North Temperate Lakes
Long Term Ecological Research Program

Core Data Collection
Quality Assurance Project Plan
VERSION 2023.1 - DRAFT

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A. Program Background

A1. Scientific Goals

North Temperate Lakes Long Term Ecological Research (NTL LTER) studies the ecology of lakes as one of a network of sites established by the U.S. National Science Foundation. We are interested in how biophysical setting, climate, and changing land use and cover interact to shape lake characteristics and dynamics over time (past, present, future). Our two field stations facilitate research in the lake districts - the Hasler Laboratory of Limnology on Lake Mendota in the Yahara Lake District of southern Wisconsin and the Trout Lake Station in the Northern Highlands of Wisconsin. Most of our data are public and available through the website (lter.limnology.wisc.edu).

For the current funding cycle, our vision is to gain a predictive understanding of the ecology of lakes at longer and broader scales than has been traditional in limnology. Our conceptual framework uses a nested set of spatial scales, from individual lakes and their watersheds, to hydrologically linked sets of lakes, entire lake districts, multiple lake districts within the Great Lakes region, and comparative studies of lakes and lake districts around the globe. Our research program is interdisciplinary and aims to understand the ecology of lakes in relation to relevant atmospheric, geochemical, landscape and human processes. Within the NTL domain, we have observed shifts in driver and response variables that are both gradual and abrupt on time scales of years to decades.

Examples of rapid changes in lake responses that have been particularly conspicuous to both researchers and many Wisconsin residents include the collapse of valuable Walleye fisheries; increasing fluctuations in lake levels; arrival of new aquatic invaders; and sudden declines in lake water clarity, among others. At the same time, we are also seeing changes in drivers that can cause abrupt change, but abrupt changes have not followed, leading us to ask why. Our research activities are inspired by these observations and are organized around the overarching question: What are the causes and consequences of abrupt ecological change in lakes and their surrounding landscapes? We address the following questions about abrupt ecological change:

- **Climate variability and lake phenology:** What are lake phenological responses to a warmer and more variable climate that may lead to abrupt ecological change?
- **Interacting drivers and abrupt change in urban aquatic ecosystems:** How do interactions of land use/land cover and climate change affect urban aquatic ecosystems?
- **Interacting drivers and water quality:** How do external drivers interact with aquatic invasive species to regulate water quality?
- **Managing for abrupt change in whole-lake experiments:** What causes intentional ecosystem manipulations to persist, revert, or lead to novel states?

A2. Lakes and the Landscape

Our primary study sites are a set of seven northern and four southern Wisconsin lakes and their surrounding landscapes. The northern lakes are in the lake-rich region of the Northern Highland Lake District (Vilas County), with watersheds mainly comprised of secondary forest and peatland. Some of the lakeshores are lined with cottages, and there is active logging in the region, but otherwise human development of the land is quite low compared to other regions of Wisconsin. The seven northern lakes have low productivity (oligotrophic, dystrophic; Table 1). The southern lakes are in an agriculturally dominated and urban area. Given the large-scale, intensive modification of the landscape in this region by humans, the four southern lakes have a much higher productivity (eutrophic; Table 2). The NTL LTER program also performs quarterly sampling of Lake Waubesa and Kegonsa, two of the lower lakes in the Yahara Chain of Lakes.

Table 1. *Characteristics of northern study lakes*

Characteristic	Crystal Bog (27-2)	Trout Bog (12-15)	Crystal Lake	Big Musk-ellunge Lake	Sparkling Lake	Allequash Lake	Trout Lake
Abbreviation	**CB**	**TB**	**CR**	**BM**	**SP**	**AL**	**TL**
Latitude	46.008	46.041	46.003	46.021	46.008	46.038	46.029
Longitude	-89.606	-89.686	-89.612	-89.612	-89.701	-89.621	-89.665
Area (ha)	0.6	1.0	37.5	363.4	63.7	164.2	1565.1
Mean Depth (m)	1.7	5.6	10.4	7.5	10.9	2.9	14.6
Max Depth (m)	2.5	7.9	20.4	21.3	20	8	35.7
Landscape Position	High	High	High	Intermediate	Intermediate	Low	Low
Ice cover (days)	152	154	138	141	136	147	135
pH*	5.2	4.8	6.1	7.3	7.4	7.6	7.6
ANC (µeq/L)*	14	11	23	390	631	831	846
Conductivity (µS/cm)*	11	23	13	50	86	92	96
Total P (µg/L)*	18.2	46.6	8.3	17.5	15.0	29.3	13.5
Total N (µg/L)*	722	961	210	441	371	379	247
SiO_2 (µg/L)*	834	1927	48	309	7217	13853	9529
Secchi Depth (m)*	1.5	1.1	7.5	6.6	6.2	3.2	4.7
Chlorophyll (µg/L)*	9.9	16.2	1.8	2.9	2.2	7.8	3.1

** Mean values from spring and fall mixis (pH, ANC, conductivity), spring mixis only (SiO2, TN, TP), or summer stratified period (chlorophyll, Secchi depth).*

Table 2. *Characteristics of southern study lakes.*

Characteristic	Fish Lake	Lake Mendota	Lake Wingra	Lake Monona
Abbreviation	**FI**	**ME**	**WI**	**MO**
Latitude	43.287	43.099	43.053	43.063
Longitude	-89.652	-89.405	-89.425	-89.361
Area (ha)	80.4	3961.2	136.2	1359.8
Mean Depth (m)	6.6	12.8	2.7	8.2
Max Depth (m)	18.9	25.3	6.7	22.5
Shoreline (km)	4.3	33.8	5.9	20.9
Landscape position	high	low	high	low
Ice cover (days)	unknown	119	120	107
pH*	8.1	8.4	8.5	8.5
ANC (µeq/L)*	2921	3665	3745	3547
Conductivity (µS/cm)*	280	412	500	434
Total P (µg/L)*	22.4	109.5	40.3	73.5
Total N (µg/L)*	835	860	933	845
SiO_2 (µg/L)*	367	711	5551	454
Secchi Depth (m)*	2.4	3.0	0.7	2.4
Chlorophyll (µg/L)*	5.1	4.8	10.5	8.1

** Mean values from spring and fall mixis (pH, ANC, conductivity), spring mixis only (SiO2, TN, TP), or summer stratified period (chlorophyll, Secchi depth).*

A3. Program History

Funding Cycle Explanation: NTL was one of the first six LTER sites funded by the U.S. National Science Foundation in 1980, with core data collection beginning in 1981 for the northern lakes. In 1995, the southern lakes were added as sites for core data collection as a part of regionalization supplement. The funding cycle for LTER sites is six years with a midterm review in Year 3 of the grant and renewal submission in Year 5. The most recent renewal was granted in 2020. Therefore, the midterm review will be held in summer 2023 and the renewal submission will be prepared in 2025. The US LTER program also undergoes a decadal review evaluating the network. The most recent decadal review was performed in 2022.

History of Limnological Research and NTL Personnel: The University of Wisconsin-Madison is generally considered the birthplace of limnology as a scientific discipline in North America, mainly due to the efforts of Edward Birge (UW-Madison faculty and university chancellor) and Chauncey Juday. A detailed history of their contributions to Wisconsin limnology and the founding of the Center for Limnology can be found at https://limnology.wisc.edu/about-cfl/history-of-limnology/.

John Magnuson was the first lead principal investigator (PI) of the NTL LTER, from 1980 – 1996. Beginning in 1996, Steve Carpenter took over as lead PI, coinciding with the inclusion of the southern lakes in the core data collection program. In 2008, Emily Stanley became the lead PI of the NTL and Carpenter moved into the Center for Limnology directorship. Stanley currently serves as the lead PI for the NTL program with 19 co-investigators, dozens of graduate and undergraduate students, and numerous administrative and research staff.

Related Research through the Years: In addition to continuing core data collection on the eleven study lakes (seven northern, four southern), in each funding cycle we pursue question-driven research based on the long-term knowledge and anticipated future changes at our site. We use a combination of long-term monitoring (the core data set), whole-lake experiments (e.g., Crystal Lake mixing, Sparkling Lake crayfish removal), theory and models, and comparative surveys to address our hypotheses. See summary of current cycle goals in section A1.

Another major research focus area is the Microbial Observatory (often referred to simply as "MO"), led by co-investigator Trina McMahon. The MO research is aimed at understanding which factors contribute to structuring microbial communities and populations, at regional and local spatial scales. They maintain a time series of microbial samples from a subset of the NTL lakes, many of which have been analyzed by shotgun metagenomics.

The NTL LTER has a long history of partnership with state and federal natural resource agencies. Some of the co-investigators for the NTL LTER program are affiliated with Wisconsin Department of Natural Resources (DNR) and the USGS Middleton office. Partnerships with the Wisconsin DNR range from collaborative sampling efforts for water quality parameters, macrophytes, and fish in the southern lakes region, to management and ecosystem experiments in both the northern and southern lakes regions.

Finally, there have been a number of long-term projects in the northern lakes region led by NTL LTER investigators, though not formally a part of the NTL program. This includes the Little Rock Lake Acidification project that was led by Trout Lake Station scientists (Tim Kratz, others) in partnership with the US Environmental Protection Agency. Today, Little Rock Lake is a site for the National Ecological Observatory Network (NEON). UW-Madison researchers have been working on Peter and Paul Lakes since the 1950s when Art Hasler divided the lake into two basins. The lakes are located at the University of Notre Dame's Environmental Research Center. Beginning in the 1980s, a series of whole-lake food web manipulations occurred to induce a trophic cascade. Since that time, a number of lake experiments have been performed as a part of the "Cascade" project (Carpenter lead PI for UW-Madison, now Wilkinson).

Regional lakes survey: The Northern Highlands Lake District (NHLD) is one of the few regions in the world with periodic comprehensive water chemistry data from hundreds of lakes spanning almost a century. Birge and Juday directed the first comprehensive assessment of water chemistry in the NHLD, sampling more than 600 lakes in the 1920s and 30s. These surveys have been repeated by various agencies and we now have data from the 1920s (UW), 1960s (WDNR), 1970s (EPA), 1980s (EPA), 1990s (EPA), and 2000s (NTL). The 28 lakes sampled as part of the Regional Lake Survey have been sampled by at least four of these regional surveys. These 28 lakes were selected to represent a gradient of landscape position and shoreline development, both of which are important factors influencing social and ecological dynamics of lakes in the NHLD. This long-term regional dataset will lead to a greater understanding of how large-scale drivers such as climate change and variability, lakeshore residential development, introductions of invasive species, or forest management have altered regional water chemistry.

B. Program Management

B1. Personnel

The NTL LTER program has ~18 investigators (faculty, research scientists), 10 full or part time staff, 12-18 graduate students, and several undergraduate and limited term employees. The core staff and investigators are listed in Table 3.

Table 3. NTL LTER Project staff descriptions and personnel (location HL = Hasler Lab, TLS = Trout Lake Station)

NTL Role	Staff (location)	Core Program Responsibilities
Lead Investigator (PI)	Emily Stanley (HL)	• Leads project management including budget, staffing • Coordinates scientific efforts among participants • Leads monthly science and PI meetings • Leads proposal and annual report drafting
Northern Lakes, Investigator	Noah Lottig (TLS)	• Project management support • Coordinates and performs fish sampling annually • Supervises Regional Lakes survey
Northern Lakes Field & Biology Manager	Carol Warden (TLS)	• Coordinates and performs field sampling • Analyzes zooplankton and macroinvertebrate samples • Assists with fish sampling • Assists with other on-site sample analysis
Northern Lakes Field & Buoy Manager	Paul Schramm (TLS)	• Coordinates and performs field sampling • Maintains northern lakes buoys • Performs other on-site sample analysis • Assists with fish sampling
Outreach Specialist	Amber Mrnak (TLS)	• Coordinates school yard program and other outreach at northern site • Project management support
Chemistry Supervisor, Investigator	Grace Wilkinson (HL)	• Supervises chemistry manager • Assists Lead PI with core program management • Led development of quality assurance project plan
Southern Lakes Field & Biology Manager	Alice Ogden-Nussbaum (HL)	• Coordinates and performs field sampling • Coordinates and performs zooplankton and chlorophyll sample analysis
Chemistry Manager	Jimmy Sustachek (HL)	• Coordinates and performs chemical analyses • Assists with other project tasks, as needed • Leads quality assurance project plan review annually • Coordinates data publishing and archiving
Information Manager (IM)	Corinna Gries (HL)	• Contributes to network-wide IM community • Assists with database maintenance • Assists with data archiving and publishing

Buoy and Database Manager	Mark Gahler (HL)	• Southern lakes buoy maintenance • Leads database maintenance, ChemLab maintenance • Leads data archiving and publishing
Outreach and Communications Specialist	Adam Hinterthuer (HL)	• Coordinates and performs outreach in southern region • Manages public communications including press releases, blog, and social media
Administrative Assistant	Kelly O'Ferrell (HL)	• Project management support • Manages logistics of monthly science and PI meetings, site reviews

B2. Meeting Schedule

***Science Meetings*:** The entire NTL LTER community (PIs, staff, graduate students) meets monthly during the academic year (September through May) at the UW-Madison Memorial Union. The purpose of these meetings is to share progress on scientific objectives for the current funding cycle, plan future research and education/outreach, identify synergies among research and education/outreach programs within the site, network across researchers and professionals within our site, and participate in training opportunities. These meetings usually last ~2 hours and are open to anyone interested in or participating in NTL LTER activities. Historically, these meetings are held on Wednesday afternoons. The meeting is run by the Lead PI (currently EH Stanley) with support from other principal investigators (PIs) and project staff.

Principal Investigator Meetings: The principal investigators (faculty, research scientists) of the NTL LTER meet monthly to discuss progress on scientific goals, project administration, and plan future research and outreach. These meetings are historically held on Friday afternoons before the monthly science meetings (see above). The PI meetings are led by the Lead PI (currently EH Stanley) and a meeting agenda is set by the Wednesday prior to the meeting.

Project Staff Meetings: Individual staff meet with their supervisors and local team on an as-needed basis, depending upon tenure with the program (newer staff should meet regularly with team and supervisors) and need. There is an annual staff meeting held in person in January to review the prior year's progress, address problems, and review protocols and documentation (e.g., this QAPP) for the coming year. All permanent project staff, the Lead PI, and select PIs (usually those in a supervisory role) participate in the meeting.

US LTER All-Scientist Meetings: These meetings are held every three years at the Asilomar Conference Center in Monterey, California. Each LTER site can bring ~10 project personnel (PIs, graduate students, staff) to the conference to present current NTL science, lead and participate in workshops, and identify synthesis efforts within the network for NTL LTER. Project personnel representing the NTL LTER will be selected by the Lead PI in consultation with the PI group. Additional project personnel may be able to attend at the discretion of the Lead PI, pending available funding.

B3. Communication

There are two email lists that are used to communicate among LTER personnel: *ntl_lter_all* is for all NTL LTER personnel whereas *ntl_lter_pi* is for principal investigators only. To join an email list, please contact Kelly O'Ferrell. In addition to emails, many members of the NTL staff use Slack to communicate while at the Hasler Lab (workspace: haslerlaboratory.slack.com) or Trout Lake Station (workspace: troutlakestation.slack.com). Contact Amber Mrnak (TLS) or Hilary Dugan (HL) to be added to the workspaces.

B4. Community Expectations

All members of the NTL LTER community are bound by the Center for Limnology Code of Conduct. The UW-Madison Center for Limnology (CFL) Code of Conduct is intended to be a living document that reflects a shared responsibility for maintaining a professional environment. This Code of Conduct applies to all members of the CFL, including visitors, whether at Hasler Lab, Trout Lake Station (TLS) or on/off campus participating in CFL activities. This policy provides guidance for safe, respectful, and inclusive collaboration as well as procedures for reporting information that alleges violation and/or general concerns. The code of conduct is available on the CFL website (limnology.wisc.edu/cfl-code-of-conduct/) and NTL LTER website (https://ntllter.wiscweb.wisc.edu/code-of-conduct/).

C. Core Data Collection

C1. Annual Sampling Schedule

Sampling efforts are led by project staff with assistance from undergraduate hourly technicians and limited term employees. The team that performs the non-chem, monthly chem, quarterly, zooplankton, phytoplankton, and macrophyte sampling events are referred to as "base crew". The team performing fish sampling is referred to as "fish crew" and usually travels between the northern and southern lakes to perform sampling. All crews are led by research specialists in the north and south who also perform the other seasonal sampling. Buoy deployment and maintenance is also performed by project staff. Water chemistry analysis from the northern and southern lakes is performed by the water chemistry research specialist and undergraduate technicians.

Table 4. *Annual sampling schedule for northern and southern lakes.*

	Sampling Activity	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
NORTHERN LAKES	Non-Chem Sampling (surface)	1	1	1	2	2	2	2	2	2	2	1	1
	Zooplankton Sampling	1	1	1	2	2	2	2	2	2	2	1	1
	Phytoplankton Sampling	1			1		1	1	1		1		
	Monthly Chemistry Sampling	1	1	1	1	1	1	1	1	1	1	1	1
	Quarterly Sampling (approx.)	1			1			1			1		
	Fish Sampling												
	Macrophyte Sampling												
	Buoy Deployment (TR, SP, CR)												
	Benthic Invertebrate Sampling												
	Sediment Trap Sampling												
	Groundwater Well Sampling												
SOUTHERN LAKES	Non-Chem Sampling	1			2	2	2	2	2	2	1	1	
	Zooplankton Sampling	1			2	2	2	2	2	2	1	1	
	Phytoplankton Sampling	1			2	2	2	2	2	2	1	1	
	Chemistry Sampling	1			1	1	1	1	1	1	1		
	Quarterly Sampling (approx.)	1			1			1			1		
	Fish Sampling												
	Macrophyte Sampling												
	Buoy Deployment (ME)												

C2. Routine Sampling Description

Sampling generally occurs bi-weekly during the summer and monthly during the non-summer season (weather permitting). Quarterly sampling events occur approximately every 3 months.

"Non-chem" Profiles and Plankton Sampling Event

- Occurs regularly at the station over the deepest point in the lake
    - biweekly during the ice-free season in both north and south
    - every six weeks during the ice-covered season in the northern lakes and monthly in the fall and once in winter in the southern lakes
    - Coincides with "Profile" sampling (monthly and quarterly) once per month during the summer and "Profile" and "Quarterly" sampling at all other times of the year.
- While phytoplankton sampling occurs monthly in the southern lakes, phytoplankton sampling only occurs six times each year in the northern lakes. See section C3 for a description of plankton sampling and analysis methods.

Table 5. *Non-chem sampling event parameters in northern and southern lakes.*

Team	Data Set	Parameter	Sampling Depth	Preservation	Sampling Equipment
North	29	Water Temperature	Every 1 m	NA	YSI Pro-ODO
North	29	Dissolved Oxygen	Every 1 m	NA	YSI Pro-ODO
North		Secchi Depth	NA	NA	Secchi disk
North	38	Chlorophyll	Discrete depths depending on lake	Filtered, frozen until analysis	Peristaltic pump
North	90	Zooplankton	Integrated net tow, depth recorded	95% EtOH, dark storage until counting	2 m Schindler Patalas trap with 53 µm mesh, vertical tow with 80 µm mesh Wisconsin net
North	88	Phytoplankton	Pooled sample	Lugol's, slide mounts	Peristaltic pump
South	29	Water Temperature	Every 1 m	NA	YSI Pro-ODO
South	29	Dissolved Oxygen	Every 1 m	NA	YSI Pro-ODO
South		Secchi Depth	NA	NA	Secchi disk
South	38	Chlorophyll	Biweekly integrated: ME & MO: 0-8 m, Other: 0-2 m; Thermal profile depths monthly	Filtered, frozen until analysis	ME, MO: composite tube sample Other: Kemmerer bottle
South	90	Zooplankton	Integrated net tow, depth recorded	95% EtOH, dark storage until counting	2 m Schindler Patalas trap with 53 µm mesh, vertical tow with 80 µm mesh Wisconsin net
South	88	Phytoplankton	Mendota: 0-8 m Other: 0-2 m	Glutaraldehyde, slide mounts	Mendota: composite tube sample Other: Kemmerer bottle

Monthly and Quarterly Chemistry Sampling Events

- Occurs monthly, ice conditions allowing (usually only once per winter for southern lakes) at the station over the deepest point in the lake
- Always coincides with a non-chem sampling event so physical profiles, zooplankton, and phytoplankton data are aligned with monthly chemistry
- Monthly Chem profile sampling depths are based on thermal stratification:
    - *When stratified:* top of epilimnion, bottom of epilimnion, mid thermocline, top of hypolimnion, middle of hypolimnion, bottom of hypolimnion
    - *When mixed:* surface, middle of the water column, bottom
- For the southern lakes only, there are a set of bottles (H1 and H2) that are taken for analysis at the State Lab of Hygiene (SLOH) for nutrient and silica concentrations in accordance with state certified methods for our DNR partners. Be sure that sampling procedures, chain of custody, and holding times are being respected for these samples.

Table 6. *Water chemistry sampling in northern lakes*

Northern Lakes MONTHLY & QUARTERLY Chemistry Sampling						
Event Type	**Bottle**	**Filtered in Field?**	**Preservative**	**Storage**	**ChemLab ID**	**Parameter**
Monthly	'S' Bottle (125 mL)	No	1 mL conc. Optima HCl	Box in 101 Lab	2	Total N unfiltered
						Total P unfiltered
Monthly	'S' Bottle (125 mL)	Yes	1 mL concentrated Optima HCl	Box in 101 Lab	3	Total dissolved N
						Total dissolved P
						Dissolved Reactive Si
Monthly	'N' vial (20 mL)	Yes	Frozen	Freezer in 101 Lab east fridge	6	Nitrite+Nitrate-N
						Ammonium-N
Monthly	Glass vial + septa	No	4°C	East fridge in 101 Lab	0	Total Inorganic Carbon
						Total Organic Carbon
Monthly	Glass vial + septa	Yes	4°C	East fridge in 101 Lab	1	Dissolved Inorganic Carbon
						Dissolved Organic Carbon
Quarterly	'V' vial (20 mL)	Yes	4°C	East fridge in 101 Lab	4	Chloride
						Sulfate
Quarterly	'S' Bottle (125 mL)	Yes	1 mL concentrated Optima HCl	Box in 101 Lab	3	Magnesium
						Calcium
						Sodium
						Potassium
						Iron
						Manganese
Quarterly	'A' vial (20 mL)	No	4°C	NA	5	Alkalinity

Table 7. *Water chemistry sampling in southern lakes.*

Southern Lakes MONTHLY & QUARTERLY Chemistry Sampling						
Event Type	**Bottle**	**Filtered in Field?**	**Preservative**	**Storage**	**ChemLab ID**	**Parameter**
Monthly	'S' Bottle (125 mL)	No	1 mL conc. Optima HCl	Box in 101 Lab	2	Total N unfiltered
						Total P unfiltered
Monthly	'S' Bottle (125 mL)	Yes	1 mL concentrated Optima HCl	Box in 101 Lab	3	Total dissolved N
						Total dissolved P
						Dissolved Reactive Si
Monthly	'H1' Bottle	No	Concentrated H_2SO_4	State Lab of Hygiene	NA	Total N unfiltered – SLOH
						Total P unfiltered – SLOH
Monthly	'H2' Bottle	Yes	Concentrated H_2SO_4	State Lab of Hygiene	NA	Nitrite+Nitrate-N – SLOH
						Ammonium-N – SLOH
						Orthophosphate – SLOH
						DRSi – SLOH
Monthly	'N' vial (20 mL)	Yes	Frozen	Freezer in 101 Lab east fridge	6	Nitrite+Nitrate-N
						Ammonium-N
Monthly	Glass vial + septa	No	4°C	East fridge in 101 Lab	0	Total Inorganic Carbon
						Total Organic Carbon
Monthly	Glass vial + septa	Yes	4°C	East fridge in 101 Lab	1	Dissolved Inorganic Carbon
						Dissolved Organic Carbon
Quarterly	'A' vial (20 mL)	No	4°C	NA	5	Alkalinity
Quarterly	'V' vial (20 mL)	Yes	4°C	East fridge in 101 Lab	4	Chloride
						Sulfate
Quarterly	'S' Bottle (125 mL)	Yes	1 mL concentrated Optima HCl	Box in 101 Lab	3	Magnesium
						Calcium
						Sodium
						Potassium
						Iron
						Manganese

C3. Plankton Sampling and Analysis Description

Phytoplankton: Phytoplankton samples are collected using a peristaltic pump and tubing, collecting a separate sample from the epilimnion, metalimnion and hypolimnion for most of the lakes. For 27-2 Bog Lake, which is only 2m deep, we collect one 0-2m composite sample. The samples are preserved with Lugol's iodine solution. We create a single hypsometrically pooled composite sample per lake from subsamples of the epi, meta, and hypo samples. The pooled samples are sent to PhycoTech, Inc., a private lab specializing in plankton analysis, to be made into permanent slide mounts.

***Zooplankton*:** For the Northern Lakes, Schindler-Patalas trap samples are collected with a 2-meter high, 45L Schindler-Patalas trap with 53um mesh net at the deepest part of the lake. Samples are collected from specified target depths to include most or all the water column,

every two weeks during open water and every five weeks during ice cover. In addition, a vertical tow taken with an 80um mesh Wisconsin net is collected from the same location. Samples are preserved in the field with cold 95 percent EtOH. For zooplankton counting, a hypsometrically pooled sample is created from subsamples of the individual Schindler Patalas samples. Subsample volumes are calculated using the hypsometric data for each lake, so that each subsample volume is proportional to the volume of lake water represented by the trap sample. A portion of the pooled sample is counted for copepods, cladocerans, and rotifers (northern lakes only), identifying individuals to species or genus. All eggs are counted, and length measurements are taken on copepods and cladocerans.

***Spiny Water Flea*:** Beginning in 2014, vertical net tows (0.5m diameter, 400um mesh net) are collected every other week from Trout Lake during the open water season specifically for the invasive spiny water flea, *Bythotrephes longimanus*. This data set includes the number of individuals seen in each tow, calculated density reported as number per cubic meter.

C4. Seasonal Sampling Description

Buoy Deployment: Four instrumented buoys are deployed on a subset of the lakes during the ice-free season. Instruments are cleaned, downloaded, and calibrated regularly.

The instrumented buoy on Lake Mendota is equipped with a thermistor chain, limnological and meteorological sensors that provide fundamental information on lake thermal structure, weather conditions, and lake metabolism. Data are collected every minute.

The instrumented buoy on Trout Lake is equipped with a thermistor chain that measures water temperature from thermistors placed throughout the water column at a frequency of 1 minute. The Trout Lake buoy is also equipped with a dissolved oxygen sensor and meteorological sensors that provide fundamental information on lake thermal structure, weather conditions, and lake metabolism.

The instrumented raft on Sparkling Lake is equipped with a dissolved oxygen and CO_2 sensors, a thermistor chain, and meteorological sensors that log data every minute. This includes micrometeorological parameters from which evaporation can be calculated using relative humidity and air temperature, wind velocity, and water temperatures combined with measurements of total long-wave and short-wave radiation data from a nearby shore.

The instrumented buoy on Sparkling Bog North is equipped with a dissolved oxygen sensor, a thermistor chain, and meteorological sensors. The buoy is also equipped with a CO2 monitor and a YSI AutoProfiler that measures several parameters including dissolved oxygen, water temperature, conductivity, pH, ORP, turbulence and chlorophyll-a.

Macrophytes: For the northern lakes, annual macrophyte sampling occurs in Trout Lake only. Four sites along the shoreline of Trout Lake have been sampled annually in August along permanent line transects. A 0.25 m^2 ring placed at 1-meter intervals (except site 7 - 0.5 to 1 M depth where the ring is placed at 2 meter intervals) along the transect designates the sampling quadrat. Using SCUBA, species data are recorded along with the total number of quadrats encountered between the following depth intervals: 0.5-1 m, 1-2 m, 2-3 m and 3-5 m. Five

replicate quadrats are harvested for all above ground biomass at each site at each of three nominal depths: 1.5, 2.5, and 4 m. Samples are removed along a line parallel to shore - located midway between sites for cover estimates. In the lab, samples are separated by species and are dried and weighed for biomass estimates.

For the southern lakes, macrophyte surveys are conducted from a boat at stations located at depths from 1 to 4 meters at 0.5 m intervals along transects perpendicular to the lake shoreline. Sampling occurs from late June through August. A weighted, double-headed garden rake is cast off the front left, front right, rear left, and rear right of the boat and then dragged approximately 2 meters across the bottom by means of an attached line. For each rake cast, filamentous algae and any aquatic macrophyte species present are assigned a density rating from 0-5 based on the extent of coverage of the upper rake head and weighed for biomass.

Fish: The same sampling sites are used each year. All sampling occurs between the 3rd week of July and Labor Day. Sampling is done at six littoral zone sites of per lake with seine at night, minnow or crayfish traps, and fyke nets; a boat-mounted electrofishing system samples three littoral transects. Vertically hung gill nets are used to obtain two pelagic samples per lake from the deepest point. A trammel net samples across the thermocline at two sites per lake. In the bog lakes only fyke nets and minnow traps are deployed. Parameters measured include species-level identification and lengths for all fish caught, and weight and scale samples from a subset. For all collecting methods, each individual fish is identified to species. The total length of the fish is measured in mm, from nose to pinched tail. Two fish are weighed for each species in each 5mm size category. A scale sample is collected from each yellow perch, rock bass, and cisco that is weighed. For gill net catches, the depth each individual is caught is also recorded.

Total pelagic fish abundance data is collected annually in mid-summer (following stratification) using hydroacoustic surveys along a set of transects in Mendota, Crystal, Sparkling, Big Muskellunge, and Trout.

Benthic Macroinvertebrates *(northern lakes only):* Modified Hester-Dendy samplers are constructed as a stack of ten plastic mesh panels and a plastic scrubbing ball bolted between hardboard end panels. They are placed in the lakes early- to mid-August and left for approximately four weeks. Each sampling site consists of three Dendy samplers spaced 3 meters apart. Shoreline samplers are place in ~1 m of water, deep sites at the deepest part of the lake. The shoreline sets are retrieved by a snorkeler who places the sampler in a container before surfacing to avoid loss of invertebrates due to disturbance, while deep sites are pulled up to the surface from a boat. Samplers are preserved in ethanol in the field, disassembled in the lab, and the invertebrates identified and counted under a dissecting microscope.

Crayfish *(northern lakes only):* Crayfish data include crayfish catch in cylindrical minnow traps baited with beef liver and occasional occurrence in other gear used to sample fish. Traps are placed at fyke net locations in the lakes. Individuals are identified to species and counted. In Trout and Sparkling Lake more detailed surveys have been conducted during the summer on an ad hoc basis to track distribution and abundance of the invading species *Orconectes rusticus*.

Pelagic Macroinvertebrates *(northern lakes only):* Pelagic macroinvertebrates are collected at night from the deepest location of each of the northern lakes by vertical tow with a 1-m

diameter, 1-mm mesh net. On Trout Lake four additional sites are sampled, where depths are approximately 10m, 15m, 20m, and 25m. Sampling is once per year in the summer, with replicate tows collected on each lake. Samples are preserved, and later counted in their entirety for *Chaoborus spp.* (differentiating between larvae and pupae), *Leptodora kindtii*, *Mysis relicta,* and *Bythotrephes longimanus*.

Groundwater Wells: Water chemistry and groundwater level is measured annually in 11 monitoring wells to characterize regional groundwater chemistry in the Trout Lake area. The chemical parameters measured include pH, conductivity, total alkalinity, dissolved inorganic and organic carbon, total nitrogen, nitrate, ammonia, total phosphorus, calcium, magnesium, sodium, potassium, chloride, sulfate, iron, manganese, total silica and dissolved reactive silica.

***Sediment Deposition*:** Settling particulate matter is collected using sediment traps deployed in the hypolimnion at the deepest part of the lake. Duplicate traps are set at one station in Trout, Sparkling and Crystal lakes. Traps are deployed during the ice-free period for four-week intervals. Mass deposition rates are calculated from the dry-weight of material collected, and mass flux is reported as mg per meter-squared per day. Sampling Frequency: every four weeks during ice-free season.

C5. Water Chemistry Analyses

Table 8. Water chemistry parameters measured in the laboratory with preservation, reporting limits, and analytical range. **LOD last calculated in 2014, new limits being calculated in 2023.

Parameter	Preservation	Sample Bottle	Holding Time	Analytical Method	**Limit of Detection	Working Range
Total N unfiltered **(totnuf)**	1% HCl, room temp	125 mL HDPE ('S' bottle)	1 year	EPA 365.1 v2, Astoria Pacific	21 µg L^{-1}	2500 µg L^{-1}
Total P unfiltered **(totpuf)**	1% HCl, room temp	125 mL HDPE ('S' bottle)	1 year	EPA 365.1 v2, Astoria Pacific	3 µg L^{-1}	800 µg L^{-1}
Total dissolved N **(totnf)**	1% HCl, room temp	125 mL HDPE ('S' bottle)	1 year	EPA 365.1 v2, Astoria Pacific	21 µg L^{-1}	2500 µg L^{-1}
Total dissolved P **(totpf)**	1% HCl, room temp	125 mL HDPE ('S' bottle)	1 year	EPA 365.1 v2, Astoria Pacific	3 µg L^{-1}	800 µg L^{-1}
Dissolved Reactive Silica **(dsrif)**	1% HCl, room temp	125 mL HDPE ('S' bottle)	1 year	Astoria Pacific	6 µg L^{-1}	15000 µg L^{-1}
Nitrite+Nitrate-N **(no2, no2no3)**	Frozen until analysis	20 mL HDPE ('N' vial)	1 year	EPA 353.2 v2, Astoria Pacific	2 µg L^{-1}	4000 µg L^{-1}
Ammonium-N **(nh4)**	Frozen until analysis	20 mL HDPE ('N' vial)	1 year	EPA 350.1 v2, Astoria Pacific	3 µg L^{-1}	4000 µg L^{-1}
Orthophosphate **(srp, drp)**	Frozen until analysis	20 mL HDPE ('N' vial)	1 year	EPA 365.1 v2, Astoria Pacific	3 µg L^{-1}	400 µg L^{-1}
Alkalinity **(alk)**	4°C without aeration	20 mL HDPE ('A' vial)	14 days	Orion 720A pH meter	5 µE L^{-1}	4000 µE L^{-1}
Total Inorganic Carbon **(tic)**	4°C without aeration	40 mL borosilicate vial	14 days	EPA 415.3 Shimadzu	0.15 mg L^{-1}	60 mg L^{-1}
Total Organic Carbon **(toc)**	4°C without aeration	40 mL borosilicate vial	14 days	EPA 415.3 Shimadzu	0.30 mg L^{-1}	30 mg L^{-1}
Dissolved Inorganic Carbon **(dic)**	4°C without aeration	40 mL borosilicate vial	14 days	EPA 415.3 Shimadzu	0.15 mg L^{-1}	60 mg L^{-1}
Dissolved Organic Carbon **(doc)**	4°C without aeration	40 mL borosilicate vial	14 days	EPA 415.3 Shimadzu	0.30 mg L^{-1}	30 mg L^{-1}
Chloride **(cl)**	4°C	20 mL HDPE ('V' vial)	1 year	EPA 300.0, Ion chromatography	0.01 mg L^{-1}	100 mg L^{-1}
Sulfate **(so4)**	4°C	20 mL HDPE ('V' vial)	1 year	EPA 300.0, Ion chromatography	0.01 mg L^{-1}	60 mg L^{-1}
Magnesium **(mg)**	1% HCl, room temp	125 mL HDPE ('S' bottle)	1 year	Perkin-Elmer 4300 DV ICP	0.03 mg L^{-1}	50 mg L^{-1}
Calcium **(ca)**	1% HCl, room temp	125 mL HDPE ('S' bottle)	1 year	Perkin-Elmer 4300 DV ICP	0.06 mg L^{-1}	50 mg L^{-1}
Sodium **(na)**	1% HCl, room temp	125 mL HDPE ('S' bottle)	1 year	Perkin-Elmer 4300 DV ICP	0.06 mg L^{-1}	50 mg L^{-1}
Potassium **(K)**	1% HCl, room temp	125 mL HDPE ('S' bottle)	1 year	Perkin-Elmer 4300 DV ICP	0.06 mg L^{-1}	10 mg L^{-1}

Iron (fe)	1% HCl, room temp	125 mL HDPE ('S' bottle)	1 year	Perkin-Elmer 4300 DV ICP	0.02 mg L^{-1}	20 mg L^{-1}
Manganese (mn)	1% HCl, room temp	125 mL HDPE ('S' bottle)	1 year	Perkin-Elmer 4300 DV ICP	0.01 mg L^{-1}	2 mg L^{-1}
Chlorophyll-a NORTHERN LAKES (spectrophotometer)	Frozen until analysis	Type A/E glass fiber filters	14 days	Beckman DU 800 spectrophotometer		
Chlorophyll-a SOUTHERN LAKES (spectrophotometer)	Frozen until analysis	Type A/E glass fiber filters	14 days			
Chlorophyll-a SOUTHERN LAKES (fluorometer)	Frozen until analysis	Type A/E glass fiber filters	14 days			

C6. Sample Archiving

Water samples: All water samples are kept for two years from the date of collection in case analyses need to be performed again. After this time, all 'S' bottles (filtered and unfiltered) from the quarterly sampling events are stored at "Warm Storage" (SWAP 1061 Thousand Oaks Trail Verona, WI 53593). At the facility, drive around to the back of the building and you will find the warehouse section. Ask one of the warehouse workers where the Limnology storage is. Should be in the back right side of the warehouse.

Zooplankton: The Wisconsin net sample and the hypsometrically-pooled sample are archived in the UW Zoology museum for each lake and sampling event.

Phytoplankton: Mounted slides produced by PhycoTech are archived in the UW Zoology Museum; no wet samples are saved.

Benthic Macroinvertebrates: All invertebrates are preserved in ethanol and archived in the UW Zoology Museum.

Pelagic Macroinvertebrates: One tow from each lake/station is archived in the UW Zoology Museum.

Fish Scales: stored in small envelopes, organized by lake, year, and species in card catalog drawers.

Macrophytes: dried and stored in boxes on open shelving.

Microbial samples: stored on 0.2 um filters that were used to capture planktonic biomass from whole water at the time of sampling. The filters are stored at -80C inside 2.0 mL screw-cap tubes.

D. Quality Objectives and Criteria

D1. Quality Control Procedures

Long-Term Method Detection Level: Long-Term Method Detection Levels (LT-MDLs) are updated annually based on the previous year's data. The LT-MDL is the predicted concentration at the upper 95th percentile of the blank values for a normal distribution (Childress et al., 1999). If the measured blank distribution is heavily skewed, outliers are removed from the data and data are normalized. For measured blank values with a meaningfully negative averaged measured blank concentration (for example NHx), LT-MDL is predicted at the 99% confidence interval. When the mean of replicate sample values are equal to or below the LT-MDL, quality control statistics are not performed as values under the LT-MDL are below the lab's limit of detection for precision and accuracy.

Field Duplicates: For quality control purposes, duplicate samples are collected at one depth for each sampling event in each lake. This entails a complete re-sampling of the sample site and depth without re-navigating to the site. The bottles are labeled with "blind" and are assigned a unique identifier in ChemLab.

Laboratory Duplicates: Samples are split and analyzed in the lab at a rate of 10%. The measurements for a split sample are averaged for reporting.

Check Standards and Independent Calibration Verification: In addition to analyzing calibration standards and sample blanks, check standards from the same stock solution and independent calibration verification standards from a separate manufacturer are analyzed periodically throughout a batch analysis.

Matrix Spikes and Recovery: Samples are spiked with calibration standard at a rate of 15%. Spike recovery is calculated based on the sample concentration from an unspiked sample and the known quantity of calibration standard added.

Proficiency Testing: The laboratory takes part in annual round-robin proficiency tests through the United States Geological Survey.

D2. Data Quality Determination

The overall project data quality objective is to provide valid environmental data of known and documented quality. Key indicators of data quality are precision and bias which when combined, communicates data accuracy (American Public Health Association, 1998).

Precision: a measure of agreement between two or more data with each other. Field sampling precision is determined by using field duplicate samples and field split samples. Laboratory analytical precision is determined by comparing the results of duplicate samples and laboratory split samples. Sampling and/or analytical precision is determined from split or duplicate samples by calculating the Relative Percent Difference (RPD) as follows:

$$RPD = (A - B) \div ((A + B) / 2) * 100$$

where ***A*** is the larger of the two duplicate samples and ***B*** is the smaller value. As a backup protocol, three or more replicate samples or measurements may be taken, and the Relative Standard Deviation (RSD) instead of the RPD is calculated as follows:

$$RSD = (s / \chi) * 100$$

where ***s*** is the standard deviation of the replicate values and ***χ*** is the mean of the replicates.

Accuracy: expresses the degree to which an observed (measured) value agrees with an accepted reference standard or differs from it due to systematic error/bias. Laboratory analytical accuracy is determined by analyzing blind proficiency testing samples. Accuracy is also determined by calculating the percent recovery of blank spikes and sample spikes.

D3. Statistics to Determine Rerun

Calculations to determine re-runs are based on the relative standard deviation (RSD) (i.e., coefficient of variation) of each set of replicates for a sample. This statistic normalizes the standard deviation and sometimes facilitates making direct comparisons among analyses that include a wide range of concentrations. The RSD can only be calculated with values above the detection limit. Calculate the RSD as follows:

$$RSD = (s / \chi) * 100$$

where s is the standard deviation of the replicate values and χ is the mean of the replicate values. The percent standard deviation should be less than 15% for replicates that fall above the limit of detection. If so, follow these guidelines:

Lab Duplicates

- If %RPD < 15%, then final reported value is the mean of the duplicate values.
- If %RPD > 15%, then sample is re-run.

Re-run Duplicates

- If %RPD < 15%, then final reported value is the mean of the re-run duplicate values to represent that the reported value is based on a re-run sample.
- If %RPD > 15%, then sample is re-run.

Sample Dilutions: If TN < NH_4 + NO_3 + NO_2, or if TP < SRP on a sample, then TN or TP must be diluted. If a sample's concentration is greater than the end point of the calibration curve, the sample must be diluted for that analyte. Dilutions are noted on the sample log sheet and the dilution factor is entered on the spectrophotometer or segmented flow analyzer as part of the sample name and in the dilution factor column. For a half-dilution, "2" is entered, and so on.

E. Data Workflow and Publishing

E1. Current Core Data Sets

The following tables contain the data set ID, title (always preceded by "North Temperate Lakes LTER:"), and link to the repository through the Environmental Data Initiative. These are only the data sets that are currently updated as a part of the core sampling program. Note that there are several data sets on core program lakes from other projects or previous core sampling efforts that are not included in the tables below.

ID	Title
1	Chemical Limnology of Primary Study Lakes: Nutrients, pH and Carbon 1981 - current
2	Chemical Limnology of Primary Study Lakes: Major Ions 1981 - current
3	Crayfish Abundance 1981 - current
4	High Frequency Meteorological and Dissolved Oxygen Data - Sparkling Lake Raft 1989 - current
5	High Frequency Water Temperature Data - Sparkling Lake Raft 1989 - current
6	Fish Lengths and Weights 1981 - current
7	Fish Abundance 1981 - current
8	Fish Length Frequency 1981 - current
9	Groundwater Levels 1984 - current
10	Groundwater Chemistry 1984 - current
11	Benthic Macroinvertebrates 1981 - current
13	Pelagic Macroinvertebrate Abundance 1983 - current
14	Pelagic Macroinvertebrate Summary 1983 - current
17	Meteorological Data - Woodruff Airport 1989 - current
21	Macrophyte Biomass - Trout Lake 1983 - current
22	Macrophyte Transects - Trout Lake 1982 - current
23	Macrophyte Rating - Madison Lakes Area 1995 - current
24	Macrophyte Biomass - Madison Lakes Area 1995 - current
25	Macrophyte Biomass in Trout Lake Summary 1983 - current
26	Macrophyte Species at Quadrat Level - Trout Lake 1993 - current
28	Macrophyte Richness - Trout Lake 1993 - current
29	Physical Limnology of Primary Study Lakes 1981 - current
30	Lake Levels 1981 - current
31	Secchi Disk Depth; Other Auxiliary Base Crew Sample Data 1981 - current
32	Ice Duration - Trout Lake Area 1981 - current
33	Ice Duration - Madison Lakes Area 1853 - current
34	Snow and Ice Depth 1982 - current
35	Chlorophyll - Trout Lake Area 1981 - current
37	Zooplankton - Trout Lake Area 1982 - current
38	Chlorophyll - Madison Lakes Area 1995 - current
40	Sediment Deposition - Trout Lake Area 1986 - current
87	Color - Trout Lake Area 1989 - current

88	Phytoplankton - Madison Lakes Area 1995 - current
90	Zooplankton - Madison Lakes Area 1997 - current
115	Pelagic Prey - Sonar Data 2001 - current
116	High Frequency Water Temperature Data - Trout Lake Buoy 2004 - current
117	High Frequency Meteorological and Dissolved Oxygen Data - Trout Lake Buoy 2004 - current
129	High Frequency Data: Meteorological, Dissolved Oxygen, Chlorophyll, Phycocyanin - Lake Mendota Buoy 2006 - current
130	High Frequency Water Temperature Data - Lake Mendota Buoy 2006 - current
133	Soil Temperature - Woodruff Airport 2006 - current
181	Lake Wingra: Fish Lengths and Weights 1995 - current
182	Lake Wingra: Fish Abundance
228	High Frequency Water Temperature Data - Sparkling Bog North Buoy 2008 - current
238	Phytoplankton - Trout Lake Area 1984 - current
245	Fish Species Richness 1981 - current
259	Light Extinction - Trout Lake Area 1981 - current
264	Physical Limnology of Lake Kegonsa and Lake Waubesa 1995 - current
338	Regional Survey Macrophytes Plant Index 2015 - current
380	Regional Survey Water Chemistry 2015 - current
381	Regional Survey Zooplankton 2015 - current
382	Regional Survey water temperature DO 2015 - current
389	Trout Lake Spiny Water Flea 2014 - present
401	Physical and Chemical Limnology of Lake Kegonsa and Lake Waubesa 1994 - current

E2. Data Validation and Flags

flag	definition	Comments
A	Sample suspect	recode to flag K
B	Standard curve/reduction suspect	recode to flag K
C	No sample taken	delete data
D	Sample lost	delete data
E	Reporting average of duplicate analyses	no change
F	Duplicate analyses in error	Keep
G	Analyzed late	Keep for NTL data only
H	Outside of standard range	no change
I	Outside of data entry constraints	Keep or figure out
J	Nonstandard routine followed	SLOH only
K	Data suspect	Keep
L	Sample and duplicate blind differ by more than 15%	for internal QC, remove for archiving, blind duplicate value receives K flag
M	More than four flags	no change
N	Sample retested	no change
O	Value suspect but total pigment(CHL + PHAEO) value accurate	no change
P	TPM uncorrected for humidity change between filter weighing	Keep
Q	Quality control comments on SLOH lab sheet	SLOH only
R	Value between LOD and LOQ	SLOH only
S	Value below detection limit; set to zero	SLOH only
T	Sample contaminated; data not reported	delete data
U	Equipment malfunction produced bad value; value set to missing;	delete data
V		
W	negative light value; set to zero	Keep
X	Value below detection limit - non SLOH data	
Y		
Z		

E3. Field Data Workflow

Sample events are created by the field team in ChemLab3. The sample event type, location, date, and depths are entered into ChemLab3 to generate unique sample IDs and barcode labels. These sample IDs are used to track samples throughout processing (e.g., in the chemistry lab) Information and observations collected on the lake are recorded on data sheets and entered by hand into ChemLab3, ,with secondary checking and approval by one other trained personnel. Once the sampling event has been completed and the event table is verified (dates, location,

depths), the field team will push "Send to Lab" in ChemLab3 to alert the laboratory that samples are incoming (although no actual alert is generated).

E4. Chemistry Lab Data Workflow

Sample events are generated by the field team and submitted through ChemLab3.0. Samples in the ChemLab3 system are either listed as "Unsubmitted" (event table has been generated by the field team but not submitted), "At Lab" (physical samples that have been stored at the lab but are currently unanalyzed or entered), "Results Entered" (samples analyzed and data entered into ChemLab3), and "Dumped" (indicating the physical sample is no longer archived).

Samples are brought into the lab and stored according to laboratory guidelines. Upon receipt, the chemistry lab manager or designated personnel create the paper ***sample tracking log*** for use by the chemistry lab. On individual instruments and/or analyses, samples are identified through the sample ID number generated in ChemLab3.0 and barcode tracking. Data files from instrument analyses are kept on the respective computer associated with that instrument and an additional copy is stored in raw format on the CFL Share Drive. Data collection that is not automated (e.g., alkalinity), standardized bench sheets are used and digitized regularly upon completion of the analysis, with secondary checking and approval by one other trained laboratory personnel.

Data entry into ChemLab3.0 is performed by the lab manager and trained lab personnel after quality control samples and practices have met requirements. Following data entry and prior to publication through the Environmental Data Initiative, the laboratory manager, in consultation with the information manager, will perform database queries in NaviCat for a final quality control and quality assurance procedure.

The database queries fall into two categories: 1) checking the blind field sample against the known sample for all analytes, and 2) checking if dissolved fractions are greater than total. Specifically, we check if dissolved organic (or inorganic) carbon is greater than total organic (or inorganic) carbon and if the sum of NO_3 + NO_2 + NH_4 is less than total nitrogen.

E5. Plankton Data Workflow

[text description in development]

E6. Buoy Data Workflow

[text description in development]

E7. Seasonal Data Workflow

[text description in development]

E8. Other Data Sources Workflow

[text description in development]

E9. Data Publishing

Following quality assurance and quality control certification for all data sets from the prior year, data are published annually in the Environmental Data Initiative repository and the NTL LTER website by the information manager.

F. Historical Method Changes

F1. Protocol Logs

pH and Air–equilibrated pH: The pH of LTER waters is measured potentiometrically in a closed system apparatus to minimize chemical exchange with the atmosphere. This pH measurement has been performed at the Trout Lake Field Station since the beginning of the project in 1982, and is still the current method. Air-equilibrated pH measurements were initiated in 1986 and are also performed at the field station laboratory. ***No longer done.***

Alkalinity: Alkalinity of the LTER waters is determined by the Gran Alkalinity titration technique. Prior to 1986, and currently, alkalinity titrations were performed manually and recording the potential measurements by hand. During the period from February 1986 - November 2001, the alkalinity determinations for Trout, Sparkling, Big Muskellunge and Allequash Lakes have been made by a Brinkmann 636 Titroprocessor.

Bicarbonate Reactive Silica: The bicarbonate reactive silica analyses were initiated in February 1986 according to the sample digestion method developed by Vigon (1976). Digested samples are analyzed according to the same procedures as the dissolved reactive silica on the Technicon Autoanalyzer II. ***This test was suspended as of 2004***.

Dissolved and Total Organic Carbon: Dissolved and Total Carbon are determined by acidification (for Inorganic carbon) and heated persulfate digestion for (for Organic Carbon) with an OI Model 700 Carbon Analyzer. Dissolved (filtered) samples were initiated in August, 1985 and Total (unfiltered) samples initiated in May, 1986. In May, 2006, carbon analysis has been performed with a Shimadzu TOC-V-csh Total Organic Carbon Analyzer. Inorganic Carbon is still determined by acidification, but Organic Carbon is determined by combustion and not a heated persulfate digestion.

Dissolved Reactive Silicon/Silicate: These determinations were performed manually using a Bausch and Lomb Spectrophotometer from the beginning of the project until April 1984. From 1984 through 2005, Dissolved Reactive Silicon was determined on a Technicon AutoAnalyzer II. Samples after January 2006, and currently, are run on an Astoria-Pacific Astoria II Autoanalyzer.

Nitrite and Nitrate – Nitrogen: Measurements of this test have been made since the beginning of the LTER project. The analyses have been performed using a Technicon AutoAnalyzer II until 2006 and analyzed simultaneously with Ammonium-N. Samples after 2006, and currently, are run on an Astoria-Pacific Astoria II Autoanalyzer.

Ammonium – Nitrogen: Measurements of this test have been made since the beginning of the LTER project. The analyses are analyzed simultaneously with Nitrite-Nitrate-N, and have been performed using a Technicon AutoAnalyzer II until 2006. Samples after January 2006, and currently, are run on an Astoria-Pacific Astoria II Autoanalyzer.

Total Phosphorus: Prior to April 1986, all samples for TP were determined manually on a Bausch and Lomb Spectrophotometer after undergoing a persulfate digestion process. From 1986 through 2005, samples were determined (and analyzed simultaneously with TN) on a Technicon AutoAnalyzer II after undergoing a modified persulfate digestion (Julian and Kroner, 1966) which was developed by Doug Lindelof in the LTER lab. Samples after January 2006, and currently, are run on an Astoria-Pacific Astoria II Autoanalyzer.

Total Nitrogen: TN determinations began in 1986, when TP started being determined using the modified digestion, which is also used for the Total Nitrogen analysis. From 1986 through 2005, samples were determined (and analyzed simultaneously with Total Nitrogen) on a Technicon AutoAnalyzer II after undergoing a modified persulfate digestion (Julian and Kroner, 1966) which was developed by Doug Lindelof in the LTER lab. Samples after January 2006, and currently, are run on an Astoria-Pacific Astoria II autoanalyzer.

Total Particulate Matter: In the southern lakes from 2000-2012, total particulate matter was measured bi-weekly during the ice free season at one station in the deepest part of each lake at the top of the epilimnion for lakes Mendota, Monona, Fish and Wingra. ***No longer sampled.***

In the northern lakes, total particulate matter was measured monthly during open water at the deepest part of the lake.

- 1986 -- began collecting TPM samples. Filters were weighed in Madison
- 2000 -- began weighing filters at Trout Lake Station using Cahn Model 31 electrobalance
- 2007 -- began using Sartorius Research analytical balance for filter weighing
- 2021 -- ***discontinued TPM measurement***

Sulfate and Chloride: Up to and including 1997 data, sulfate was determined (simultaneously with chloride) by a Dionex model DX10 Ion Chromatograph using a Bicarbonate/Carbonate eluent and a fiber suppressor. From January 1998 to December 2010, sulfate is determined by a Dionex model DX500, still using a Bicarbonate/Carbonate eluent but with an electro-chemical suppressor. From January 2011 to present sulfate is determined by a Dionex ICS-2100 using an eluent generation cartridge to yield KOH. An electro-chemical suppressor and a continuously regenerated trap column are also used.

Calcium and Magnesium: Until January 2002, calcium was determined on a Perkin-Elmer model 503 Atomic Absorption Spectrophotometer. Lanthanum at a 0.8% concentration added as a matrix modifier to suppress chemical interferences. From January 2002 to present calcium is determined on a Perkin-Elmer model 4300 DV Inductively-Coupled Plasma Spectrophotometer.

Sodium and Potassium: Until January 2002, potassium was determined on a Perkin-Elmer model 503 Atomic Absorption Spectrophotometer. Lithium at a concentration 1800-2000 ppm was added as a matrix modifier to suppress ionization. From January 2002 to present potassium is determined on a Perkin-Elmer model 4300 DV Inductively-Coupled Plasma Emission Spectrophotometer.

Iron and Manganese: Until January 2002, iron was determined on a Perkin-Elmer model 503 Atomic Absorption Spectrophotometer. From January 2002 to present iron is determined on a quarterly basis instead of monthly on a Perkin-Elmer model 4300 DV Inductively-Coupled Plasma Emission Spectrophotometer.

Phytoplankton: several taxonomic updates were implemented in February 2013, including name changes to currently accepted names, changes from genus level to species based on long term expertise from PhycoTech, and revisiting some slides to resolve taxonomic uncertainty.

- Converted all *Melosira* entries to *Aulacoseira* and changed species names appropriately.
- Converted all *Oscillatoria* entries to *Pseudanabaena*, changed species names appropriately
- Converted all *Synedra tenera* to *Synedra filiformis*.
- Converted all *Phacotus* entries without a species name to *Phacotus lendneri*.
- Converted all *Phormidium mucicola* to *Pseudanabaena*
- Converted *Glenodinium* entries without a species name to *Glenodinium quadridens*
- Assume that all other entries with genera but no species cannot be resolved to species
- Converted all *Chrysococcus* entries to *Chrysocccus minutus*
- Changed some single-celled *Microcystis* entries so that they would match the format of the colonial entries (genus + species)
- Resolved some entries to species that were previously coded incorrectly by genus.
- Added in *Cylindrospermopsis raciborskii* entries that were recently recounted and changed from *Anabaenopsis raciborskii*.
- Converted all entries of genus *Erkenia* to *Erkenia subaequiciliata*

Zooplankton:

- 1981 – May1984: used a 0.5m high, 31L Schindler Patalas trap with 80um mesh net was used. Two Wisconsin Net tows were collected. Preservative was 12% buffered formalin.
- June 1984: changed to 53um mesh net on Schindler trap
- July1986: began using the 2m high, 45L Schindler Patalas trap. Changed WI Net collection to take only one tow
- 2001: changed zooplankton preservative from 12% buffered formalin to 95% EtOH. The number of sample dates per year counted varies with lake and year, from 5 dates per year to 17 dates per year.
- 1981-1983: pooled samples are of several types: Total Pooled (TP) were created using equal volume subsamples of the Schindler samples. Epi, Meta, Hypo pooled used equal volume subsamples from the Schindler samples collected from each of the thermal strata. Strata Pooled used equal volume subsamples from the Epi, Meta, Hypo pooled samples to create an entire lake sample. Hypsometrically Pooled (HP) is our standard, which uses subsample volumes weighted to represent the hypsometry of the lake.

Macroinvertebrates (northern lakes): The modified Hester-Dendy samplers were set in all seven lakes in 1981-1989,1992 and 1993. Only Trout, Sparkling, and Crystal Lakes were sampled in 1990, 1991, and 1994 to present. No lakes were sampled in 2020.

Crayfish: Crayfish trapping ceased in 2003 in the southern lakes. In Trout and Sparkling Lake more detailed surveys have been conducted during the summer on an ad hoc basis to track

distribution and abundance of the invading species *Orconectes rusticus*. In Sparkling Lake, Rusty Crayfish (*Orconectes rusticus*) was removed from 2001 to 2008. Additional data sets consist of pre-LTER sets (initiated in late June 1972) gathered by Capelli (Ph.D. dissertation) and Lorman (Ph.D. dissertation). Most of pre-LTER data is detailed distribution in Trout Lake, and community composition in other area lakes.

Fish:

- 1983 Discontinued fyke nets and trammel nets on Lake Mendota
- 1984 Discontinued crayfish on Lake Mendota. Only gillnet and seines on Lake Mendota.
- 1995 Resumed sampling Lake Mendota with the full suite of sampling gear.
- 1995 Began sampling Lakes Wingra, Monona, and Fish.
- 1997 Two fish are weighed for each fish species in each 5mm size category. Previously, five fish were weighed for each fish species in each 10mm size category.
- 1997 Data recording switched from manual field sheets to an electronic system.
- 1997 Changed from 4 to 3 electrofishing runs per lake.
- 1997 Changed from 18 to 12 seine hauls per lake.
- 1998 Changed from 30 to 18 crayfish or minnow traps per lake.
- 2004 Discontinued crayfish or minnow traps on southern lakes.
- 2020 Fish sampling very limited due to pandemic.
- 2021 discontinued all night seining.

Mendota Buoy:

- 2017 - a boating mishap caused the loss of air temperature, relative humidity, and wind sensors between May 28 and July 11. The dissolved oxygen sensor had significant biofouling from algae and zebra mussels.
- 2018 - A PME MiniDOT logger replaced the D-Opto dissolved oxygen sensor that had been in use since 2006. The PME sensor was used only one year.
- 2019 - A YSI EXO2 sonde was added to buoy and includes DO, chlorophyll, phycocyanin, specific conductance, pH, fDOM, and turbidity sensors. The chlorophyll and phycocyanin sensors replace Turner Cyclops 7 fluorometers that had been in use in prior years. Both sets of sensors output RFU, but have significant magnitude differences. The YSI pH, DO, and specific conductance sensors were cleaned and calibrated every two weeks.
- 2020 - Cleaning and calibration of the YSI sensors occurred nearly every week. The dissolved CO2 sensor was not operating between July 2 and September 17.

Sediment Deposition:

- 1986 -- began sampling sediment deposition, using one trap per lake. Analysis is done in Madison.
- 1986-1991 -- sediment was analyzed for carbon, nitrogen, and phosphorus.
- 1988 -- began deployment of two traps per lake.

- 1996 -- began to measure the mass of particles in the trap water above the collection bottle in addition to the sediment in the bottle.
- 2000 to present -- analysis is done at Trout Lake Station.
- 2011-2015 -- Crystal Lake was not sampled.
- 2020 -- no lakes were sampled.

F2. Analytical Equipment Log

Table X. Log of analytical equipment use over the course of the program.

Parameter	Type	Make and Model	Start	End	Location
Anions	Analytical	Dionex DX10 - chemical fiber suppressor	1981	1998	lab
Anions	Analytical	Dionex DX500 - electrochemical suppressor	1998	2011	lab
Anions	Analytical	Dionex ICS - 2100 - electrochemical suppressor	2011	present	lab
Nutrients	Analytical	Technicon autoanalyzer	1981	2005	lab
Nutrients	Analytical	Astoria-Pacific Astoria II autoanalyzer	2006	present	lab
Silicon	Analytical	Bausch and Lomb Spectrophotometer	1981	1984	lab
Silicon	Analytical	Technicon autoanalyzer	1984	2005	lab
Silicon	Analytical	Astoria-Pacific Astoria II autoanalyzer	2006	present	lab
Carbon	Analytical	OI Model 700 Carbon Analyzer	1981	04/2006	lab
Carbon	Analytical	Shimadzu TOC V-csh	05/2006	03/2016	lab
Carbon	Analytical	Shimadzu TOC-L-cph	04/2016	present	lab
Cations	Analytical	Perkin-Elmer model 503 AAS	1981	2002	lab
Cations	Analytical	Perkin-Elmer 4300 DV ICP	2002	04/2019	lab
Cations	Analytical	Agilent 5110 VDV ICP-OES	05/2019	present	lab
Chlorophyll	Analytical	Beckman DU800 spectrophotometer		present	southern
Chlorophyll	Analytical	Beckman Allegra x-14 centrifuge		present	southern
pH	Analytical	PHM 84	1981	1988	northern
pH	Analytical	Orion 720	1988	2010	northern
pH	Analytical	Orion 4-Star	2010	present	northern
conductivity	Analytical	Sybron Barnstead	1981	1988	northern
conductivity	Analytical	YSI Model 32	1989	present	northern
color	Analytical	Kontron	1990	2008	northern
color	Analytical	Beckman DU800	2008	present	northern
DO	Handheld	Montedoro-Whitney DOR-2A	1982	1982	northern
temp	Handheld	Montedoro-Whitney CTU-3B	1982	1982	northern
DO/temp	Handheld	YSI-57	1982	1984	northern
DO/temp	Handheld	YSI-58	1985	2010	northern
DO/temp	Handheld	YSI Pro-ODO	2011	present	northern
DO/temp	Handheld	YSI Pro-DSS	2023	present	southern
DO	Buoy	Zebra Tech D-Opto	2016	2018	Mendota
DO	Buoy	PME miniDOT	2018	2018	Mendota
DO	Buoy	YSI EXO2 sonde	2019	present	Mendota
CO2	Buoy	Turner Designs C-sense	2017	present	Mendota
pH	Buoy	YSI EXO2 sonde	2019	present	Mendota
fDOM	Buoy	YSI EXO2 sonde	2019	present	Mendota
Turbidity	Buoy	YSI EXO2 sonde	2019	present	Mendota
Chlor/Phyco	Buoy	Turner Designs Cyclops	2006	2019	Mendota
Chlor/Phyco	Buoy	YSI EXO2 sonde	2019	present	Mendota
Conductivity	Buoy	YSI	2006	2006	Mendota
Conductivity	Buoy	YSI EXO2 sonde	2019	present	Mendota

Water Temp	Buoy	Apprise Templine	2006	2006	Mendota
Water Temp	Buoy	RBR Concerto	2007	present	Mendota
Temp/RH	Buoy	RM Young 41382	2006	2017	Mendota
Temp/RH	Buoy	Vaisala HMP110	2017	present	Mendota
Wind	Buoy	Vaisala (ultrasonic)	2006	2012	Mendota
Wind	Buoy	RM Young 05106	2013	present	Mendota
IR Skin Temp	Buoy	Apogee SI-111	2013	present	Mendota
PAR-above	Buoy	Apogee SQ-110	2016	present	Mendota
PAR-below	Buoy	Apogee SQ-111	2016	present	Mendota

F3. Lake Manipulation Log

Fish Stocking: Performed by the DNR, annual fish stocking information can be found: https://infotrek.er.usgs.gov/doc/wdnr_biology/Public_Stocking/StateMapHotspotsAllYears.htm For the LTER lakes, the DNR is regularly stocking species such as Muskellunge, Walleye, Lake Trout in Allequash, Big Muskellunge, Sparking, Trout, Crystal (though not since 2006), Fish, Mendota, Monona, and Wingra Lakes.

Little Rock: A curtain was installed and the lake in 1983, and the lake was acidified on one half from 1984-1989. In 2002 there was a tree removal from the lake paired with a whole-tree removal from Camp Lake. There was potentially a fish removal in 2007 performed by Jereme Gaeta. In 2013 the curtain dividing the lake was removed. In 2017, the National Ecological Observatory Network (NEON) established a monitoring site on Little Rock Lake, including an instrumented buoy.

Crystal Lake: From 2012-2013 the water column of Crystal Lake was mixed to eradicate the invasive rainbow smelt from the lake (Lawson et al. 2015, Smith et al. 2018). Additional smelt removal efforts have been ongoing. In 2020, there was a reintroduction of cisco, a native planktivore, to the lake.

Lawson, Z. J., Zanden, J. V., Smith, C. A., Heald, E., Hrabik, T. R., & Carpenter, S. R. (2015). Experimental mixing of a north-temperate lake: testing the thermal limits of a cold-water invasive fish. Canadian Journal Of Fisheries And Aquatic Sciences, 72, 926-937. http://doi.org/10.1139/cjfas-2014-0346

Smith, C. A., Read, J. S., & Zanden, J. V. (2018). Evaluating the "Gradual Entrainment Lake Inverter (GELI) artificial mixing technology for lake and reservoir management. Lake And Reservoir Management, 34, 232-243. http://doi.org/10.1080/10402381.2018.1423586

Sparkling Lake: An intensive crayfish removal effort was undertaken to eradicate the invasive rusty crayfish from Sparkling Lake (Hein et al. 2006, 2007). Efforts included walleye stocking, fishing regulations, and trapping. In the early 2010s, overharvest of fishing regulations were used to control invasive rainbow smelt (Gaeta et al. 2015). In 2020, cisco, a native planktivore, was reintroduced to the lake.

Gaeta, J.W., Hrabik, T.R., Sass, G.G. et al. A whole-lake experiment to control invasive rainbow smelt (Actinoperygii, Osmeridae) via overharvest and a food web manipulation. Hydrobiologia 746, 433–444 (2015). https://doi.org/10.1007/s10750-014-1916-3)

Hein, C. L., B. M. Roth, A. R. Ives, and M. J. Vander Zanden. 2006. Fish predation and trapping for rusty crayfish (Orconectes rusticus) control: A whole-lake experiment.Canadian Journal of Fisheries and Aquatic Sciences 63:383–393.

Hein, C. L., M. J. Vander Zanden, and J. J. Magnuson. 2007. Intensive trapping and increased fish predation cause massive population decline of an invasive crayfish. Freshwater Biology 52:1134–1146.

South Sparkling Bog: From 2019-2021 snow was removed from the ice overlying South Sparkling Bog (Socha et al. 2023).

Socha, E, A Gorsky, NR Lottig, G Gerrish, EC Whitaker, HA Dugan (2023) Under-ice plankton community response to snow removal experiment in bog lake. 10.1002/lno.12319

Wingra: Carp removal occurred under the ice in 2008/2009 (Lathrop et al. 2013).

Lathrop, R.C., D.S. Liebl, and K. Welke. 2013. Carp removal to increase water clarity in shallow eutrophic Lake Wingra. LakeLine, 33(3):23-30. https://www.nalms.org/wp-content/uploads/2018/09/33-3-8.pdf

Mendota: From 1987-1999 there was an intensive food web manipulation in the lake with the goal of inducing a top-down trophic cascade (Lathrop et al. 2002).

Lathrop, R.C., Johnson, B.M., Johnson, T.B., Vogelsang, M.T., Carpenter, S.R., Hrabik, T.R., Kitchell, J.F., Magnuson, J.J., Rudstam, L.G. and Stewart, R.S. (2002), Stocking piscivores to improve fishing and water clarity: a synthesis of the Lake Mendota biomanipulation project. Freshwater Biology, 47: 2410-2424. https://doi.org/10.1046/j.1365-2427.2002.01011.x