sup>3</sup>	20	44° 51. 22'	73° 09.05'
<b>New York</b>			
Saranac (SARA01)	1,575	44° 41.52'	73° 27.19'
Ausable (AUSA01)	1,323	44° 33.63'	73° 26.95'
Mettawee (METT01)	1,098	43° 33.33'	73° 24.10'
Great Chazy (GCHA01)	769	44° 58.81'	73° 25.96'
Bouquet (BOUQ01)	712	44° 21.84'	73° 23.41'
Little Ausable (LAUS01)	189	44° 35.65'	73° 29.79'
Salmon (SALM01)	175	44° 38.40'	73° 29.70'
Putnam (PUTN01)	160	43° 57.35'	73° 25.99'
Little Chazy (LCHA01)	139	44° 54.12'	73° 24.88'
<sup>1</sup> Station codes used in the database are indicated in parentheses.			
<sup>2</sup> Added in 2007.			
<sup>3</sup> St. Albans Bay tributaries added in October 2008.			

## 10.4 Selection of Tributary Sampling Frequency

The primary purpose of the tributary sampling program is to assess the status and trends in loadings of total phosphorus and other materials to the lake using methods described in Vermont DEC and New York State DEC (1997) and Medalie and Smeltzer (2004). The tributary sampling frequency for the monitoring program was originally designed to include 10 samples per year, with sampling events targeted to high flow conditions in order to maximize the precision of annual mean loading estimates (Vermont DEC and New York State DEC, 1997).

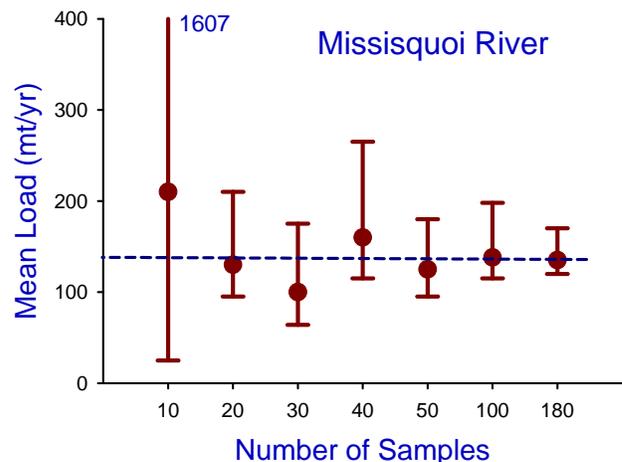
In 2000, a review of the monitoring program was conducted by the Lake Champlain Basin Program to ensure that the sampling effort was sufficient for the key purpose of estimating annual phosphorus loads from the tributaries. As part of this review, the tributary phosphorus data collected from 1990 to 1999 was statistically analyzed to empirically determine the relationship between the number of samples and the precision of the annual mean loading estimates.

All total phosphorus sample results during 1990-1999 for selected rivers were used with corresponding average daily flow data to calculate mean phosphorus loads for the period using load estimation procedures provided by the FLUX program (Walker, 1987, 1996). Then, individual phosphorus results were randomly eliminated from the data set and the mean loads were recalculated using progressively smaller sample sizes. The precision of the mean load estimates (expressed as 95% confidence intervals) were examined as a function of sample size.

The results of this analysis are shown in Figure 4 for the Missisquoi River. Similar findings were produced using data from other tributaries. When sample size increases from 10 to 20, there is a large improvement in the precision of the mean loading estimate. Samples sizes greater than 20 yield diminishing returns of improved precision. Furthermore, experience has shown that the actual number of high flow days available for sampling each year limits the annual sample size to around 20 high flow events. Based on this analysis, a target of 20 high flow total phosphorus samples per year from each tributary was established for the monitoring program design, beginning in 2001. In addition, 4 low flow total phosphorus samples per year will be obtained in order to define the concentration vs. flow relationship over the full range of flow conditions for each tributary.

This analysis was based on samples collected predominantly under high flow conditions, and achieving adequate precision of annual mean load estimates is dependent on continuing to sample primarily at high flow times. The target sampling frequency for parameters other than total phosphorus remained at 10 per year because precise loading estimates for these parameters were not considered essential on an annual basis, and data from multiple years could be combined to produce adequate precision for means loads over longer time intervals.

**Figure 4. Precision of mean total phosphorus loading estimates as a function of sample size for the Missisquoi River**



Note: Error bars are 95% confidence intervals for the mean loads. Dotted line indicates the mean load estimated using all available samples during 1990-1999 (N=180).

means loads over longer time intervals.

## 10.5 Selection of Zebra Mussel Monitoring Stations and Sampling Frequency

Zebra mussel monitoring will include veliger (larvae) and settled veliger (juvenile) life stages at openwater and nearshore stations. Specific monitoring objectives encompass the following:

- Determine the occurrence and density of zebra mussel veligers in selected openwater and nearshore areas of Lake Champlain.
- Determine the occurrence and density of full growing season settled juvenile mussels in selected nearshore areas of Lake Champlain.
- Determine the occurrence of zebra mussels in Lake Champlain tributaries and inland lakes within the basin.

Using a plankton net, occurrence and density of veligers will be determined at five northeast Lake Champlain openwater stations as shown in Table 5 and Figure 5. Veliger sampling at other openwater lake stations conducted during previous years was discontinued in 2006 because the infestation in these areas appeared to be fully developed. Openwater stations are co-located with stations of the LTMP. Co-location of these stations will allow for comparison of zebra mussel monitoring results with other water quality and biological data, and improved overall sampling efficiency. Occurrence and density of veligers will also be determined at four nearshore northeast lake stations located in shallow water areas near marinas or in bays.

Occurrence and density of season settled juveniles will be determined at 9 nearshore stations, as shown in Table 5 and Figure 5, on both the Vermont and New York sides of the lake by deploying polyvinyl chloride (PVC) settling plates which will be left in the lake for the entire sampling season.

Six Vermont tributaries (Figure 6), including the Missisquoi River, Lamoille River, Winooski River, Otter Creek, Castleton River and Poultney River will be sampled upstream above Lake Champlain influence. The Lake Champlain Native Mussel Working Group recommended these tributaries for sampling, as they support diverse native mussel populations including some rare species. In addition, samples collected from nine New York tributaries, including the Great Chazy River, Little Chazy River, Saranac River, Salmon River, Little Ausable River, Ausable River, Bouquet River, Putnam Creek and the Mettawee River by New York State Department of Environmental Conservation staff will be analyzed by the VTDEC. The Connecticut River will also be sampled.

Veliger sampling at public access areas or lake outlets will be performed in ten Vermont inland lakes (Figure 6) with high boating activity and close proximity to Lake Champlain. These lakes include: Lake Carmi, Fairfield Pond, Arrowhead Mountain Lake, Shelburne Pond, Lake Iroquois, Cedar Lake, Lake Dunmore, Lake Hortonia, Lake Bomoseen, and Lake St. Catherine. Eleven additional lakes in Vermont outside the Lake Champlain Basin with high boating activity will be sampled for veligers, including Lake Memphremagog, Lake Salem, Seymour Lake, Lake Willoughby, Island Pond, Crystal Lake, Caspian Lake, Joe's Pond, Harvey's Lake, Lake Morey, and Lake Fairlee. Shoreline surveys for the presence of adult zebra mussels will be conducted using diving mask and snorkel as time allows in any lake where veligers were found in plankton net tow samples.

**Table 5. Openwater and nearshore sampling site locations for zebra mussel and mysid monitoring in Lake Champlain**

Location	Description	Parameter	Latitude Longitude Coordinates
STA 25	Co-located with Lake Station 25, outer Malletts Bay, VT	Openwater Veligers	N 44° 34.92' W 73° 16.87'
STA 34	Co-located with Lake Station 34, "Inland Sea," VT	Openwater Veligers	N 44° 42.49' W 73° 13.61'
STA 40	Co-located with Lake Station 40, St. Albans Bay, VT	Openwater Veligers	N 44° 47.12' W 73° 09.73'
STA 50	Co-located with Lake Station 50, Missisquoi Bay, VT	Openwater Veligers	N 45° 00.80' W 73° 10.43'
STA 51	Co-located with Lake Station 51, Missisquoi Bay, Quebec	Openwater Veligers	N 45° 02.50' W 73° 07.78'
SH 05	Burlington Boathouse, VT @ dock	Season Settled Juveniles	N 44° 28.57' W 73° 13.39'
SH 06	Marble Island Club, Colchester, VT @ dock	Nearshore Veligers, Season Settled Juveniles	N 44° 34.24' W 73° 13.83'
SH 08	Tudhope Sailing Center, Grand Isle, VT in "the Gut" @ dock	Nearshore Veligers, Season Settled Juveniles	N 44° 45.98' W 73° 17.50'
SH 09	St. Albans Bay, VT, town pier	Nearshore Veligers, Season Settled Juveniles	N 44° 48.39' W 73° 08.45'
SH 10	Missisquoi Bay Bridge, VT in bay	Nearshore Veligers, Season Settled Juveniles	N 44° 57.85' W 73° 13.23'
SH 11	Lighthouse Point Marina, near Rouses Point, NY @ dock	Season Settled Juveniles	N 44° 49.95' W 73° 21.00'
BAHA	Basin Harbor, VT @ dock	Season Settled Juveniles	N 44° 11.48' W 73° 21.53'
CHIP	Chipman Point Marina, VT @ dock	Season Settled Juveniles	N 43° 48.01' W 72° 22.35'
WILL	Willsboro Bay Marina, Willsboro, NY @ dock	Season Settled Juveniles	N 44° 24.30' W 73° 23.30'
10	North of Thompson's Point	Mysids only	N 44° 18.25' W 73° 19.32'
STA19	Main Lake	Mysids only	N 44° 28.26' W 73° 17.95'
62	South of Diamond Island	Mysids only	N 44° 12.30' W 73° 22.00'

Figure 5. Open-water and nearshore sampling site locations for Lake Champlain zebra mussel and mysid sampling

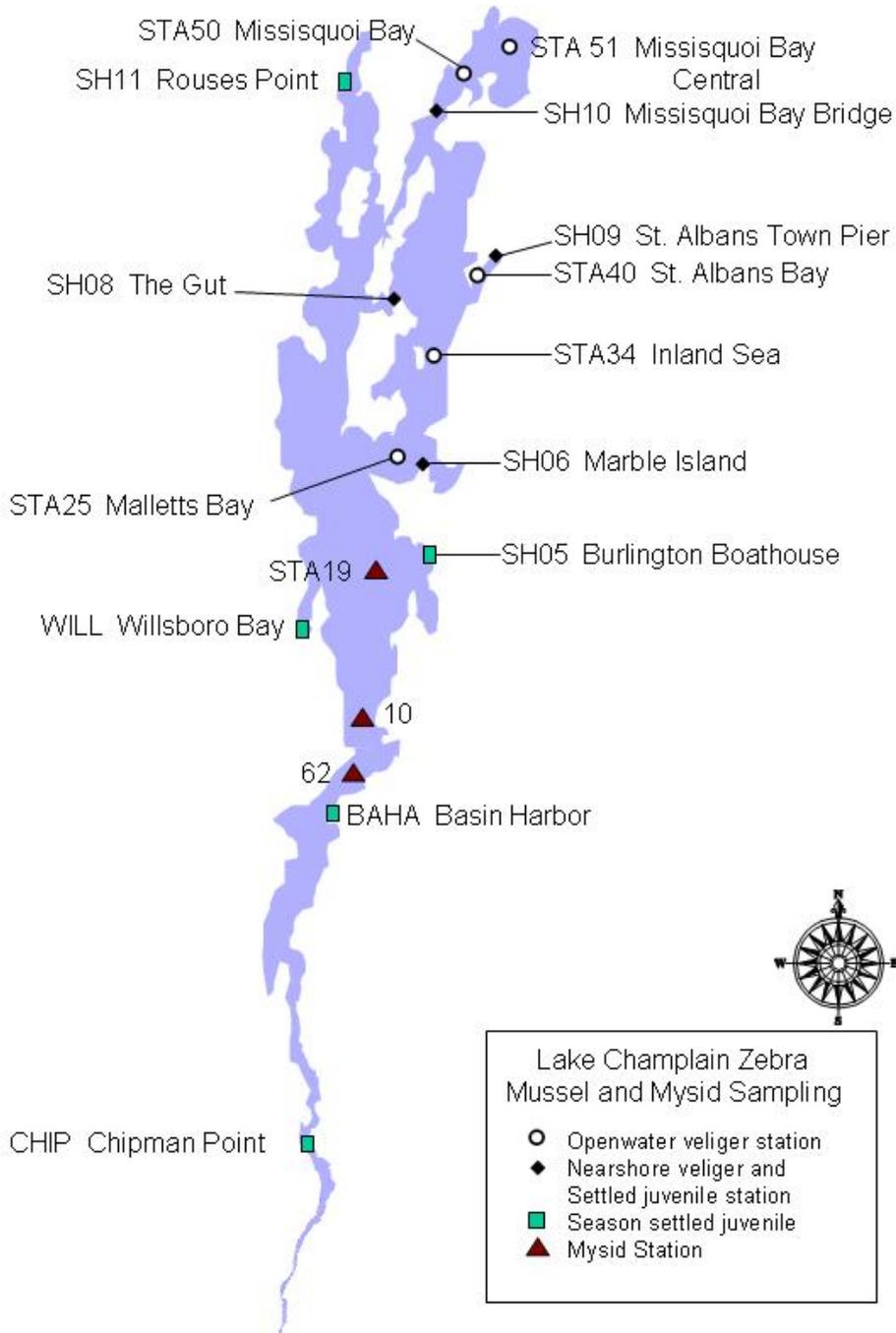
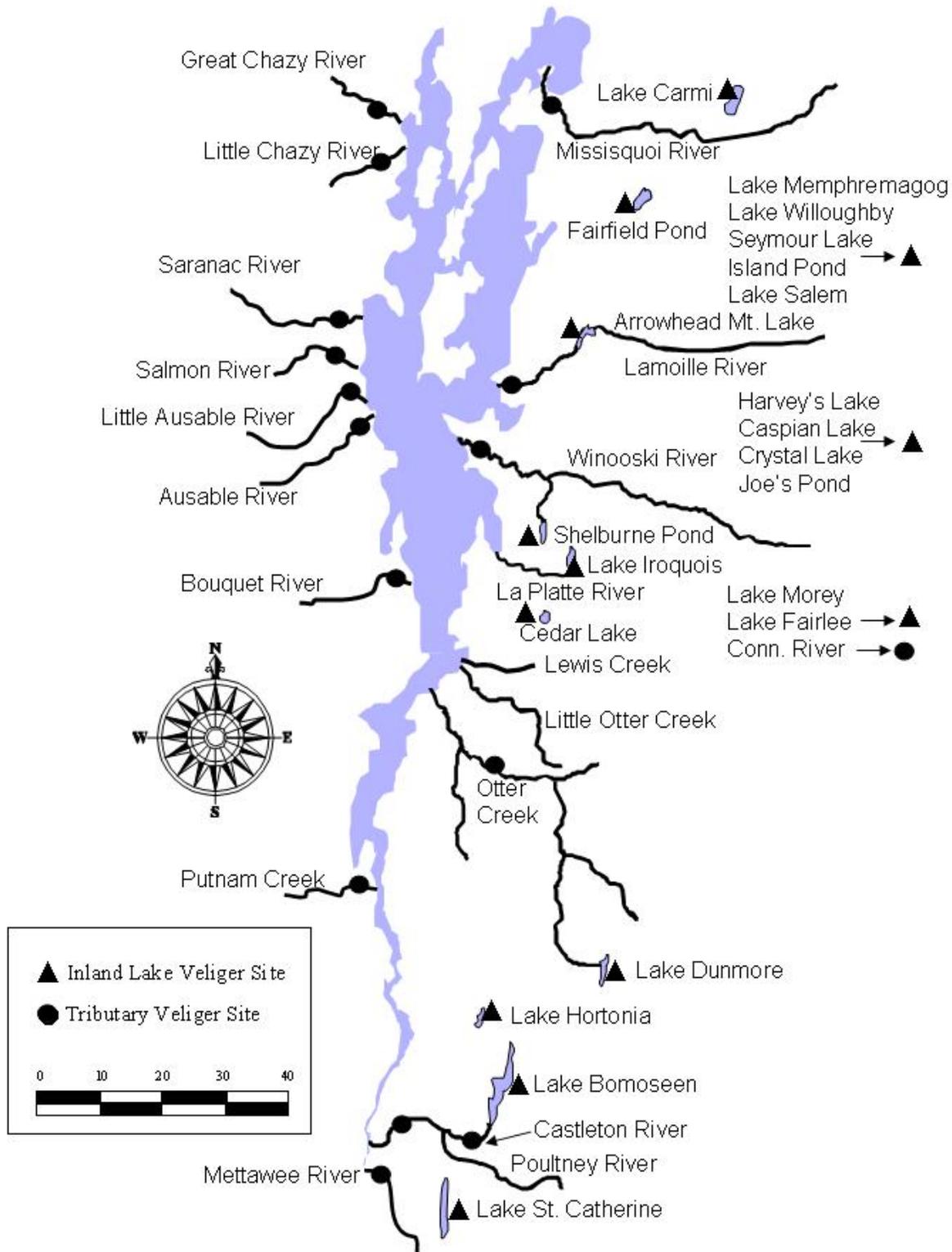


Figure 6. Inland lake and tributary sampling site locations for zebra mussels



Openwater and nearshore veliger monitoring will commence with deployment of season settling plates in late April. Veliger net tows will occur approximately every two weeks in conjunction with other sampling programs. Sampling for veligers will be discontinued in the fall when counts decrease to low values indicating that reproduction has ceased. Season settling plate retrieval will occur in October. Lake tributaries will be sampled during a period of two to three weeks in mid-summer. Inland lake veliger samples will be collected during the summer.

## **10.6 Selection of Mysid Stations and Sampling Frequency**

Mysids (*Mysis relicta*) are sampled at the very deepest parts of the lake (100 meters or deeper). Previous work indicated very few mysids were found above 100 meters during daytime hours (Siegfried, 2006). Sites deeper than 100 meters are limited to an area spanning from Station 19 in the Main Lake segment southward to the vicinity of Station 9 in the Otter Creek segment. Historical mysids sampling, therefore, was constrained to a relatively small area of the lake in a spatial context. The clustering of the original mysids sampling sites was to ensure an adequate baseline of information about the mysids population in the lake. With a baseline dataset now available, the sampling network has been reduced to 3 long term sites spatially separated to monitor for both trends and shifts in seasonal patterns (Figure 5, Table 5).

## **10.7 Champlain Canal Monitoring for Invasive Species**

The zooplankton spiny waterflea, *Bythotrephes longimanus/cedarstoemi* was confirmed as being present in Great Sacandaga Lake in the Hudson River system of NY in fall of 2008. This lake drains to the Hudson River upstream of the Glens Falls Feeder Canal which carries flow augmenting water from the Hudson River to the Champlain Canal. Given the connectivity of these drainages, there is potential for movement of the spiny waterflea into the Champlain system via the Feeder Canal and Champlain Canal. Zooplankton sample collection will be conducted in the Champlain Canal beginning in 2009 in each of the canal segments between Locks 8 & 9, 9 & 11, and 11 & 12. Samples will be collected on a monthly basis in accordance with methods described elsewhere in this document. Samples will be screened for the presence/absence of spiny waterflea, as well as, other zooplankton species not documented as being found in Lake Champlain. In the event an invasive is confirmed, a complete count of the sample will be conducted and species density will be determined. Lab analysis will be in accordance with methods described elsewhere in this document. Additional follow-up sampling will be conducted to determine extent of distribution.

## **11.0 Sampling Methods Requirements**

### **11.1 Lake collections**

#### *Chemical/Physical*

The 15 lake stations identified in Table 3 will be located using a global positioning system. Measurements and collections will be made at each station at the frequencies listed in Table 6. Sampling and field processing methodologies follow the Vermont Water Quality Division's Fields methods manual (VTDEC, 2006) ([www.anr.state.vt.us/dec/waterq/bass/docs/bs\\_fieldmethodsmanual.pdf](http://www.anr.state.vt.us/dec/waterq/bass/docs/bs_fieldmethodsmanual.pdf)) and the New York Division of Water's Lake Champlain LTM SOP NYSDEC SOP 401-07, 2007 hereafter referenced as NYDEC 2007.

Hydrolab® MS-5 multi-probe units will be utilized at the stations to record temperature, oxygen, pH, and conductivity at 1m increments. Dissolved oxygen will be measured using a luminescent optical probe. Also, the iodometric (Winkler) titration method will be used to measure water column dissolved oxygen at 5-11 discrete depths at deep lake stations 4, 7, 19, 25, 34, and 36 (VTDEC, 2006; NYDEC, 2007).

Visual transparency using a Secchi disk will be measured and recorded (VTDEC, 2006; NYDEC, 2007).

Alkalinity, total and dissolved phosphorus, total nitrogen, chloride, dissolved reactive silica, and metals (Ca, Mg, Na, K, Fe) will be analyzed from composited samples collected with a horizontal VanDorn bottle or Kemmerer bottle. During nonstratified conditions, a single composite sample will be collected representing three discrete depths in the water column: 2 meters below the lake surface, mid-depth, and approximately 2 meters above the lake bottom. During stratified conditions, two samples will be obtained, representing the epilimnion and hypolimnion, respectively. Within the epilimnion, 3 discrete samples will be collected and then composited: 2 meters below the lake surface, mid-epilimnion, and approximately 2 meters above the upper knee of the thermocline. Within the hypolimnion, 2 discrete samples will be collected and then composited: mid-hypolimnion, and approximately 2 meters above the lake bottom. The dissolved reactive silica and metals analyses are on 5 year rotations with the next series of analyses scheduled for 2010. Table 7 summarizes the field processing procedures for the water chemistry parameters.

Chlorophyll-a samples will be collected using a vertically-integrated hose-sampler beginning at the lake surface to a depth representing twice the Secchi depth (VTDEC, 2006). Samples for chlorophyll-a will be filtered in the field; 100 ml on 47mm diameter GF/A glass fiber filters wrapped in 90 mm No.3 glass fiber filters and placed in a dark container on ice for transport to the laboratory. At Station 19, an aliquot of sample will be saved in a 50 ml centrifuge tube, preserved with Lugols solution and retained in the phytoplankton archive. At the Vermont laboratory, samples will be frozen and stored until analyzed.

#### *Phytoplankton*

Vertically-integrated phytoplankton samples will be collected using a 63 µm mesh plankton net with a 13 cm opening, towed upwards at a rate of 0.5 m/sec from a depth of twice the Secchi disk depth (VTDEC, 2006). The net will be rinsed with lake water, the concentrate will be collected into a 50 ml centrifuge tube and preserved with Lugols solution. In addition to identifying each sample by date, location, and sample type (e.g. net phytoplankton), a line will be drawn on the tube indicating sample level. This will allow us to assess whether significant evaporation or leakage has occurred in storage. Samples will be stored at room temperature, in the dark, until analysis.

**Table 6. Lake monitoring parameters**

Parameter	No. of Stations	No. of Samples / Site / Visit	Sampling Frequency	No. of Samples / Year	Sample Parameter Analysis
<b>Physical/Chemical</b>					
Temperature (meter)	15 Baseline	Profile	bi-weekly(12)	--	Field measure
Dissolved Oxygen (electrode method)	15 Baseline	Profile	bi-weekly(12)	--	Field measure
Dissolved Oxygen (iodometric method)	Sites: 4, 7, 9, 19, 25, 34, 36	5 - 11	bi-weekly(12)	840	VTDEC lab
Secchi transparency	15 Baseline	1	bi-weekly(12)	--	Field measure
Specific conductance	15 Baseline	1 - 2 <sup>1</sup>	bi-weekly(12)	--	Field measure
pH	15 Baseline	1 - 2 <sup>1</sup>	bi-weekly(12)	--	Field measure
Alkalinity	15 Baseline	1 - 2 <sup>1</sup>	Seasonally(3)	45 - 90 <sup>1</sup>	VTDEC lab
Total phosphorus	15 Baseline	1 - 2 <sup>1</sup>	bi-weekly(12)	180 - 300 <sup>1</sup>	VTDEC lab
Dissolved phosphorus	15 Baseline	1 - 2 <sup>1</sup>	bi-weekly(12)	180 - 300 <sup>1</sup>	VTDEC lab
Total nitrogen	15 Baseline	1 - 2 <sup>1</sup>	bi-weekly(12)	180 - 300 <sup>1</sup>	VTDEC lab
Dissolved reactive silica <sup>2</sup>	15 Baseline	1 - 2 <sup>1</sup>	bi-weekly(12)	180 - 300 <sup>1</sup>	VTDEC lab
Chloride	15 Baseline	1 - 2 <sup>1</sup>	bi-weekly(12)	180 - 300 <sup>1</sup>	VTDEC lab
Metals (Ca, Mg, Na, K, Fe) <sup>2</sup>	15 Baseline	1 - 2 <sup>1</sup>	Seasonal(3)	45 - 90 <sup>1</sup>	VTDEC lab
<b>Biological</b>					
Chlorophyll	15 Baseline	1	bi-weekly (12)	180	VTDEC lab

Phytoplankton	15 Baseline	1	bi-weekly (12)	192 <sup>3</sup>	VTDEC
Zooplankton	15 Baseline	1	bi-weekly (12)	180	NYSDEC / SUNY
Mysids	Sites:10, 19, 62	6 (3 tows w/ paired bongo nets)	Monthly (6)	108	NYSDEC
Zebra mussel – Veligers	8	1	Biweekly (12)	96	VTDEC
Settled juveniles	11	1	Annual (1)	11	
Tributaries	14	2	Annual (1)	28	
Inland lakes	10	2	Annual (1)	20	

Note: <sup>1</sup>Number of samples will vary with duration of thermal stratification.

<sup>2</sup>Collection on 5 year cycle. Next field collection will be in 2010.

<sup>3</sup>Includes 1 wholewater sample collected at Sta 19 per sampling trip.

**Table 7. Summary of processing, preservation, and storage containers for water quality parameters**

Parameter	Processing	Preservation	Container	Holding Time
Total phosphorus	b	E	4	4
Total dissolved phosphorus	a	E	4	4
Total nitrogen	b	C	1	4
Chloride	a	E	1	4
Metals (Fe, Ca, Mg, Na, K)	b	B	3	5
Dissolved reactive silica (lake)	a	A	1	4
Alkalinity	b	A	2	3
Dissolved oxygen	c	D	5	1
Total suspended solids	b	A	6	2
Chlorophyll a	d	F	7	6

Processing:  
 a - filtrate (through 0.45µ cellulose nitrate filter)  
 b - whole sample  
 c - fix in field w/ 2 ml MnSO<sub>4</sub>, followed by 2 ml of iodide-azide  
 d - filter through glass fiber filter GF/A(1.6µm). Wrap in clean filter

Preservation:  
 A - no addition, sample kept cooled at 4°C  
 B - 0.5 ml concentrated HNO<sub>3</sub> / 250 ml of sample.  
 C - 0.1 ml concentrated H<sub>2</sub>SO<sub>4</sub> / 50 ml of sample, sample kept cooled at 4°C. Use Reagent Grade Sulfuric Acid with Low Level Nitrogen Total Nitrogen (N) <0.0005%  
 D - after fixing with D. O. reagents, sample kept cooled at 4°C, store in dark  
 E - no addition, sample kept at room temperature  
 F - freeze

Containers:  
 1 - 50 ml polyethylene centrifuge tube  
 2 - 250 ml polyethylene bottle  
 3 - 250 ml polyethylene bottle  
 4 - 60 ml glass vial  
 5 - 300 ml BOD bottle  
 6 - 1 liter polyethylene container (Tributaries)  
 7 - Wrap in clean filter, store in black jar

Holding times:  
 1 - 8 hours  
 2 - 7 days  
 3 - 14 days  
 4 - 28 days  
 5 - 6 months  
 6 - 21 days

### *Zooplankton*

Zooplankton samples will be collected by vertical net tows using a 30cm diameter, 153µm mesh net fitted with a 153µm screened cod end. (NYDEC, 2007). Tows will begin just above the sediments and hauled vertically to the water surface. The net will remain still for approx. 30 seconds just above the bottom before start of retrieval. Net retrieval rate will be 1 meter per second. Station, date, net size, and tow depth will be recorded on sample bottles and field sheets.

Nets will be gently rinsed from the outside with the onboard garden hose fitted with a spray head to wash organisms that may be stuck to the net down into the cod end. The cod end will be detached from the net and the screening and sides of cod end will be washed with a spray bottle, concentrating the samples into the bottom. The samples will be washed into 125 ml. bottles. The cod end will be washed into the sample bottle until bottle is filled ½ full (approx. 65 ml). If resulting sample volume does not allow for adequate preservative, further concentrating of the sample will be necessary.

The sample will be narcotized by adding 10 to 15 ml of cold club soda or ½ of an antacid tablet. Cold club soda may also be used when performing the final rinse from the cod end.

After about 5 minutes (or if using antacid tablet, after fizzing stops), buffered 10% formalin-sucrose-rose bengal solution will be added to bring volume up to the shoulder to create a final approx. 5% formalin solution concentration (approx 2.5% formaldehyde concentration). The samples will be placed into coolers with ice. Samples will be transported to the laboratory for further processing.

### *Mysids*

Mysids will be sampled by vertical net tows using paired bongo nets (0.5 m diameter, 253 µm mesh) at Stations 19, 10, and 62 (see Figure 5, Table 5), independent of the water chemistry and biological parameters, on a monthly basis (NYDEC, 2007). Triplicate vertical tows of the whole water column from just above the sediments to the surface will be performed. The net will remain still for approx. 30 seconds just above the bottom before start of retrieval. Net retrieval rate will be 1 meter per second. Station, date, net size, tow depth, and replicate will be recorded on sample bottles and field sheets.

Nets will be gently rinsed from the outside with the onboard garden hose fitted with a spray head to wash organisms that may be stuck to the net down into the cod end. The cod end will be detached from the net and the screening and sides of cod end will be washed with a spray bottle, concentrating the samples into the bottom. The samples will be washed into 125 ml. bottles. The cod end will be washed into the sample bottle until bottle is filled ½ full (approx. 65 ml). If resulting sample volume does not allow for adequate preservative, further concentrating of the sample will be necessary.

A buffered 10% formalin-sucrose solution will be added to bring volume up to the shoulder to create a final approx. 5% formalin solution concentration (approx 2.5% formaldehyde concentration). The samples will be placed into coolers with ice. Samples will be transported to the laboratory for further processing.

In the laboratory, samples will be washed and picked to separate the mysids from the other organisms. Mysids will be placed in glass scintillation vials with 95% ethyl alcohol, labeled by station, date, and replicate and stored in the dark at room temperature for further processing.

## *Zebra mussels*

Openwater zebra mussel veliger samples will be collected using vertical plankton net tows (VTDEC, 2006). A 13 cm aperture size Wisconsin style plankton net with a 63  $\mu\text{m}$  net mesh size will be towed vertically to the lake surface from a depth of 10 m, or 1 m from the lake bottom in areas where the bottom depth is less than 10 m, at a 0.5 m/sec retrieval rate for optimal veliger entrapment. Veliger samples will consist of five composited net tows of equal length. Length of net tow, surface temperature, and Secchi depth will be recorded for each sample. Once out of the water, the net contents will be concentrated and transferred to a 50 ml plastic sampling container and preserved with a 95% ethanol solution in a 1:1 ratio of sample to ethanol. Samples do not need refrigeration while stored at the laboratory for analysis. After sampling, the net will be rinsed vigorously three times in the lake.

Nearshore zebra mussel veliger samples will be collected using a horizontal plankton net tow (VTDEC, 2006) in the upper 1 meter of the water column. A 13 cm aperture size Wisconsin style plankton net with a 63  $\mu\text{m}$  mesh size will be towed horizontally at a 0.5 m/sec retrieval rate for optimal veliger entrapment. Net tow samples and field duplicates will be composites of five tows of equal length. Length of net tow, surface temperature, and Secchi disk depth will be recorded for each sample. Net cleaning protocol, sample preservation, and storage will be the same as that specified for open water sampling.

Occurrence and density of season settled zebra mussel juveniles will be determined using a 15 x 15 cm dark colored PVC settling plate. The plate will be arranged horizontally along a stainless steel threaded eyebolt. The plate will be suspended vertically in the water column by attaching a rope to the eyebolt to a marina dock, bridge abutment, or float. The plate will be submerged so that the plate is 2-3 m below the lake surface and can be adjusted during the summer as lake levels drop. The bottom of the eyebolt will be attached to a rope with a weight. The plate will remain in the water for the entire sampling season to estimate seasonal accumulation. The plates will be transported to the laboratory where they will be stored in a refrigerator at 4° C (40° F) and counted within 3 days.

Tributary zebra mussel veliger samples will be collected using a horizontal plankton net tow (VTDEC, 2006) in the upper one meter of the water column. A 13 cm aperture size Wisconsin style plankton net with a 63  $\mu\text{m}$  mesh net size will be towed horizontally at a 0.5 m/sec retrieval rate for optimal veliger entrapment. Net tow samples and field duplicates will be composites of five tows of equal length. Length of tow and surface water temperature will be recorded. The veliger tow will be taken in each tributary during the summer using a plankton net that was not used in Lake Champlain. Horizontal veliger tow samples will be preserved as described in the openwater veliger section. When traveling between sampling areas the plankton net will be stored in a 95% ethanol solution to kill any veligers that may be entrained in the net.

Inland lake zebra mussel veliger samples will be collected using the same method as described in the tributary sampling section. Horizontal plankton net tows will be taken at public access areas or lake outlets. Veliger tow samples will be preserved as described in the openwater veliger section. The veliger tow will be taken in each lake during the summer using a plankton net that was not used in Lake Champlain. When traveling between sampling areas, the plankton net will be stored in a 95% ethanol solution to kill any veligers that may be entrained in the net.

## **11.2 Tributary collections**

The stream sampling procedures used by the Lake Champlain Diagnostic-Feasibility Study and the LTMP have proven to be practical in the field and successful in supporting accurate loading estimates (Vermont DEC and

New York State DEC, 1997). These procedures will continue to be used for a long-term monitoring program on Lake Champlain tributaries.

Samples will be obtained at the downstream-most bridge crossings for each tributary at the same locations used for the previous studies. Depth and velocity integrated samples will be obtained using either the DH-48 or DH-59 suspended sediment samplers. Samples will be obtained under a full range of flow conditions each year, but with a strong emphasis on high flow conditions. Beginning in 2006, collections of four low flow events will also be conducted. The following measurements and collections will be made at each station (summarized in Table 8).

Temperature will be measured directly and recorded using a thermometer or electronic thermistor.

pH and conductivity will be measured directly and recorded using an ion selective electrode method.

Alkalinity, total and dissolved phosphorus, total nitrogen, total suspended solids, chloride, and metals (Ca, Mg, Na, K, Fe) will be analyzed from composite samples. Samplers will be lowered to the bottom of the water column and slowly raised so as to collect a composite bottom-to-top sample. This procedure will be performed at 3 points across the stream on wider rivers, or at the centroid of flow on narrower ones. The collected samples will be composited into a single sample for chemical analysis. The metals analyses are on a 5 year rotation with the next series of analyses scheduled for 2010.

When traveling between sampling areas the suspended sediment sampler and rope will be stored in a 95% ethanol solution to kill any aquatic invasive species that could be entrained in the rope or in the water left on the device. The sampler will be rinsed in ambient water after immersion in the ethanol prior to sample collection at each sampling site.

**Table 8. Tributary monitoring parameters**

Parameter	No. of Stations	No. of Samples / Site / Visit	Annual Sampling Frequency	No. of Samples / Year	Sample Parameter Analysis
Temperature (meter)	21	1	14 <sup>2</sup>	294	Field measure
pH	21	1	14 <sup>2</sup>	294	Field measure
Specific conductance	21	1	14 <sup>2</sup>	294	Field measure
Total phosphorus	21	1	24 <sup>2</sup>	294	VTDEC lab
Dissolved phosphorus	21	1	14 <sup>2</sup>	294	VTDEC lab
Total nitrogen	21	1	14 <sup>2</sup>	294	VTDEC lab
Total suspended solids	21	1	14 <sup>2</sup>	294	VTDEC lab
Chloride	21	1	14 <sup>2</sup>	294	VTDEC lab
Alkalinity	21	1	Seasonally(3)	63	VTDEC lab
Metals (Ca, Mg, Na, K, Fe) <sup>1</sup>	21	1	Seasonally(3)	63	VTDEC lab

Notes: <sup>1</sup>Metals collection on 5 year cycle. Next field collection will be in 2010.

<sup>2</sup>Tributary sampling increased to 20 high flow events, half of which are TP only, and 4 low flow, full suite of parameters beginning in 2006.

## **12.0 Sample Handling and Custody Requirements**

Samples are collected by field teams from Vermont and New York. Vermont samples remain in the team's custody until reaching the laboratory, where they are entered into the Laboratory's Information Management System (LIMS). Log-in is normally completed on the day of collection. The VTDEC Laboratory QAP (VTDEC 2009, <http://www.anr.state.vt.us/dec/lab/htm/QualityControl.htm>) provides additional detail on log-in and laboratory custody.

Water quality samples collected by the NY team remain in the team's custody, under proper storage conditions, until arrangements can be made for transfer by VT staff to the VTDEC Laboratory, typically within 2-5 days. NY samples are entered into the LIMS by VT staff when they reach the Laboratory. Copies of the field data collection sheets accompany the samples.

Each sample is assigned a unique accession number. Accession numbers are sequential, and identify the team that collected them (e.g., NY samples are 41xxx, VT are 42xxx). All water quality containers filled from the sample use the same accession number. Plankton are assigned a number corresponding to the epilimnion or unstratified layers. All containers carry labels identifying station, accession number and parameter. In addition, the LIMS generates new labels identifying each container for water quality analysis by a unique laboratory identifier as well as the project-specific information.

Table 7 documents sample container type and processing procedures for water quality samples. Table 11 documents this information for the biological samples.

## **13.0 Analytical Methods Requirements**

### **13.1 Water Sample Analytical Methods**

Table 9 summarizes the field and laboratory analytical methods that will be used for water quality samples collected as part of the project

**Table 9. Analytical procedures for parameters and field measurements**

<b>Parameter</b>	<b>Method [Reference]</b>
Phosphorus (all forms)	APHA 4500-P H[b]
Total nitrogen	APHA 4500-NC.[b]
Chloride	APHA 4500-Cl G[b]
Dissolved reactive silica	APHA 4500-Si O <sub>2</sub> F [b]
Metals (Fe, Ca, Mg, Na, K)	USEPA 3005A (Fe digestion) [a], 6020A [a]
Alkalinity	APHA 2320-B [b]
Total suspended solids	APHA 2540-D [b]
pH, <u>in situ</u> and laboratory	Hydrolab [c], YSI [d], VTDEC [f] NYDEC [g]
Dissolved oxygen, <u>in situ</u> and laboratory	Hydrolab [c] APHA 4500-OC [b] VTDEC [f] NYDEC [g]
Temperature, <u>in situ</u>	Hydrolab [c], YSI [d], VTDEC [f], NYDEC [g]
Specific conductance, <u>in situ</u>	Hydrolab [c], YSI [d], VTDEC [f], NYDEC [g]
Chlorophyll a	USEPA 445.0 [e], VTDEC [f]

[a] U. S. Environmental Protection Agency. Test methods for evaluating solid wastes. Office of Research and Development, Washington, D. C.

[b] American Public Health Association, American Water Works Association, and Water Pollution Control Foundation. 2005. Standard Methods for the Examination of Water and Wastewater. 21st Edition. American Public Health Association, Washington, D.C.

[c] Hydrolab Corporation. 1991 and Rev B 1997. Operations and Maintenance Manuals for Hydrolab Surveyor III and IV, Austin, TX.

[d] YSI, Inc. 1998 Operations Manual for YSI Model 63

[e] U.S. Environmental Protection Agency. Method 445.0 In Vitro Determination of Chlorophyll a and Pheophytin in Marine and Freshwater Algae by Fluorescence. Revision 1.2 Sept. 1997.

[f] Field Methods Manual. Vermont Department of Environmental Conservation, Water Quality Division. March 2006.

[g] Lake Champlain LTM SOP. New York DEC Division of Water. 2007.

### 13.2 Biological Analytical methods

Detailed procedures for the biological analyses are located in Appendix A. Short descriptions are presented here and summarized in Table 11.

#### *Zooplankton*

In the laboratory, counts will be made of all zooplankton (rotifers and crustaceans) in 1 ml subsamples. Subsamples will be drawn off using a 1ml Henson-Stempel pipette and counted in 1ml Sedgwick rafter cells under an inverted microscope at appropriate (40X to 100X) magnification. Additional 1 ml subsamples will be counted until at least 100 individuals of each dominant species are counted, or the entire sample has been examined. Identification will be made to lowest possible taxon. Zooplankton size will be measured and recorded. Up to 15 individuals of each taxon from each sample will be measured. For crustaceans, length will be measured from the tip of the head to the base of the tail spine (cladocerans) or caudal rami (copepods) (Johnson *et al.*, 2004). For rotifers, length will be measured from the corona to the opposite end at the base of the spine or to the opposite end and excluding any extensions (USEPA, 2003). Size distributions will be recorded as counts per 0.1mm size categories. Abundance estimates will be converted to biomass estimates using literature values. Samples will be scanned for rare or non-indigenous species such as *Bythotrephes longimanus* (spiny waterflea) and *Cercopagis pengoi* (fishhook waterflea). Analysis will be performed at the Lake Champlain Research Institute at SUNY Plattsburgh.

#### *Phytoplankton*

Phytoplankton net samples will be prepared and analyzed utilizing Sedgewick Rafter cells following APHA (2005), identifying taxa to the lowest feasible level and measuring ten representative individuals for use in biomass calculations using standard geometric formulae (Wetzel and Likens, 2000). Counting will continue until at least 10 fields or 100 of the most abundant phytoplankter have been evaluated, or up to three 1 mL aliquots have been examined. Counts will be made on an Olympus CKX41 inverted microscope. Whole water samples will be analyzed using Utermohl settling chambers. Counting, measuring and identification criteria are identical to those for net plankton. Counts will be typically completed using a single aliquot. Samples will be archived for five years.

Analysis of historical phytoplankton samples from four of the five priority stations previously identified by the Lake Champlain Technical Advisory Committee (4, 19, 34, and 36) was initiated in 2006 and is still underway. Analyses on available samples from station 50, the fifth priority station, were completed in 2005. Sample collection began in 1992 at these stations, but many vials are missing from earlier years during this period. The time series is most complete from 1996 through 2003, when wholewater phytoplankton sampling was discontinued, and analysis will focus on these years. Table 10 summarizes phytoplankton samples in archive by year at the priority stations.

**Table 10. Numbers of archived phytoplankton samples at priority stations remaining to be analyzed.**

	1996	1997	1998	1999	2000	2001	2002	2003	Total
Station 4	11	14	7	6	4 (2)	3 (3)	2 (4)	3 (2)	50 (11)
Station 19	11	12	6	8	2 (3)	3 (3)	2 (3)	2 (1)	46 (10)
Station 34	13	12	5	7	2(3)	2 (4)	1 (4)	3 (1)	45 (12)
Station 36	15	11	6	7	2 (3)	3 (3)	3 (2)	2 (1)	49 (9)
									190 (42)

Note: Numbers in parentheses indicate samples that have already been analyzed

Because these samples are old, sample condition is highly variable and will be evaluated for each individual tube. Sample volume varies considerably among centrifuge tubes and it is unclear whether evaporation or leakage has occurred. Samples will be analyzed utilizing settling chambers, identifying taxa to the lowest feasible level. Quantitative cell counts will be made for all samples in reasonable condition. A sample will be considered in reasonable condition if there are no large masses of fungal hyphae or large numbers of spores, liquid still retains some coloring from the preservative, and organisms are adequately preserved (e.g. cell walls are intact, cellular contents are visible, a reasonable variety of taxa and/or a reasonable density of cells are present). Questionable samples will be scanned for the species present, but no counts will be made. Because of uncertainties regarding the length of storage on cell size, no cell measurements will be made. The resulting data will provide estimates of species richness, and relative abundance. Determination of cell densities will be made only for those samples that show minimal reduction in volume (e.g., tubes with at least 47 mL of liquid).

#### *Mysids*

Mysid density and size distributions will be determined for each sample. Mysids will be measured using digital calipers under a binocular microscope. Total length will be determined by measuring from behind the eyes to the cleft in the telson and will be recorded to the nearest 1 mm. Individuals will be classified as juvenile, female, or male and recorded in appropriate 1 mm size classes. The brood pouches of ovigerous females will be examined and brood size recorded. Young will be assigned to one of four development classes: stage 1 (egg), stage 2 (comma), stage 3 (eyes developed), stage 4 (fully developed 1-2 mm). (Balcer *et al.*, 1984).

#### *Zebra mussel veligers*

Analytical procedures and calibration follow methods detailed in Marsden (1992). A dissecting stereomicroscope at 30X magnification will be used with a cross-polarization light technique to enhance veliger detection for counting purposes. Veliger identification will be verified using a compound microscope with assistance from taxonomists at the Biomonitoring and Aquatic Studies Section of the VTDEC. For samples containing relatively few veligers (100 per sample), all veligers will be counted. If veliger samples are too numerous to count in full (>100 per sample), the sample will be diluted quantitatively as necessary and three 1 ml subsamples will be extracted into a Sedgewick-Rafter cell and counted.

#### *Zebra mussel settled juveniles*

Settled juvenile densities will be determined using methods described by Marsden (1992). The 15 X 15 cm (225 cm<sup>2</sup>) settling plate will be placed under a dissecting stereo-microscope at 30X magnification and all juveniles that have settled on the undersides of the plate will be counted. Only one side of the plate will be examined, as mussel shells on the bottom would be crushed while under the microscope. If settled juvenile densities are too abundant to count accurately, five 1 cm<sup>2</sup> blocks will be counted using a 1 cm<sup>2</sup> counting cell randomly placed on the plate. On season settling plates with dense encrustations and uniform distribution of individuals, ¼ of the plate will be counted.

Comparisons of veliger and settled juvenile densities between lake stations and/or between years are based on seasonal time-weighted mean density estimates. Simpson's integral was used to calculate the area under the density vs. time plots for each year, and the areas were divided by the duration of the sampling season. Seasonal weighted mean estimates were based on equal sampling season lengths of 150 days starting and ending with zero density values at the beginning and end of the sampling seasons.

Seasonal weighted mean densities were considered more appropriate than geometric means, arithmetic means, or single peaks because of the extreme within-season variation in veliger and settled juvenile densities. Veliger production and juvenile settlement occur during discrete time periods, causing densities to increase from zero upwards over several orders of magnitude within a short time interval during a season at some stations. Mean values would therefore be too strongly biased by the number of samples obtained during non-reproductive periods. Seasonal time-weighted mean density values provide a better index of the overall larval and juvenile production at each site.

**Table 11. Parameter table for biological monitoring**

Parameter	Chlorophyll-a	Phytoplankton	Zooplankton	Mysids	Zebra mussel young
Number	180	192	180	108	140 Veligers 11 Settled Juv.
Pretreatment	100 ml sample retained on a 47mm GF/A filter (1.6µm)	None	Narcotize, preserve in 10% formalin solution	preserve in 10% formalin solution	none
Preservation	freeze	Acid Lugols, store in the dark until analysis	10% formalin	10% formalin	Veligers: 95% ethanol Settled juveniles: Refrigerate
Container	glass container, wrapped with aluminum foil	50 ml centrifuge tubes	125 ml polyethylene bottles	125 ml polyethylene bottles	Veligers: 50ml centrifuge tubes
Laboratory pretreatment	90% acetone	Subsample	Subsample concentrate, dilute	Separate, move to 90% ethyl alcohol	Subsample if high density
Type of sample	Filter residue, ground and extracted	Concentrated lake water	total / 1-5 ml aliquots	total sample	1-50ml veligers ¼ or whole plate for settled juveniles
Apparatus	fluorometer	Sedgewick Rafter cell, inverted microscope @ 200 – 400X	Sedgewick Rafter cell, inverted microscope @ 40-100X	gridded dish, binocular microscope @ >15X, digital caliper	Petri dish or sedgewick rafter cell 30x binocular scope with polarization
Data recorded	calculate chl-a concentration, based on fluorescence	species abundance, biovolume	Taxa, species abundance, size	Abundance, size frequency, sex ratio, brood size	Abundance Settled juveniles average size
Criteria for completion of analysis <sup>1, 2, 3</sup>	total chl-a in µg/l	> 100 of dominants counted or up to 3ml concentrate examined, scan for rare forms	> 100 of dominants counted, scan for rare forms in counted aliquots	Total Count	Total count

<sup>1</sup>Evaluation of sampling and analysis

- 1) counting error - mean of 2 replicate counts, S.E., analyst comparisons
- 2) site error - mean of replicate samples, 95% confidence error
- 3) taxonomic error - analyst comparisons, confirmations by external investigators, voucher specimens

4) pretreatment error - repeat examinations by other analysts

<sup>2</sup>Criteria of acceptance

- 1) S.E. < 10%, analyst comparisons within 2%
- 2) S.E. < 25%
- 3) Confirmed agreement on all determinations
- 4) No additional specimens found

<sup>3</sup>Response if unacceptable

- 1) Increase number of replicate counts, additional training for analyst(s)
- 2) Increase number of replicate samples, modify sampling apparatus
- 3) Additional training for analyst(s)
- 4) Increase time/repeats for pretreatment examination, additional analyst training

## **14.0 Quality Control Requirements**

### **14.1 Field Duplicates and Blanks**

Field QC samples represent approximately 10% of the water and biological collections made. Field duplicates are a second sample collected on-station and not a split of a sample. To generate a blank for water analytes, an aliquot of deionized water is run through the sampling equipment (ie: depth integrated sampler, horizontal VanDorn, churn splitter) after the equipment has been rinsed.

### **14.2 Laboratory Quality Assurance**

Table 12 summarizes the analytical quality assurance information for analytes measured as part of this project. Approximately 10 percent of all samples analyzed by the VTDEC Laboratory will be laboratory spikes or laboratory duplicates. Also refer to the Laboratory QAP(VTDEC, 2009).

**Table 12. Quality assurance information for analytes**

<b>Parameter</b>	<b>Reporting Limits (PQLs)<sup>a</sup></b>	<b>Units</b>	<b>Precision (RPD)<sup>b</sup></b>	<b>Accuracy (% Recovery)<sup>c</sup></b>
Reactive silica	0.2	mg/L as SiO <sub>2</sub>	5	85-115
Chloride	2.0	mg/L	5	85-110
Total nitrogen	0.1	mg/L	10	85-115
Total phosphorus	5.0	µg/L	15	85-115
Dissolved phosphorus	5.0	µg/L	15	85-115
Calcium	.05	mg/L	5	80-120
Magnesium	.01	mg/L	5	80-120
Potassium	0.05	mg/L	5	80-120
Sodium	0.05	mg/L	5	80-120
Iron	0.05	mg/L	7.5	80-120
Alkalinity	1.0	mg/L as CaCO <sub>3</sub>	5,15 <sup>d</sup>	N/A
Total suspended solids	1.0	mg/L	15	N/A

Footnotes:

<sup>a</sup>Practical Quantitation Limits (PQL) are 2 to 10 times the calculated MDL. PQL will increase when sample dilution is necessary.

<sup>b</sup>Relative Percent Difference (RPD) of laboratory duplicates. Average RPD's from historical data are approximately 1/2 to 1/5 these values and will vary due to sample matrix and concentration. RPDs will likely be higher for values at or near the PQL.

<sup>c</sup>Percent recovery of matrix spikes calculated as a percent of known addition recovered. Percent Recovery ranges are laboratory control limits.

<sup>d</sup>Conc. range 1 (<20 mg/CaCO<sub>3</sub>/L)=15  
2 (>20 mg/CaCO<sub>3</sub>/L)=5

### 14.3 Quality control checks for biological analyses

#### *Zebra mussels*

For openwater veligers and nearshore veligers, two field duplicate samples will be collected per sampling cycle so that roughly 10% of the samples are dedicated to QC. Also, one sample from each field duplicate pair will be reanalyzed by the project manager or designee as laboratory analytical duplicates and the RPD values will be reported. Duplicate season settling plate arrays will be placed at 1 station and used as a field duplicate. In the laboratory, 10% of all plate counts will be duplicated. Accuracy of veliger and settled juvenile identifications will be accomplished by comparison with reference samples and through consultation with taxonomists in the Biological and Aquatic Studies Section of the VTDEC. Data comparability will be achieved by using standardized methods as defined in the VTDEC Field Methods Manual (VTDEC, 2006) and in Marsden (1992). Calculation of data quality indicators is detailed in the Laboratory Quality Assurance Plan (VTDEC, 2009). Data quality objectives for this project are given in Tables 13 and 14.

**Table 13. Field data quality objectives for veliger, settled juvenile and adult density duplicate samples for zebra mussels**

Parameter	Units	Density	Precision (RPD)	Detection Limit
Veligers	N/m <sup>3</sup>	0-100	200%	0.66
		>100-1000	100%	0.66
		>1000	50%	0.66
Season Settled Juveniles	N/m <sup>2</sup>	0-100	200%	44
		>100	50%	44

**Table 14. Laboratory data quality objectives for veliger, season settled juvenile and adult density duplicate samples for zebra mussels**

Parameter	Units	Density	Precision (RPD)	Detection Limit
Veligers	N/m <sup>3</sup>	0-10	100%	0.66
		>100	50%	0.66
Season Settled Juveniles	N/m <sup>2</sup>	0-100	100%	44
		>100	50%	44

#### *Phytoplankton*

In the laboratory, 10% of the counts will be duplicated and RPD values reported (Table 15). Identifications will be made using available taxonomic references including but not limited to Prescott (1982) and Ettl and Gärtner (1988). Phytoplankton biologists with the Biological and Aquatic Studies Section of the Vermont DEC will also be consulted. A digital photographic archive will be initiated.

**Table 15. Data quality objectives for phytoplankton analyses**

Sample Type	Parameter	Units	Precision (RPD)
Field Collection	Total cell density	Cells/L	200%
Field Collection	Total biovolume	µg/L	200%
Laboratory Analysis	Total cell density	Cells/L	100%
Laboratory Analysis	Total biovolume	µg/L	100%

*Zooplankton and Mysids*

Reference collections, drawings, and photographs have been made of Lake Champlain zooplankton to assist in maintaining accuracy and consistency in taxonomic identification.

10% of the samples will be recounted by a second laboratory technician, or other project designee. Recounts will be conducted on the same subsamples as originally counted to remove bias except that associated with taxonomic identification and enumeration.

To verify taxonomic identification, the Percentage Similarity of Community Index (PS<sub>c</sub>) (Barbiero, 2003) will be used to compare identification on a single sub-sample by two different analysts. The formula is given as:

$$PS_c = 1 - 0.5 \sum_{i=1}^k |a - b|$$

Where *a* and *b* for a given species represent the relative percents of the total samples *A* and *B* respectively from a single sub-sample examined by two different analysts. The absolute value of their difference is summed over all *k* species. The sample is considered to pass if the PS<sub>c</sub> is 0.9 (90%) or greater.

To verify sample enumeration, the Relative % Difference (RPD) between two total counts on a single sub-sample conducted by two different analysts will be determined. The formula is given as:

$$RPD = \frac{[|\text{count\#1} - \text{count\#2}|]}{\text{average}(\text{count\#1}, \text{count\#2})} \times 100$$

Where count#1 and count#2 represent duplicate counts of total zooplankton on a single sub-sample conducted by two different analysts. The sample is considered to pass if the RPD is 5% or less.

The list of species between the original and recount samples will be compared to verify both taxa and counts are similar. In the event of gross differences or QC that is outside limits as described above, the sample will be re-examined to determine the cause of differences. Appropriate corrective actions will be taken.

**15.0 Instrument/Equipment Testing, Inspection, and Maintenance Requirements**

Tables 16 and 17 list the field equipment used by the LTMP. Field equipment will be inspected prior to use at each station for cleanliness and needed repairs or adjustments. Equipment will be rinsed with ambient water at each station prior to use. After use at each station all field equipment will be thoroughly washed, and then rinsed again prior to use at the next station. Water sampling equipment will be inspected for smooth operation, and adjusted, maintained, or repaired as necessary. Worn parts will be repaired or replaced. Small holes in nets will be sealed with clear fingernail polish, larger holes by patching with appropriate mesh material. Damaged equipment that cannot be satisfactorily repaired will be replaced. Field instruments will be maintained in working order and calibrated in accordance with manufacturer’s specifications. Field instrument log books will be maintained with each instrument indicating dates of calibration, maintenance, and notes regarding abnormalities or problems, and corrective actions. Chemical reagents will be checked for contamination and expiration date. Contaminated or outdated reagents will be replaced with fresh. Project field team members are responsible for the maintenance and calibration of field equipment and instruments, as well as, the logs associated with this.

**Table 16. Water quality sampling equipment**

<b>Water Sample Collection Gear</b>	<b>Multiprobe Unit</b>
Secchi disk with sounding line weight	Hydrolab™ MS 5
Van Dorn sampler with messenger	Calibration cup with cover
Kemmerer bottle with messenger	Hydrolab Surveyor 4 datalogger
Sample filtration apparatus	130-meter cable (NY) 100-meter cable (VT)
Hose for integrated sampling of chlorophyll-A	
Sample compositing container	

**Table 17. Biological sampling equipment**

<b>Phytoplankton</b> Phytoplankton net with depth-marked line Secchi disk with depth-marked line	<b>Zooplankton</b> 30 cm diameter 153 µm Puget Sound style nets w/ screened cod ends, depth-marked line.
<b>Mysids</b> 50 cm diameter 253 µm mesh paired bongo net assembly, Speed calibrated hydraulic winch	<b>Zebra Mussels</b> 13 cm diameter 63 µm mesh Wisconsin plankton net 15cmx15cm gray PVC plates

Laboratory equipment is maintained following the VTDEC Laboratory QAP (VTDEC, 2009).

**16.0 Instrument Calibration and Frequency**

Calibration of Secchi, Kemmerer/Van Dorn, and net lines are checked each spring with a calibrated ruler. New markings are made or old markings are verified and darkened.

The Hydrolab multiprobe is calibrated routinely by each team. Typically, the pH and conductivity probes are calibrated at the beginning of each week, and checked periodically. The depth sensor is calibrated at the start of sampling as well. The LDO dissolved oxygen sensor is calibrated weekly using HYDRAS3 LT software [http://www.hydrolab.com/products/ldo\\_sensor.asp](http://www.hydrolab.com/products/ldo_sensor.asp)

The Project teams keep a log of the calibration records for the field equipment. Calibration failures and drift are recorded in the log, so that the data from the affected parameters can be flagged or deleted in the database

accordingly. Summarized in Table 18 are the calibration schedules, procedures, standards and acceptance criteria for the field measurements.

All calibration standards used for calibrating the Hydrolab field instrument are Vendor certified. They are used directly from the vendor without dilution or further preparation. Between standards, deionized water is used to rinse sensors and calibration cup. Sensors and calibration cup are air dried and/or rinsed with the calibration standard prior to calibration with a standard. Standards are used for two consecutive calibrations before being discarded.

**Table 18. Calibration frequency, procedures, standards and acceptance criteria for major measurement systems**

Instrument	(parameter)	Frequency	Procedure	Standards	Acceptance Criteria
Hydrolab multiprobe	(Dissolved Oxygen, LDO method)	Weekly calibration using HYDRAS3 LT software	Barometric Pressure Calibration LDO instruction method 2	Barometer (uncorrected at elevation)	Comparison with Winkler data
	(Depth)	Daily	Calibration (1 point)	1 m depth marking on instrument line	
	(pH)	Weekly	Calibration (2 point)	Vendor certified 7 & 10 buffers	+/- 0.2 @ 25°C
	(Conductivity)	Weekly	Calibration (2 point)	Vendor certified 10 & 500 Nist-Traceable	Within 5% of certified value
	(Temperature)	Weekly	Check against laboratory thermometer		Within 0.5 degrees
Kemmerer/Van Dorn Lines Phytoplankton Lines Zooplankton Lines		1/yr	Calibrate 1m markings on line	Meter stick	New markings made and any incorrect ones removed.
Secchi Line		1/yr	Calibrate 0.5m markings on line	Meter stick	New markings made and any incorrect ones removed.
Hose		1/yr	Calibrate 1m markings on line. 10% sulfuric acid rinse 3 times followed w/ tap water flush	Meter stick	New markings made and any incorrect ones removed.

All instruments and equipment used within the VTDEC Laboratory are routinely calibrated by Laboratory personnel. Many small instruments and measurement devices are also annually calibrated by an external calibration service following ISO Guide 25 protocol. A summary of calibration procedures for individual instruments and tests is provided in Section 8 of the Lab QAP (VTDEC, 2009).

Stock Standards used for calibration are purchased from a reputable dealer or prepared at the Laboratory using reagent grade material. All purchased primary standards are certified by the vendor for purity and identity. Calibration Standards (working standards) are dilutions or mixtures of stock standards used to calibrate an instrument. These standards are prepared or re-standardized frequently. NIST traceable reference materials are used when available. A second source standard is routinely analyzed to verify the primary standard. To insure that instruments remain calibrated throughout analysis, it is Laboratory practice to run a Calibration Check Standard or a Quality Control Reference Sample immediately following calibration, after every 10-20 samples for extended runs and after the last sample analyzed (VTDEC, 2009).

## **17.0 Inspection and Acceptance Requirements for Supplies**

Sample containers are provided by the VTDEC Laboratory to both field teams and shall be certified clean by the Laboratory. Sampling containers will be stored and maintained in a manner ensuring their integrity prior to their use. All sample containers and associated supplies will be visually inspected for cleanliness and potential contamination prior to use. Suspect containers will be set aside and replaced with new, clean containers. Sampling containers will be kept closed until time of sample collection. Project field team members are responsible for coordinating with laboratories for procuring and maintaining sampling supplies.

## **18.0 Non-direct measurements**

### **18.1 Phosphorus loading from Wastewater Treatment Facilities**

Loads of phosphorus, as metric tons per year (mt/yr), will be reported annually for the 60 Vermont and 29 New York wastewater treatment facilities which have individual waste load allocations specified in the Lake Champlain Phosphorus TMDL. The data will be obtained from monthly discharge monitoring reports submitted by the wastewater facility operators to the Vermont DEC and the New York State DEC according to the monitoring specifications in their discharge permits.

Total phosphorus concentrations (mg/l) are reported monthly for most facilities based on an average of one or more samples taken from the effluent each month. Most samples are composites (e.g., 8-hour or 24-hour). Monthly average effluent flow rates, as million gallons per day (mgd), are also reported.

Monthly effluent flow and total phosphorus concentration measurements will be either transcribed manually or transferred electronically from the discharge monitoring reports into the project database. Data validation will occur by checking any values that are inconsistent with permit requirements or data from previous years and verifying that the data value in question is consistent with the original submission by the facility operators.

The annual average flow rate (mgd) from each facility will be calculated as the mean of the monthly average flow rates (mgd). The annual average effluent phosphorus concentration from each facility will be calculated as the mean of the monthly concentration values. The annual phosphorus load (mt/yr) discharged from each facility will be calculated as the product of the annual average flow rate (mgd), times the annual average phosphorus concentration (mg/l), times a units conversion factor of 1.381.

Vermont wastewater treatment facilities are required under the terms of their discharge permits to conduct total phosphorus and other laboratory analyses according to test procedures published in the Code of Federal Regulations (40 CFR Part 136). The Vermont Department of Environmental Conservation provides a Laboratory Manual and Quality Assurance Guidelines for Wastewater Treatment Facility Laboratories for use by facilities conducting their own analyses (Fish, 1995, 1996). Facilities using external laboratories send samples to laboratories certified by the National Environmental Laboratory Accreditation Conference ([www.epa.gov/nelac/](http://www.epa.gov/nelac/)).

New York wastewater treatment facilities are required to monitor and test wastewater samples in accordance with procedures approved in the Code of Federal Regulations (40 CFR Part 136). Additionally, the Environmental Laboratory Approval Program (ELAP) of the Wadsworth Center of the New York State Department of Health (NYSDOH) is responsible for the certification of laboratories performing analyses on environmental samples. All laboratories analyzing environmental samples must be certified. ELAP currently grants certification to commercial, facility self-monitoring and government operated environmental laboratories, in categories covering Public potable (drinking) water, Non-potable water, Solid/hazardous Waste and ambient Air and Emissions. To become certified a laboratory must be directed by an individual who is qualified through

education and experience, perform satisfactorily in at least semi-annual proficiency testing and a biennial on-site inspection. Certified laboratories are required to use state-approved analytical methods and adhere to a program of mandated quality assurance/quality control procedures. The NYSDOH provides a Laboratory Certification Manual detailing procedures and protocols for certification: <http://www.wadsworth.org/labcert/elapcert/certmanual/index.html>.

## **18.2 Invasive Species Documentation**

A variety of invasive species, including zebra mussels and alewife, are currently present in Lake Champlain. Numerous others are present in watersheds abutting Lake Champlain. The LCBP Aquatic Nuisance Species Subcommittee was created in 2005 to facilitate communication among partner agencies within the Basin with respect to identification and response to aquatic invasive species. The VTDEC and NYSDEC field personnel are members of this subcommittee and receive notification of potential and confirmed occurrences of new species. A tabulation of aquatic invasive species will be included in the LTMP annual report.

## **19.0 Data Management**

### **19.1 Field collection data**

Field collection data are noted on paper forms in the field. These forms are reviewed by each team. Originals from the NY field teams are maintained by the NY project manager at the NYDEC with copies accompanying samples sent for analysis to the VTDEC Laboratory in Vermont. Field documents are stored in a paper file in the Lakes and Ponds section of the Vermont Division of Water Quality. These data receive a final evaluation at the end of each year, before incorporation into the project's main database. Procedures for the year-end review of field data are located in Appendix B.

### **19.2 Water Chemistry Data**

Data management procedures for the VTDEC Laboratory are outlined in Section 10.0 of the Laboratory QAP (VTDEC, 2009). This section covers data reduction, validation, reporting and storage procedures for the laboratory. Results of WQ analyses are reviewed by laboratory staff and periodically by the VT field team. Discrepancies and possible quality issues are addressed, and transmitted to the NY team as necessary. At the end of the year, approved water quality data is downloaded electronically and reviewed by VT staff prior to inclusion in the project database. Procedures for the year-end review of laboratory data are located in Appendix B.

### **19.3 Biological Data**

Biological data acquisition is overseen by NY staff (mysids, and zooplankton) or VT staff (phytoplankton and zebra mussels). Each team is responsible for sample analysis, data compilation, necessary calculations, and final review. Upon completion of this process, summary data are added to the Project database. Original analytical data reside with the respective project team. Summary data will be added to the project's main database and will be accessible via the webpage. The complete databases will be available upon request.

#### *Phytoplankton*

Phytoplankton data are overseen by VT staff. Counts are recorded with a commercially available program "Counter" ([www.bioware.co.nz](http://www.bioware.co.nz)). Biomass measurements are recorded in an ACCESS file. Data are evaluated for accuracy and completeness by the analyst during the course of the analysis and receive a second review

prior discarding the sample. Data are backed up each day. Completed analytical data receive a final check for completeness and are added to main phytoplankton data table. Final data storage and calculations of cell density/L and biomass/L are accomplished using Microsoft Access™. Summary data for each site are added to the project's main database and are accessible via the webpage. The complete phytoplankton database is available upon request.

### *Zooplankton and Mysids*

Zooplankton and Mysid data are maintained by the NYDEC project manager. All raw counts are recorded by the examining laboratory technicians into Microsoft® Office Excel 2003 spreadsheet templates. Data are evaluated for completeness and accuracy. Formulas for conversion to density values are re-verified. All data are backed up on redundant hard-drives, as well as, written to compact disc. Final approved data will be migrated to Microsoft® Office Access 2003 to be merged with the Long Term Project dataset. Zooplankton datasets are available upon request.

### *Zebra mussels*

Zebra mussel data are overseen by VT staff. Counts are recorded on laboratory datasheets and entered into EXCEL spreadsheets. Data are evaluated by the analyst during the course of the analysis and data entry process. Final data storage and calculations are accomplished using EXCEL. Summary data will be added to the project's main database and will be accessible via the webpage. The complete data are available upon request.

## **19.4 Electronic Data storage**

The project master database and associated datasets are stored on VTDEC and NYSDEC computer servers. Daily and monthly tape back-ups are performed and the tapes are stored separately in fireproof cabinets in a locked room. See also Section 10.4 of the VTDEC Laboratory QAP for a complete description (VTDEC, 2009). Additional data back-ups are stored on redundant servers as well as written to compact disc. The data are available to other government agencies, researchers, consultants, students, and the general public on request in either electronic, paper copy form or on the web at [www.anr.state.vt.us/dec/waterq/lakes/html/lp\\_longterm.htm](http://www.anr.state.vt.us/dec/waterq/lakes/html/lp_longterm.htm)

## **20.0 Assessments and Response Actions**

The VTDEC Laboratory is accredited through The National Environmental Laboratory Accreditation Institute-TNI (formally NELAC). A TNI on-site audit is conducted every two years. A USEPA Region I Office of Environmental Measurement and Evaluation representative is a member of the TNI audit team. EPA Region I accepts TNI accreditation which is through the NH ELAP. Additional Laboratory Systems and Performance Audits are described in Section 12.0 of the Laboratory QAP (VTDEC, 2009). Corrective actions associated with the LTMP data are reported to project staff according to protocols outlined in Section 15 of the Laboratory QAP (VTDEC, 2009).

Significant sources of errors may include analytical and equipment problems, and deviations from intended plans and procedures. Refer to the quality assurance plan (VTDEC, 2009) for procedures addressing problems encountered within the analytical laboratory. Additionally, the entire project will be available for inspection and review at any time. The project managers will conduct reviews with the project QA officers and other team members, as necessary, to check for project deficiencies, irregularities, or other problems. Deficiencies, irregularities, or other problems observed shall be reported to the project staff responsible for the element in

question. Appropriate project personnel shall then develop and implement a corrective action to ensure the integrity of the project.

## **21.0 Reports**

Quarterly progress reports will be issued to the Lake Champlain Basin Program during the course of the study. Project managers and/or field team members will report on current status of on-going work, accomplishments, and problems encountered. Reports will be submitted through each state's Lake Champlain Coordinator. An annual report is the joint responsibility of the co-investigators. An annual report will consist of a summary of the history and purpose of the LTMP, description of the sampling network, summary of field sampling and analytical methods, parameter listings, and data tables. The purposes of this annual report will be achieved by maintaining an up-to-date Program Description document, graphical statistical presentations of the data, and an interactive database on the project website:

[http://www.anr.state.vt.us/dec/waterq/lakes/htm/lp\\_longterm.htm](http://www.anr.state.vt.us/dec/waterq/lakes/htm/lp_longterm.htm). In addition, the quarterly report produced in April each year will provide a summary of program accomplishments for the calendar year just ended, including the number of samples obtained and analyzed at each site by parameter.

The project website will provide the ability for data users to selectively view the original data for specific sampling stations, time periods, and analytical tests using simple, interactive query forms. The tabular data displayed on the website can then be readily transferred to standard spreadsheet programs for further analysis.

The tributary stations were sampled during 1990-1992 for total phosphorus, dissolved phosphorus, and chloride by the Lake Champlain Diagnostic-Feasibility Study (Vermont DEC and New York State DEC, 1997) using the same sampling and analytical methods employed by the current long-term monitoring program. These earlier tributary data have been added to the project database and will be included in the graphical statistical summaries well.

Graphical displays of the chemical data on the project website will be updated annually to include each year's data in a time series. These data are depicted as annual box plots for each test at each lake and tributary station over the entire monitoring period, showing median, 10<sup>th</sup>, 25<sup>th</sup>, 75<sup>th</sup> and 90<sup>th</sup> percentiles. Long-term medians will be graphically compared among the various stations, as well. The lake graphs will include only data from epilimnion and unstratified samples. When results are below analytical detection limits, the detection limit will be used (i.e., 'less than signs' will be ignored). When simultaneously obtained field duplicates exist, only the first member of a duplicate pair will be used.

Biological data will be summarized on the project website and will be updated annually thereafter. Graphical formats to best present abundance, composition and biomass of phytoplankton and zooplankton will be developed for the long term cumulative dataset and updated annually. Graphs will also be developed for the cumulative dataset for mysid abundance at each station and subsequently updated with each year's data. Presentations of veliger and settled juvenile zebra mussel densities for Lake Champlain, major tributaries and inland lakes will follow the formats used in previous annual reports of zebra mussel monitoring efforts, incorporating existing historical data. The biological data are integrated into the interactive web-based dataset. These data are available for viewing and querying similarly to the water chemistry data. Additionally, the data from the biological monitoring effort will be available in paper format, electronically in spreadsheet format, or on CD.

## **22.0 Data Review, Validation, and Verification Requirements**

Water chemistry analysis is being conducted by the Vermont DEC laboratory. Reference may be made to the quality assurance plan (VTDEC, 2009). Each data package generated is peer reviewed by a second staff analyst. The data are electronically imported into laboratory management system, and checked by a second analyst. The laboratory supervisor or designee reviews all parameters associated with a sample prior to authorizing the results.

The zooplankton analysis is being conducted by the Lake Champlain Research Institute (LCRI) at the State University of New York at Plattsburgh. The phytoplankton and zebra mussel analyses are being conducted at the Biological Aquatic Assessment Laboratory of the Vermont DEC by Vermont project personnel. Similarly to the Vermont DEC lab process, the biological data will be peer reviewed in house before final review and release.

## **23.0 Validation and Verification Methods**

Final data validation is the responsibility of the Project Manager before reporting. Results of field blanks and duplicates are tracked during the sampling season to identify potential field-related problems. In the event that poor duplication or blanking is evident, data for the corresponding parameter is evaluated to ensure its quality. Poor replication or blanking may be cause for rejecting an entire run of data, although this is not a necessity. Field data are also reviewed periodically during the season to ensure quality. At the close of the field season, data quality metrics are calculated and compared to data quality objectives.

In addition to the review conducted by the VT DEC Laboratory to verify laboratory data quality, data are examined for accuracy prior to inclusion in the master database. This includes a review of laboratory-flagged data and outlier evaluation as outlined in Appendix B1. The Project Manager works with the field staff and Laboratory QA officer to eliminate transcription error or to identify the source of the problem prior to any wholesale rejection of data.

## **24.0 Reconciliation with Data Quality Objectives**

Data quality will be assessed following protocols outlined in this document and erroneous values will not be incorporated into summary materials. A final report will be generated annually and summary materials will be posted to the project webpage. Summary materials will present the data in a variety of formats to facilitate evaluation of long-term changes, local conditions, and emerging concerns.

Data generated by the LTMP are an integral component of the Lake Champlain Basin Program's *Opportunities for Action*, the overall management plan for reducing and preventing pollution and restoring full ecological health in the Lake Champlain Basin. The data provide the baseline information necessary to evaluate water quality conditions in the Basin and assess effectiveness of management programs. An oral presentation will be made to the LCBP's Technical Advisory Committee (TAC), which reviews and comments upon the report. Questions and concerns regarding the data will be discussed by the TAC and the project managers prior to final acceptance. Modifications and new directions for the program structure will be addressed at that time, and again as the work plan is developed for the upcoming year.

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## **Appendix A. Laboratory Methods for Biological Samples**

### **A 1. Zebra mussel veligers**

Analytical procedures and calibration follow methods detailed in Marsden (1992). A dissecting stereo-microscope at 30X magnification will be used with a cross-polarization light technique to enhance veliger detection for counting purposes. Veliger identification will be verified using a compound microscope with assistance from taxonomists at the Biomonitoring and Aquatic Studies Section of the VTDEC. For samples containing relatively few veligers (<100 per sample), all veligers will be counted. If veliger samples are too numerous to count in full (>100 per sample), the sample will be diluted quantitatively as necessary and three 1 ml subsamples will be extracted into a Sedgewick-Rafter cell and counted.

### **A 2. Zebra mussel settled juveniles**

Settled juvenile densities will be determined using methods described by Marsden (1992). The 15 X 15 cm (225 cm<sup>2</sup>) settling plate will be placed under a dissecting stereo-microscope at 30X magnification and all juveniles that have settled on the undersides of the plate will be counted. Only one side of the plate will be examined, as mussel shells on the bottom would be crushed while under the microscope. If settled juvenile densities are too abundant to count accurately, five 1 cm<sup>2</sup> blocks will be counted using a 1 cm<sup>2</sup> counting cell randomly placed on the plate. On season settling plates with dense encrustations and uniform distribution of individuals, ¼ of the plate will be counted.

### **A 3. Setting up Phytoplankton Samples in Settling Chambers**

#### 1. Equipment

- Settling chamber unit: cylinder, baseplate, 2 glass plates
- Vaseline
- Small removable labels
- Pencil
- Graduated cylinder
- DI water

#### 2. Procedure

- Using Vaseline, attach cylinder and baseplate together. Fill partially with DI to verify seal is adequate. Chambers should be placed in a quiet, vibration-free location out of direct sun.
- Mix sample by inverting gently several times.
- Measure an appropriate sample aliquot in a graduated cylinder. (The volume will vary with the amount of particulate and algal material likely to be in the sample. In general, 25 – 30mls provide sufficient organisms for counting. Too much material creates a 3 dimensional matrix that cannot be adequately counted.)
- Pour sample into the settling chamber and rinse the graduated cylinder three times with a small amount of DI.
- Using a DI squirt bottle, fill the chamber to the top and cap with a glass plate. There should be no more than a few drops forced out the chamber when the glass is put in place. There should be no leakage at the bottom of the cylinder. If there is leakage, the chamber will need to be drained, rebuilt and re-filled.
- Using a removable label, note sample date, station and volume settled. Also note the date and time the chamber was filled. Place it on the base plate, on the side opposite the drainage hole.

- Allow plankton to settle for 96 hrs.

#### A 4. Retrieving and Counting Settled Phytoplankton Samples

##### 1. Equipment

- Catch basin (anything that will catch and retain the cylinder volume and allow the chamber to be securely supported while draining. Small steep-sided bowls work well.)
- Filled settling chambers
- Glass cover plates
- Small disposable pipettor
- Microscope
- Analysis sheet

##### 2. Procedure

- Transfer the column from the settling location to the catch basin, placing the drainage hole over the basin. Do this CAREFULLY to avoid setting up currents in the chamber that will re-distribute the settled material.
- Slowly slide the column over the drainage hole while holding it securely to the baseplate.
- While holding the base of the column steady and not disturbing the now-exposed sample in the baseplate, slowly slide the glass plate at the top of the column to one side, releasing the liquid contained in the column into the catch basin. Slide the empty cylinder free of the base plate.
- Look at the sample in the baseplate. To be able to place the glass cover plate with a minimum amount of disturbance, it should rise slightly above the level of the baseplate. If it does not, carefully add several drops of DI to the baseplate using the disposable pipettor.
- Place one edge of the glass cover plate on the baseplate. Lower the cover plate so that it descends at an angle rather than parallel to the liquid surface, reducing the likelihood of trapping air under the cover. Do this gently – if large bubbles or currents are formed during this process, the uniform distribution of the algae on the bottom of the chamber is destroyed and the sample must be discarded.
- Transfer the baseplate to the microscope. Using 200-400x, count the algae observed within the Whipple Grid boundaries using the following guidelines.
  - Cells touching the upper and right boundaries of the grid are counted as falling within the grid. Those touching the lower and left boundaries are not.
  - Count only those organisms that are within the grid. If a colony or filament lies partially outside of the grid, only record the number of cells that are within the grid.
  - Cell counts must be estimated for dense colonies. Evaluate the colonial structure and the number of cells visible at the surface to estimate the numbers in the colony (e.g. if 10 cells are visible at the surface and the colony appears to have 4 layers, estimate 40 cells in the colony being counted.)
  - Record both natural units and cell counts for each organism observed.
  - Count until at least 10 fields have been evaluated or 100 of the most abundant phytoplankter have been counted. If the most abundant phytoplankter is a colony or cell, count at least 25 natural units and at least 100 cells.
  - Identify each organism to the lower feasible taxonomic level.
  - After counting is completed, change to 200x and scan the chamber for taxa that were not observed during counting. Record these as “present” on the data sheet.
  - Randomly select 10 individuals from each taxon. Measure them at 400x using the ocular micrometer. Each taxon will have an assigned geometric figure and

measurements correspond to the axes needed for calculating the volume of the assigned figure.

- Clean the chambers, base plates and glass cover plates thoroughly with hot water and soap, followed by a final cleaning ethanol to remove any traces of Vaseline.
- Following APHA (2005),  $\text{cells/mL} = (C * A)/(F * AF * V)$

Where C = number of cells counted

A = area of the chamber,  $\text{mm}^2$

F = number of fields counted

AF = area of the field,  $\text{mm}^2$

V = volume settled, mL

## A 5. Preparation and Counting of Net Phytoplankton Samples Using a Sedgewick Rafter Cell

### 1. Equipment

- Clean Sedgewick Rafter cells and cover slips
- Microscope with ocular micrometer and a Whipple Grid
- Small disposable pipettes
- Analysis sheet
- Graduated cylinder

### 2. Procedure

- Place cover slip on the empty Sedgewick Rafter cell so that  $\frac{3}{4}$  of the cell is covered. If the cell is filled properly, surface tension will pull the cover slip over the remaining portion of the cell and form a bubble-free seal.
- Gently invert net plankton sample until well mixed.
- Using a clean disposable pipette, withdraw an aliquot of sample from the centrifuge tube.
- Dispense sample into the Sedgewick Rafter cell at the open end at a steady rate. Continue to add liquid until the cover slip swings into place, sealing the cell. The chamber must be bubble-free and the material evenly dispersed. Otherwise, density calculations will be inaccurate. Discard the aliquot and repeat the procedure if the cell has not filled properly.
- Using the graduated cylinder, measure the volume of the remaining concentrate. Add 1mL to this value (to account for the material used in the Sedgewick Rafter cell) and record on the data sheet.
- Transfer the Sedgewick Rafter cell to the microscope. Using 200x, count the algae observed within the Whipple Grid boundaries using the following guidelines.
  - Because samples were collected with a  $63\mu\text{m}$  mesh net, do not count small cells (< than  $50\mu\text{m}$  in length) because their densities are not accurately represented in the samples. Record them on the data sheet as “present”.
  - Cells touching the upper and right boundaries of the grid are counted as falling within the grid. Those touching the lower and left boundaries are not.
  - Count only those organisms that are within the grid. If a colony or filament lies partially outside of the grid, only record the number of cells that are within the grid.
  - Cell counts must be estimated for dense colonies. Evaluate the colonial structure and the number of cells visible at the surface to estimate the numbers in the colony (e.g. if 10 cells are visible at the surface and the colony appears to have 4 layers, estimate 40 cells in the colony being counted.)
  - Record both natural units and cell counts for each organism observed.
  - Count until at least 10 fields have been evaluated or 100 of the most abundant phytoplankter have been counted. If the most abundant phytoplankter is a colony, count at least 25 natural units and a minimum of 100 cells. If this cannot be achieved

with a single aliquot, rinse the chamber and count a second aliquot. Record the number of aliquots counted for each sample on the analysis sheet.

- Identify each organism to the lower feasible taxonomic level.
- After counting is completed, scan the chamber for taxa that were not observed during counting. Record these as “present” on the data sheet.
- Randomly select 10 individuals from each taxon. Measure them at 400x using the ocular micrometer. Each taxon will have an assigned geometric figure and measurements correspond to the axes needed for calculating the volume of the assigned figure.
- Clean the cells thoroughly before adding the next aliquot.
- Following APHA (2005), cell density/L = number in concentrate/net volume (liters)
  - Cell number =  $(C * V)/(A * D * F)$   
 where C = number of cells counted  
 V = volume of the SR cell =  $1000\text{mm}^3$   
 A = area of the field,  $\text{mm}^2$   
 D = depth of the field, 1 mm  
 F = number of fields counted
  - Number in concentrate = cell number/concentrate volume
  - Net volume ( $\text{meters}^3$ ) = tow length (m) \*  $\pi(0.065)^2$
  - Net volume (liters) = net volume ( $\text{m}^3$ ) \* 1000

#### A6. Zooplankton laboratory workup

- Laboratory glassware is cleaned according to standard laboratory protocols. Laboratory equipment is maintained, calibrated, and operated according to manufacturers' specifications.
- Gently invert sample bottle several times to mix the sample
- Using a 1ml Henson-Stempel pipette, withdraw a subsample from the sample bottle. Rinse the outside of the pipette. Dispense the subsample into a Sedgwick Rafter Counting cell.
- Samples will be examined under an inverted microscope at appropriate magnification (40X to 100X).
- Crustaceans and Rotifers will be identified to the lowest possible taxon. Additional 1ml subsamples will be similarly processed until at least 100 individuals of the dominant taxa are counted or the entire sample has been examined.
- Zooplankton size will be measured and recorded. Up to 15 individuals of each taxon from each sample will be measured. For crustaceans, length will be measured from the tip of the head to the base of the tail spine (cladocerans) or caudal rami (copepods) (Johnson *et al.*, 2004). For rotifers, length will be measured from the corona to the opposite end at the base of the spine or to the opposite end and excluding any extensions (USEPA, 2003). Size distributions will be recorded as counts per 0.1mm size categories. Abundance estimates will be converted to biomass estimates using literature values.
- Raw counts will be entered into Microsoft ® Office Excel 2003 spreadsheet templates. Density information will be calculated for each raw data entry using the formula:

$$[ ( TSV / SSV ) X RC ] / NVF$$

Where:

TSV = Total Sample Volume

SSV = # of sub-samples X sub-sample volume

RC = Raw Data Count

NVF = Net Volume Filtered given as area of net mouth opening in square meters X net tow depth in meters.

#### **A7. Mysids laboratory workup**

- Samples are received in the laboratory preserved in a 10% formalin solution. Samples will be washed on a 200 $\mu$ m sieve and mysids will be picked and placed in 90% ethyl alcohol in glass scintillation vials for archiving. Samples will be labeled with station, date, tow depth, replicate.
- Mysids will be examined under a binocular microscope and identified as male, female, or immature. A developed (or evidence of developing) 4<sup>th</sup> pleopod on the 4<sup>th</sup> abdominal segment will signify a male while the presence of a marsupial pouch (or evidence of developing pouch) ventrally located beneath the carapace and between the swimming legs will signify a female. Indistinguishable individuals will be labeled as immatures.
- Ovigerous females and brood sizes will be recorded. Brood stage will be noted as one of four;
  - Stage 1: spherical egg
  - Stage 2: elongated, but indistinguishable parts
  - Stage 3: elongated, but distinguishable parts (eyes visible)
  - Stage 4: fully developed and ready for release.
- Length measurements will be from just behind the eyes to the base of the telson using digital calipers.
- Laboratory workup will be recorded on bench sheets. Following review and verification, data will be recorded into Microsoft ® Office Excel 2003 spreadsheet templates.

## **Appendix B: Data review procedures**

### **B 1. Adding annual data to the Project database**

1. Begin this process after all data has been approved for release by the laboratory, usually not until mid-January.
2. Download data from SampleMaster using the Paradox format. Name this file “Prog63” and import into the Access 2008\_Chem. Ask the WQ Data Technician to run through the QC Check queries in the database, which include the following:
  - Check that the CustomerSampleNumber field is parsed correctly and has correct values for StationIDs, Time, Depth, QA code, Stratum, and FieldID. Check for duplicate FieldIDs.
  - Look over data that has been flagged by the chemists (e.g. overholds or other violations of QA/QC). Review the data, confer with the project manager and delete the codes or bad data as necessary.
  - Enter field data (temperature and Secchi for lakes; temperature, conductivity, pH for tributaries)
  - Run the query to check the minimum and maximum for each lake and parameter. Check for data that are anomalously high or low compared to previous years or unusual for the current year.
  - Export the data to EXCEL and use the PivotTable and Chart function to plot the current year’s data and compare it to past years and to itself.
  - Make a final review of blanks data.
  - Note any discrepancies or anomalies and discuss with the Project manager. Make appropriate changes to the temporary data tables before appending to the main tables.
3. Have the WQ Data Technician run the queries to store the lake and tributary data in the WQ Data database, where it will then be available on the WQ Division website.
4. A similar process is completed for the plankton data
  - Data are checked for accuracy and duplicate FieldIds. Plankton FieldIds are compared with water quality FieldIds. Minimum and maximum values are evaluated for accuracy.
  - Discrepancies are discussed with the Project Manager.
  - The appropriate queries are run to complete the summary statistical calculations, store the data and upload it to the WQ Division webpage.

### **B 2. Updating Web Figures to include new data**

1. Generate a query to collect all analyte data and sample date from the core lake stations
  - Stratum = “E” or “U”
  - QA = “A”
  - Year = current year
2. Export the query table to EXCEL.
3. Open a lake webpage figure file in SigmaPlot. Each parameter has its own file, with a separate section for the cumulative and annual data. The data page for each should have dummy data in column 1 as the new version of SigmaPlot has internal errors that periodically delete the data from this column.
  - In the cumulative figure data sheet, add the new year to the “year name” column for each station. Add a new column for the new data, name it. Copy the new data from the EXCEL file into the new column. Take care not to overwrite previous years’ data and check for the correct placement of new data. There must be empty rows between the data for the individual stations – add them if you need to.
  - In the annual figure data sheet, data are organized by station without regard to year. Copy the new data from EXCEL and paste into the appropriate station column. Take care not to override previous years’ data and to put data in the correct column.

- To update the figures with the new data, double-click the graph. For the cumulative figure, double-click the “graph wizard” tab and verify that all stations are selected. For the annual figure, this requires you to edit the row list under “data sampling” in the plots to include the number of rows encompassing the data for the graph. Usually, this is the current row plus 1. If you had to add rows to keep data from overlapping, the difference may be much more.
  - Verify that the figures accurately represent the new data. Figures should be compared to the previous year and checked for discrepancies. Update the title of the figures to include the new year. Update the name of the file to include the new year. Print the figures to facilitate checking.
4. Repeat these steps for the tributary figures. Use QA=”A” and the current year to generate the EXCEL file with a query. At this time, webpage tributary figures represent only cumulative data.







**Lake Champlain Mysids Bench Tally Sheet**

Station	Date	Depth	Tally By	Sheet	of
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<b>Mysid Size Distribution</b>																				
Replicate	Body Length in Millimeters as measured from behind the eyes to base of telson																			
Male	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A																				
B																				
C																				
D																				
E																				
F																				
Female	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A																				
B																				
C																				
D																				
E																				
F																				
Juvenile	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A																				
B																				
C																				
D																				
E																				
F																				

<b>Female Brood Data</b>																				
Replicate (A-F)																				
Female Size																				
Brood Stage																				
Brood Size																				

Notes:

Brood Stage	
1	Egg
2	Comma
3	Eyes Developed
4	Fully Developed

Other Observations: