Ambient Water Quality Monitoring Plan for Kingfish Maine, Land Based Aquaculture Project Jonesport, Washington County, Maine, USA

Contents

Background

Kingfish Maine, was issued a Maine Pollutant Discharge Elimination System (MEPDES) Permit #ME0037559 and Maine Waste Discharge License (WDL) (W009238-6F-A-N) on June 25, 2021, for a proposed land-based aquaculture project in Jonesport, Maine. Special Condition H of these approvals requires that Kingfish Maine, starting in 2022, monitor ambient water quality both prior to and continuing through the buildout and operation of the permitted facility. This effort will significantly increase the ambient water quality dataset for Chandler Bay, as little background water quality data has been collected prior to this effort. The pre-buildout ambient monitoring will provide an assessment of the background nutrient levels of the ambient waters surrounding and expected to be affected by the facility outflow. This document describes the Ambient Water Quality Monitoring Plan (AWQMP) which will be conducted to fulfill the permit requirement.

Project Organization

Tom Sorby	Tom Sorby Operational Manager Kingfish Maine 33 Salmon Farm Road Franklin, ME. 04634 (502) 614 9078, t.sorby@kinfish-maine.com	Representative of the MEPDES/WDL permittee. Responsible for oversight of the AWQMP and vendor management
Damian Brady	Damian C. Brady, PhD School of Marine Sciences Ira C. Darling Marine Center University of Maine 193 Clarks Cove Road Walpole, ME 04573 207-312-8752, Damian.brady@maine.edu	Responsible for AWQMP implementation, including oversight of sample collection and analysis, data management and security, report production.
Angela Brewer	Section Leader, Marine Unit, Bureau of Water Quality Maine Department of Environmental Protection 17 State House Station, 32 Blossom Lane Augusta, ME 04330 (207) 592-2352, angela.d.brewer@maine.gov	Acts as MEDEP Program lead for all monitoring and data management activities covered under the AWQMP.
Clarissa G. Trasko	Wastewater Compliance Supervisor, Maine Department of Environmental Protection, 106 Hogan Road Bangor, ME 04401, (207) 592-1389, clarissa.trasko@maine.gov	Maine DEP compliance staff person for Kingfish Maine permit.

Other personnel: Trained and experienced University of Maine staff will carry out all field sampling, sample collection, sample analysis, data compilation, data visualization and report production. Staff may be assisted by trained interns, graduate students, or other personnel, but in all cases, those assisting will be properly trained for the tasks performed and will act in accordance with the AWQMP.

Data Distribution Statement

Only data that has undergone quality testing and has been verified ready for distribution, will be distributed. An exception may be made for the entities listed in the above “Project Organization” section. Any data distributed to those entities before quality testing and verification would be watermarked “unverified, preliminary data, not for distribution”.

Ambient Water Quality Monitoring Plan Overview

Stations:

The University of Maine team will sample ambient water quality at a minimum of 4 pre-determined stations designated by Maine DEP (Figure 1, Table 1). Stations established in the MEPDES permit are shown on the map in Figure 1. One station (R1-3) is a previously established ambient monitoring station and is located off Chandler Bay. Station 1 is located near Great Bar. Station 2 is located near Bar Island, Station 3, off the southwest shore of Ballast Island Ledge, near the proposed outfall, and Station 4 is near Bay Ledge. These sites will be monitored in the same order on each sampling date; Station 4 will be sampled first, then Station 3, 2 and 1 in that order. These stations may be altered by MEDEP before the start of the 2022 sampling season. If so, this AWQMP will be updated to reflect the changes.

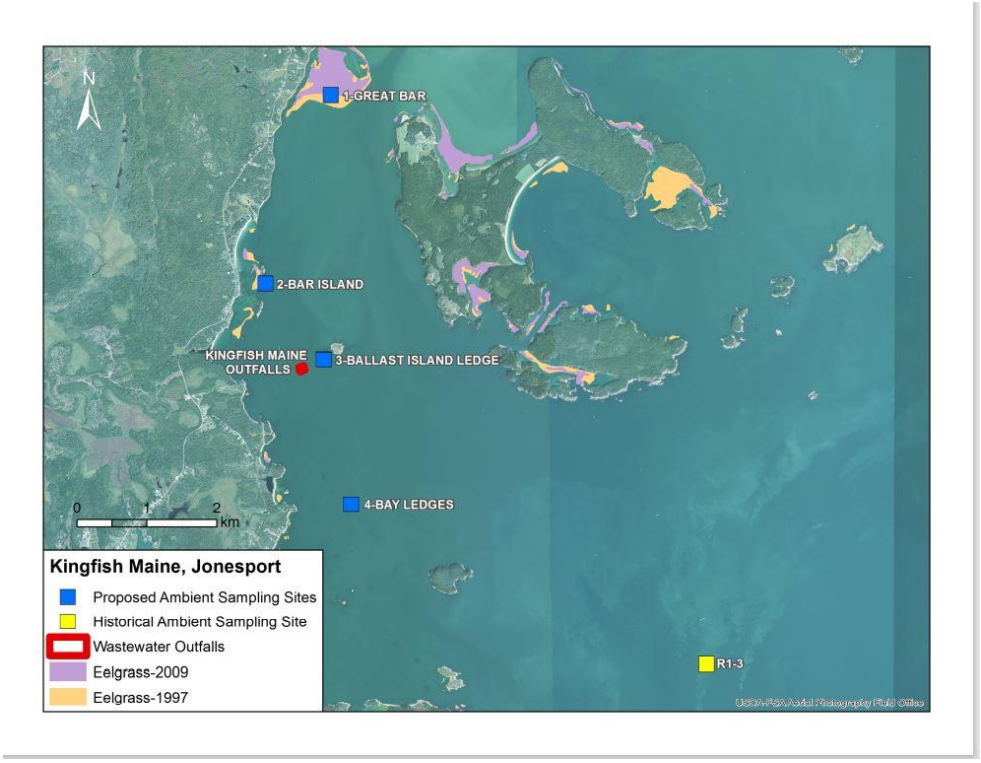


Figure. 1. Ambient monitoring stations established in the MEPDES permit.

Station	Latitude	Longitude
1-GREAT BAR	44.59209	-67.55317
2-BAR ISLAND	44.569433	-67.565643
3-BALLAST ISLAND LEDGE	44.559541	-67.556639
4-BAY LEDGES	44.540773	-67.550927

Table 1. Coordinates of Kingfish Maine ambient water quality monitoring stations

Time of Sampling

The stations will be sampled at 3-week intervals beginning in May of 2022 and extending through October, 2022. Each sampling will occur on the second half of alternating ebb and flood tides within a four-hour sampling window, including 1 hour of slack , ideally during morning hours. Kingfish Maine or the University of Maine (UM) team will provide for a vessel and captain for the sampling trips. For schedule of planned sampling dates, see Table 2. For Field Data Sheet, see Appendix F.

Date	Window	Back up Date	Window
May 10	9:15-13:15	May 11	10:13-14:13
May 31	9:20-13:20	June 1	9:57-13:57
June 21	8:05-12:05	June 22	9:08-12:08
July 13	7:41-11:41	July 14	8:37-12:37
August 4	6:30-10:33	August 5	7:24-11:24
August 25	7:28-11:28	August 26	8:05-12:05
September 20	10:30-14:30	September 21	11:24-15:24
October 11	9:11-13:11	October 12	9:52-13:52

Table 2. Planned primary and backup sampling dates for 2022 ambient monitoring. The samplings are scheduled to alternate the 4-hour sampling window between the 2nd half of ebb and flood tides. Every effort will be made to maintain the sampling schedule. In the event of a major weather event or other circumstance which would prevent sampling on both the scheduled date and the backup date, staff of UM will consult with Angela Brewer before scheduling an alternate date.

Parameters measured

Temperature, depth, salinity, dissolved oxygen, pH, chlorophyll *in situ* fluorescence, and turbidity will be measured by multiparameter sonde. Total Kjeldahl Nitrogen (TKN), Total Phosphorus (TP), Nitrate plus Nitrite (NOx) and extracted chlorophyll *a* and phaeopigment samples will be collected as grab samples and will be analyzed in the Marine Water Quality Laboratory (MWQL) at the Darling Marine Center (DMC) which is accredited by the State of Maine to perform the above laboratory analyses. If, for any reason, the MWQL is unable to analyze the samples, they will be transferred to a laboratory that is also accredited by the State of Maine for the required analysis. The sum of TKN and NOx will provide a measure of Total Nitrogen (TN). A Secchi measurement of water clarity will also be made at each station. For specific SOPs of analytical methods, see Appendix B, C and D. For secchi depth SOP, see Appendix G.

Reporting Summary

Prior to December 31st of 2022, Kingfish Maine will submit a report prepared by the University of Maine team and Kingfish Maine to MEDEP. The report will present the sample data and supporting documents as well as a narrative of results noting any trends or patterns in the data. Grab sample data will be submitted via EDD format to Angela Brewer for review as well as sonde data, notes of field and weather conditions, total water depth at each site at the time of sampling in Excel format.

Sampling Methods

Sampling methods will follow established SOPs (See Appendices). New SOPs will be developed and appended as necessary. Deviations from or amendments to existing SOPs will be identified in the required annual reports, along with justification for such changes.

Preparation for sampling

Sonde Calibration

Prior to and within 24 hours of deployment, the multiparameter sonde will be calibrated following the procedure in Appendix A. Multiple back-up sondes are available for use should the dedicated instrument require service. If a back-up sonde is needed due to malfunction of the primary instrument, a similar procedure will be followed based on the manufacturer's protocol for the model of sonde that is used and may vary slightly due to differences in manufacturer's instructions for calibration. Any deviations from the SOP for sonde calibration presented here, in the instance of the use of a back-up sonde, will be noted in the annual report.

Labware preparation for TKN, TP, NOx, and chlorophyll a samples

For TKN. TP and NOx, grab sample bottles and caps, tubes and caps, syringes and plungers will be acid washed, rinsed with milliQ water (> 18.0 megohm resistivity) and dried. Preservative (sulfuric acid) will be added to the sample vessels for TKN, TP and NOx in the laboratory, prior to sampling. Extracted chlorophyll samples will be collected in brown, HDPE or LDPE bottles which have been cleaned with Sparkleen, a general use laboratory cleaner, and rinsed a minimum of 6 times with milliQ water. Cleaning procedures for all labware are documented in the laboratory records. Coolers will be cleaned and dried. Sample bottles and tubes will be labeled with the project name, station name or #, depth (surface (S), just below thermocline (T), bottom (B)), date, analyte, and the samplers initials.

Enroute to the site, ice will be purchased for coolers to maintain the proper temperature of the samples.

Sonde profiles

Instrumentation

The University of Maine will provide an experienced technician to conduct sonde profiles and collect grab samples for each sampling. A UM owned YSI EXO II multiparameter sonde with handheld computer for user manipulation of settings, parameter readouts, data logging and display, SN: 20E101357, will be used for profiles of water column parameters. The YSI EXO II is equipped with the following sensors (QC specifications from manufacturer):

Parameter	Range	Resolution	Accuracy
Pressure/Depth	0-100 m	0.001 m	± 0.004%.
Temperature (SN: 20D101816)	-0.5 – 50 °C	0.001 °C	0.01 °C
Conductivity (SN: 20D101816)	0-200 mS/cm	0.1 mS/cm	±0.5 % or 0.001 mS/cm w. i. g.
Turbidity (SN: 19M102348)	0 - 4000 FNU	0.01 FNU	± 2% or 0.1 FNU
Chlorophyll fluorescence (SN: 18C105672):	0 - 400 µg/L	0.1 µg/L	0.1 µg/L
pH (SN: 19B102402)	0 - 14	0.01	± 0.1
Dissolved Oxygen	0 - 500 % Air Saturation/0 - 50 mg/l	0.1% Air Saturation/0.01 mg/l	± 1% Air Saturation/± 0.1 mg/L

Table 3. Manufacturers specifications for sonde parameters.

Profile Details:

At each site, the sonde will be submerged and allowed to equilibrate at approximately 1 meter depth. The sonde will then be raised to just below the surface to begin the profile. Measurements will be taken at the following depths at each site, allowing the readings to stabilize a minimum of 2 minutes at each depth sampled:

- 0.1 meters (just below surface),
- 0.5 meters
- 1 meter followed by 1-meter increments to 10 meters total depth.
- At depths greater than 10 meters measurements will be taken in 2-meter increments to a total depth of 20 meters or within 2 meters of the bottom.
- If the water column is very shallow at a station, data may be recorded in 0.5-meter increments instead of 1-meter increments to provide more data points.

Care will be taken not to disturb the bottom, as resuspended sediment can interfere with readings and the sonde can be damaged if it contacts the bottom. A weight will be attached to sonde line and will hang below the sonde to provide an indicator of proximity to the bottom by touching the substrate before the sonde. Total depth at the station will be determined by the boat depth finder.

Sonde data will be saved in real time on the attached handheld control. Each profile will have its own file and be labelled with the station name, date and time. All readings will be taken on the descent of the sonde. A field log will record weather conditions, sea conditions, sampling personnel, sample time and will provide an independent verification of the time, as well as any unusual circumstances surrounding the station sampling. In the lab, environmental as well as calibration data will be uploaded on a MWQL computer using YSI KOR or similar software, to be eventually manipulated in Microsoft Excel or other spreadsheet software.

Grab Sample Collection

Equipment

A weighted 4-liter HDPE Niskin bottle with a metered nylon line and tripping weight will be used for collecting grab samples.

Niskin preparation

The niskin bottle will be rinsed with milliQ water after use and will be stored dry in the lab. The bottle will be rinsed with ambient water before samples are collected.

Depth of Sampling

The Field Data Sheet will be used to record sampling related data and conditions (see Appendix F). Samples will be collected at 1 m and at 0.5-2 meters above the bottom. The goal is to sample 0.5 meters from the bottom, but in rough seas where it is difficult to maintain that distance, samples may be taken at up to 2 meters above the bottom to prevent the bottle from coming in contact with the bottom or with resuspended sediment directly above the bottom. At stations with a bottom depth of more than 20 meters, bottom samples will be taken at 20 meters. The actual depths that the surface and bottom samples were taken will be recorded on the field data sheet. In addition to surface and near bottom samples, if a thermocline is observed in the sonde samples, a grab sample will also be taken just below the thermocline. The thermocline for the purposes of this plan is defined as a drop of more than one degree Centigrade in temperature from one meter to the next. This grab sample will be labelled depth

"T" and the actual depth will be recorded in the field data sheet. The thermocline sample will be analyzed for the same parameters as the surface and bottom samples. Note that for Site 4, if there is a thermocline, a bottom sample need not be taken.

Collection of Grab Samples

TKN, TP, NOx, and chlorophyll *a* samples will be collected from the niskin bottle, which will be gently mixed between draws from the bottle to resuspend any settling particles. Sonde profiles will be collected first. The profile will be examined to determine if a thermocline is present which is defined as a change of more than 1 degree Centigrade per meter of depth. If no thermocline is present, then grab samples will be collected only at the surface and bottom. If a thermocline is present, a sample will be collected just below the thermocline. Water samplers will wear gloves and will take care not to touch other surfaces while wearing gloves. If gloves are contaminated, they will be removed, and a new pair utilized. When handling sample bottles and tubes, the caps and bottle will be handled in such a way as to prevent gloves from touching the inside of the cap or bottle or the rim of the bottle. Bottles will be filled as quickly as possible and capped immediately. TKN and TP samples will be collected in the same bottle. The bottle will contain preservative and will not be rinsed with sample. The NOx samples will be collected in acid washed syringes and filtered through 0.45-micron cartridge filters into acid washed 50 ml centrifuge tubes. For NOx, the syringes will be rinsed with the sample water 3 times before collecting the sample. 5 ml of sample will then be passed through the filter to waste, before collecting 40 ml of sample in the 50 ml tube. The tube will contain preservative and will not be rinsed. Tubes will be capped immediately, and sample will be stored in the cooler on ice. Chlorophyll bottles will not contain a preservative. Chlorophyll sample bottles will be rinsed three times with sample water before collecting the sample. To rinse chlorophyll bottles, up to 50 ml of sample water will be collecting in the bottle. Bottle will be capped and agitated to mix the sample. The rinse water will be dumped downstream of the sampling site. The rinsing will be repeated two more times, after which the bottle will be filled, capped, and stored in a dark cooler on ice.

Labelling of Grab Samples/Recording of Field Data

The project name, station, date and time of sampling, the depth of sampling, weather, sea conditions, names of staff collecting samples. volume of samples collected, and any unusual circumstances will be recorded in the field notebook. Field records will be recorded on water-proof paper. Data from field sheets will be scanned and manually digitized in the laboratory. Bottles will be labelled with project, date, station, time of sampling, depth, sampler's initials, and parameter.

Replicate Samples

For each parameter, one duplicate sample will be taken for every ten samples collected. If there is not enough water in one Niskin cast to collect duplicates for all parameters at one station, the duplicates may be split amongst 2 stations. For instance, TKN, TP duplicates collected from station 1 and chlorophyll a and NOx duplicates from station 3.

Garb Sample Field Storage and Transport (see Appendix E for MWQL Sample Handling SOP)

Immediately after collection in the field, sample bottles will be placed in Ziploc bags and stored in coolers on ice, in the dark. Samples will remain in the coolers until arrival at the MWQL. Loose ice will surround

the samples in the cooler. Temperature of the coolers will be monitored by either measuring the temperature of a water sample that has been stored with the environmental samples during transit. Samples will be stored between 0 ^{o}C and 6 ^{o}C.

Sample Handling in the MWQL

Standard operating procedures (SOPs) will be followed for the sampling, preservation, transportation, and storage of surface water grab samples (Appendix B, C, D & E). Special permission has been granted for the MWQL to allow an 8-hour period between collection and filtration for the Kingfish Maine (Chandler Bay) chlorophyll samples due to the long travel distance required and the availability of test data showing no significant differences in chlorophyll or phaeophytin data obtained from test samples filtered at 6 and 8 hours after collection(see Appendix H). Each sample will have a unique MWQL identification which will consist of MWQL batch code which includes the date and project, station code, depth, and replicate number (if more than 1 sample is taken). This unique identification can be traced to the field data sheet and the Chain of Custody form, which will contain additional information. Samples will be kept within temperature parameters specified by the laboratory SOPs and this document, until analysis. Temperatures of refrigerators and freezers used to store MWQL samples will be monitored on each day that the MWQL is in operation, and the readings will be recorded in a log.

Method	Analytes	Holding Time (days)	MDL (12/31/21)	RL (12/31/21)	Units
SM10200H	Chlorophyll a and Phaeophytin	21	0.003	0.010	µg/l
EPA 351.2	Total Kjeldahl Nitrogen	28	0.153	0.172	mg/l
EPA 365.4	Total Phosphorus	28	0.074	0.083	mg/l
EPA 353.2	Nitrate plus Nitrite	28	0.010	0.020	mg/l

Table 3. Methods, holding times, minimum detection limit (MDL0 and reporting limit (RL) of MWQL methods.

Chain of Custody

A chain-of-custody form will be completed the day of sampling and will document the following:
Date and time of collection, person collecting the samples, station, depth, parameter to be analyzed, project name, unique MWQL batch code, filtered/not filtered, volume of sample collected, temperature of cooler upon arrival at the laboratory, time and date of arrival at the laboratory, receiving and relinquishing staff members, Storage location in laboratory, time of placement in laboratory storage, date of any processing or analysis of the samples (details of each action will be contained in laboratory notebooks or forms specific to the actions being performed), date and time of any transfer of samples to outside facilities, any deviations from the SOPs or unusual field conditions which may impact the quality of the sample as well as identifying information for any instruments, such as thermometers used in samples monitoring.

Quality Objectives

Sonde data

All uploaded sonde data spreadsheets will be quality-checked and formatted, at which point the raw data files on the handheld computer or sonde will be deleted from the file directory. Criteria used to validate raw profile data from discrete sampling will follow Table 6 of MDEP's marine monitoring program (MDEP 2017). For raw profile data from discrete sampling, if a data value falls outside of the relevant acceptable range, best professional judgment may override these criteria when supporting data or information suggests a real aberration. If no reasonable explanation exists for an aberration, the data value will be flagged in the raw data file and not included in subsequent analyses and reporting.
Sonde data will be checked for extreme values using conditional formatting in an Excel spreadsheet. See Table 5 for ranges. In the event data is outside of these limits, it will be examined further and flagged.

Parameter	Minimum Value	Maximum Value	
Depth	0.05 m	20 m	Range based on expected conditions within Maine marine surface waters and protocol maximum
Temperature	5 ^{o}C	25 ^{o}C	Range based on expected seasonality of sampling
Salinity	0 psu	35 psu	Range based on expected location of sampling in Maine marine waters
Dissolved Oxygen	20% saturation	150% saturation	Range based on expected seasonality and location of sampling in Maine marine waters, including extremes based on primary productivity
pH	6.5	9.5	Extremes based on algal bloom conditions, should be verified with dissolved oxygen data and time of da
Turbidity	0 FNU	25 FNU	Negative values should be corrected to 0.0, high values should be verified with adjacent data values and proper functioning of probe wiper
Chlorophyll a	0 µg/L	25 µg/L	Max. value based on algal bloom

Table 5. Ranges of acceptable data for sonde measurements. Data outside of these ranges will be flagged if no reasonable explanation exists for the aberration.

Grab Samples

Precision: For activities covered under this AWQMP, precision will be measured through an assessment of laboratory sample duplicates, replicate blank matrix samples and duplicate spiked matrix samples. The variability of laboratory blanks and standards will be used to evaluate the precision of the analytical method. The variability of both the blank and spiked matrix sample will be used to calculate the minimum detection limit and the reporting limit of the analytical method. Specific procedures are available in the specific protocol SOPs.

Accuracy: To determine the accuracy of the laboratory procedures, certified standard solutions (laboratory control samples (LCS)) will be analyzed with each sample run, with the exception of chlorophyll

a in which a solid standard is analyzed with each run, to determine the if the readings have drifted from the values obtained during the last calibration with certified standard. The chlorophyll fluorometer is calibrated with liquid certified standard two times per year. For all parameters except chlorophyll a, a certified standard will also be used to spike environmental samples to assess the recovery of the certified standard.

Representativeness: Sampling stations are chosen based on the current knowledge of the movement of water in the vicinity of the proposed outflow. MEDEP will select stations based on the monitoring goals and objectives. The sampling will take place in a 4-hour window around the second half of alternating ebb and flood tides with 1 hour of slack tide in the sampling window. This will allow for sampling at both high and low tide to document any variability with the tide cycle. Sampling will take place in May through October which will capture seasonal variability. The assessment of duplicate grab samples for each sampling event (1 duplicate for each 10 samples collected) will provide and assessment of sampling variability.

Comparability: Comparability will be addressed by making and recording observations and collecting samples using methodology approved by the MDEP, by using field staff trained and experienced in the sampling methods, and by using methods approved by the MDEP performed by a laboratory accredited by the State of Maine for those methods. Additionally, comparability with historical MDEP datasets will be maintained as much as possible by maintaining regular communication with the MDEP Marine Unit. MDEP Marine Unit staff will be welcome to participate in any or all sampling events throughout the year.

Reporting QC Data

Quality control data included in the laboratory reports will include, at a minimum, equipment blanks, field duplicates, laboratory control samples, and matrix spikes for nutrient analyses. Laboratory reports will also contain a case narrative or equivalent notation, list of qualifiers and definitions, copies of COC form that includes a cooler receipt and preservation check form indicating sample temperature. The samples will be in the possession of University of Maine staff from the time of collection to arrival in the laboratory. When quality control objectives are not met and best professional judgement determines possible field operator or analytical laboratory error, the responsible party will be contacted, and resolution sought. For field readings, data may be flagged as estimated (“J”) or rejected (“R”) if no correction is possible. For laboratory concerns, the laboratory analyst will be asked to address specific questions, and samples will be rerun if sufficient sample remains and holding times can still be met

Special Training/Certification

Field sampling staff using sonde equipment will be trained and monitored by Damian C. Brady, PhD, or qualified staff member under his supervision and in accordance with University of Maine requirements. All data analysis and reporting will be subject to the review and approval of Damian C. Brady, PhD, University of Maine. The surface water grab samples will be analyzed at the University of Maine Marine Water Quality Laboratory (MWQL), a Maine State certified laboratory. All staff will be trained in relevant SOPs and protocols, all required UM safety training as well as data integrity training.

Documents and Records

Changes to AWQMP

Proposed changes this AWQMP may result from field conditions, equipment failure or other extenuating circumstances. If a sampling date must be changed, every attempt will be made to reschedule as soon as

possible and to ensure that the new date complies with the above stated conditions. Changes in field procedures may be agreed upon as MDEP staff participate in field sampling events, in which case such changes will be documented in a revision to this AWQMP. Other recommended or necessary changes will be proposed as a revision to this sampling plan and subject to MDEP review and approval. Revised Sampling Plans will be identified by a unique version number.

Standard Operating Procedures

Standard Operating Procedures will be a part of Kingfish Maine's and UM's quality assurance program. For vertical sonde profiling, instruments must be calibrated according to manufacturer's instructions prior to each sampling event and the SOP (Appendix A) must be followed. The MWQL will follow specific SOP procedures included in Appendix B-G.

Data Management

Record Keeping

Records will include equipment calibration information and logs, field data sheets, chain-of custody forms, internal laboratory documents, forms and notebooks, lab reports, field notes, sonde field data and field notes. All information, including lab-generated data, will be kept in digital files maintained by the University of Maine MWQL. Lab-generated grab sample data will be submitted in EDD format to MEDEP. Files may not be deleted or removed from hand-held devices until an electronic or hard copy of the data has been saved appropriately and subjected to data QA/QC screening. Anomalous or inconsistent field or lab results will not be deleted but will, instead, be flagged in data sets.
All data will be backed up to secure cloud storage. The MWQL uses role-base data access control principles which limit access to data and documents to those specifically authorized as outlined it the MWQL Quality Assurance Manual.

All data resulting from this monitoring program will be managed by the University of Maine. The University of Maine field measurements and the lab results will be maintained at the Darling Marine Center.
All monitoring information will be recorded onto field datasheets that will be developed prior to field sampling. All hard copies of lab field notebook will be electronically duplicated and preserved. Sonde data are logged to internal memory on either the handheld computer or sonde, and then raw files are uploaded to spreadsheets upon return to the office and prior to a subsequent sampling event

Reporting

Kingfish Maine will provide the MDEP with an annual report of the monitoring results on or before December 31st of each year. In addition to data tables, graphs, and figures, the report will compare results with previous sampling data and note any anomalous or unusual results. As appropriate, the annual report will identify necessary changes to field sampling methods, this AWQMP, or other elements of the monitoring program and, potentially, exclusion from reporting results. Flagged data, if any, will be described in annual reports submitted to MDEP.

Appendix A

UM MWQL Multiparameter sonde data collection Standard Operating Procedure (SOP) – YSI EXO II

Document Code: MWQLSOPSonde Rev. 2, 2/24/2022

Table of Contents

Specifications:

This method is applicable to marine and freshwater samples.

Applicable concentration ranges:

- **Temperature: 0-40°C**
- **Salinity: 0-40psu**
- **Dissolved oxygen: 0-150%**
- **pH: 4-10**
- **Chl a: 0-100µg/L**
- **Turbidity: 0 – 1000FNU**

Definitions:

The definitions and purposes below are specific to this method but have been conformed to common usage as much as possible.

CALIBRATION -- A set of operations that establish, under specified conditions, the relationship between values of quantities indicated by a measuring instrument or measuring system, or values represented by a material measure or a reference material, and the corresponding values realized by standards. In calibration of support equipment, the values realized by standards are established through the use of reference standards that are traceable to the International System of Units (SI).
In calibration according to methods, the values realized by standards are typically established through the use of reference materials that are either purchased by the laboratory with a certificate of analysis or purity or prepared by the laboratory using support equipment that has been calibrated or verified to meet specifications.

CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

MilliQ WATER - Reagent water that has been treated to remove impurities through reverse osmosis and filtration and has a resistivity above 18.0

Quality control measures:

All laboratory personnel using this SOP will also comply with all general laboratory QA and QC measures as stated elsewhere in the MWQL QAM. QA and QC procedures specific to this protocol are documented in this SOP.

The sonde will be calibrated according to manufacturer's specifications for each parameter the business day prior to being used to collect data. If any calibration is not successful, another calibration will be performed in order to ensure sensor is working correctly. Sensors have a limited lifetime of 1-3 years depending on type, sensor age will be monitored and replaced when life span is exhausted. If sondes area deployed, they will be calibrated prior to and after deployment.

Summary of Method:

Application:

This method is used to obtain in situ water quality measurements for depth, temperature, salinity, dissolved oxygen, pH, chlorophyll *a*, and turbidity. The sonde will be powered by internal batteries and controlled using a battery powered handheld device.

Summary of Method:
A multiparameter sonde is used to collect in situ water quality parameters at discreet depths throughout the water column. Typical parameters include temperature, salinity, dissolved oxygen, pH, chlorophyll *a*, and turbidity.

Interferences:

- The turbidity at the bottom depth can be artificially increased by the instrument hitting the bottom. To avoid this, a window weight is attached to the sonde such that when the window weight touches the bottom and lays on its side, the sonde is 0.5m above the bottom. When lowered gently this greatly reduces the incident that the bottom sediment is stirred up, artificially increasing turbidity.

Safety:

Hazards: This protocol does not require the use of any hazardous materials. All calibration solutions are non-hazardous waste. There are no known hazards associated with this protocol.

Equipment and Supplies:

- YSI EXO 2 multiparameter sonde with temperature and conductivity, dissolved oxygen, pH, total algae, and turbidity sensors installed.
- Calibration solutions
    - Specific conductivity solution (50,000µS/cm for salt water, 1,000µS/cm for fresh water)
    - pH 4, 7, and 10 buffers (for both salt and fresh water)
    - Turbidity standard (12.4FNU or 124FNU depending on expected range in situ)
    - Rhodamine WT
    - MilliQ water
    - Tap water
- Supplies
    - Aquarium stone and pump
    - YSI EXO2 calibration cup
    - Computer with KOR software
    - 1-liter volumetric flasks
    - Magnet wand to turn on sondes
    - 10 ml pipettor with tip
    - 0.5 - 1-liter beakers or jars
    - Dedicated 50 ml and 1-liter flask for rhodamine calibration

In situ water quality parameter measurements:

1. The multiparameter sonde is turned on 10 minutes prior to taking measurements to allow instrument to warm up and stabilize readings. The sonde will activate automatically when it is plugged into the handheld and the handheld is turned on.
2. Once on site at anchor, the sonde is lowered into the water and deployed to 0.5 meters above the bottom or just below the surface where it is held until all reading are stabilized. Although casts may be taken from the bottom up or the top down, for the purposes of this sampling plan, measurements will be taken from surface to bottom.
    1. If the overall water depth is greater than 5 meters, a measurement is recorded at 0.5 meters depth, then at 1 meter depth, then every meter until a depth of 10 meters. At depths greater than 10 meters, a measurement is taken every 2 meters finishing at 0.5 m below the surface.
    2. If the water column is less than 5m deep, a measurement is recorded at 0.5 meters, then every half meter, finishing with 0.5m above the surface. Actual depths may vary according to project goals.
3. At each depth interval, the sonde is held at the specific depth until all sensor readings have stabilized, then the measurements are recorded to the electronic handheld computer.
4. Each profile is given a unique name including the station # and date. Files are stored on internal memory of the handheld until return to the laboratory.
5. Field data is also recorded in a waterproof notebook.

Reagents and Standards:

All calibration solutions are purchased from the manufacturer and are used as is. No solutions are created in the lab.

Calibration:

The instrument is calibrated one business day before collecting measurement.

Calibration Procedure:

- A 5l bucket is filled with 4l of tap water and an aquarium pump and stone is used to bubble air through the water to supersaturate it for at least 1 hour prior to calibration.
- The sonde is turned on and connected to the KOR software. If the sonde is connected directly to the laboratory computer, it will activate and the magnetic wand is not needed. If the sonde is connected to the computer via Bluetooth, then users activate Bluetooth wireless by holding a magnet at the magnetic activation area on the sonde. When the blue LED on the sonde is off, the Bluetooth is disabled. When the light is on continuously, the Bluetooth is enabled, but no link has been established. When the blue LED blinks at 2 Hz, the sonde's Bluetooth is on, and has established a link."
- The sonde is placed with guard on into the bucket of bubbling water. The barometric pressure is noted (from the handheld computer) and used to calibrate the percent saturation of the water at the current atmospheric pressure. Once the readings are stable, the calibration is accepted.

- The conductivity sensor is calibrated using specific conductance. The sensor is rinsed with the old conductivity solution of the appropriate strength (50,000µS/cm for salt water, and 1,000µS/cm for fresh water) by submerging the sensors in a jar of the standard. The sonde is then submerged in a jar of new calibration solution of the correct strength. Once readings are stable, the calibration is accepted.
- For pH calibration, the sonde is first placed in the calibration cup with a pH 4 rinse (old standard solution) and submerged 3 times to avoid cross contamination between standards. The sonde is then placed in the freshly opened calibration solution and allowed to stabilize. The calibration is accepted once stable, then the user can proceed to pH 7 and 10 using the same process following the directions on the handheld.
- The turbidity is calibrated using a two-step process. First the turbidity sensor is calibrated in milliQ water for a zero reference. If a copper guard is being used during sampling (only used for long-term deployments), then the reference is 0.5. The sonde is placed in milliQ water, and the readings are allowed to stabilize before calibration point is accepted. Depending on the expected turbidity range of the sampling location, and the upper limit of either 12.4 FNU or 124 FNU will be used. The sensors are submerged in a rinse of old solution of the same strength, then submerged in fresh calibration solution until readings are stable.
- The total algae sensor is calibrated using a 1-point calibration with MilliQ or distilled water as a zero reference. The sensor is calibrated using relative fluorescence units (RFUs). The sensors are rinsed then submerged in fresh MilliQ/distilled water and readings are allowed to stabilize before accepting the calibration. In the instance where very high chlorophyll *a* concentration is expected (>30µg/L), a two-point calibration can be used with a Rhodamine WT solution with a value of 66µg/L for the upper limit. The sensor should be calibrated with rhodamine WT at least two times per year.
    - Two step-Calibration (follow instructions in software)

        - Zero
            - Use MilliQ for zero blank
        - Second Point
            - Use 125 mg/l Rhodamine WT in a dark bottle located in the chemical fridge in BGC as primary stock.
            - To make working solution stock: pipet 5 ml of the primary stock of rhodamine WT into a 1 L flask
                - Note: there is a dedicated labeled volumetric flask for Rhodamine kept on the shelf to the upper left before entering the microscope room. Do not use any other flasks for Rhodamine.
                - Put stock, pipette and flask in glass tray to catch any spills or drips.
                - Leave flask in the in the dark while running the zero blank to allow it to come to room temperature (microscope room/cabinet until you use it)
            - Fill the flask to 1 L with MiliQ water
            - Cap the flask and invert a couple of time to mix, the working solution can be kept for 24 hours
            - Pour the dilute Rhodamine directly in to the Sonde cup and rinse the sensor
            - Dispose of the rinse liquid and refill for calibration
            - Calibrate, use “RFU” instead of “Chlorophyll in ug/L”

- Refer to the total algae calibration table in the manual (p. 136) to get the temperature dependent value to input into the calibration
- When finished dispose of the Rhodamine working solution into the waste container in the lab.

Pollution Prevention and waste management:

Although none of the solutions used in this procedure are hazardous, they are still treated as waste according to the following procedures. All wastes will be handled in accordance with University of Maine Safety and Environmental Management Guidelines. Waste will be stored in properly labeled containers with secondary containment. Waste will be located in approved areas and will be inspected weekly. When waste containers are full, they will be transported by the Darling Marine Center Safety Officer to a secure dedicated waste storage facility on the DMC campus. At the point of pick up and transport to the DMC waste storage facility, the responsibility for monitoring of the waste is transferred to the DMC safety officer. Waste will be stored until pickup by a University of Maine approved waste disposal company.

Appendix B

UM MWQL Nitrate Plus Nitrite (NOx) Standard Operating Procedure (SOP)

Document Code: MWQLSOPNOX Rev. 3, 2/18/21

EPA Reference: EPA 353.2

Table of Contents

Specifications:

This method is applicable to marine and freshwater samples.

Applicable concentration ranges:

.006-0.4 mg NOx - N/l

DEFINITIONS

The definitions and purposes below are specific to this method but have been conformed to common usage as much as possible.

ANALYST - The designated individual who performs the “hands-on” analytical methods and associated techniques and who is the one responsible for applying required laboratory practices and other pertinent quality controls to meet the required level of quality.

ANALYTICAL BATCH -- An analytical batch is a group of environmental samples that are prepared and/or analyzed together with the same process and personnel, using the same lot(s) of reagents. A batch is composed of at least one and no more than 20 environmental sample(s) of the same quality systems matrix, meeting the above-mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 24 hours. An analytical batch is composed of prepared environmental samples (extracts, digestates or

concentrates) which are analyzed together as a group. An analytical batch can include prepared samples originating from various quality system matrices and can exceed 20 samples.

CALIBRATION -- A set of operations that establish, under specified conditions, the relationship between values of quantities indicated by a measuring instrument or measuring system, or values represented by a material measure or a reference material, and the corresponding values realized by standards. In calibration of support equipment, the values realized by standards are established through the use of reference standards that are traceable to the International System of Units (SI).

In calibration according to methods, the values realized by standards are typically established through the use of reference materials that are either purchased by the laboratory with a certificate of analysis or purity or prepared by the laboratory using support equipment that has been calibrated or verified to meet specifications.

CALIBRATION BLANK (CB) -- A volume of reagent water in the same matrix as the calibration standards, but without the analyte.

CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

CERTIFIED REFERENCE MATERIAL -- (CRM) means reference material, accompanied by a certificate, having a value, measurement of uncertainty and stated metrological traceability chain to a national metrology institute

CHAIN OF CUSTODY FORM (COC) -- A record, either paper-based or electronic, that documents the possession of the samples from the time of collection to receipt in the laboratory in accordance with chain-of-custody protocol. This record, at a minimum, must include the sample location, the number and types of containers, the mode of collection, the collector, the date and time of collection, preservation, and requested analyses. See also Legal Chain of Custody Protocols.

FIELD BLANK (FB) -- An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, including exposure to a sample bottle holding time, preservatives, and all pre-analysis treatments. The purpose is to determine if the field or sample transporting procedures and environments have contaminated the sample.

FIELD DUPLICATE (FD) -- Two samples taken at the same time and place under identical circumstances which are treated identically throughout field and laboratory procedures. Analysis of field duplicates indicates the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

LABORATORY BLANK (LB) -- An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, except that it is not taken to the sampling site. The purpose is

to determine if the if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

LABORATORY CONTROL STANDARD (LCS) -- A solution prepared in the

laboratory by dissolving a known amount of one or more pure compounds in a known amount of reagent water. Its purpose is to assure that the results produced by the laboratory remain within the acceptable limits for precision and accuracy. (This should not be confused with a calibration standard).

LABORATORY DUPLICATE (LD) -- Two aliquots of the same environmental sample treated identically throughout a laboratory analytical procedure. Analysis of laboratory duplicates indicates precision associated with laboratory procedures but not with sample collection, preservation, or storage procedures.

LABORATORY FORTIFIED SAMPLE MATRIX (LFSM)-- An environmental sample containing an analyte spike of known concentration at least 4 times the MDL. Used to evaluate the effect of the sample matrix on the analytical results.

LIMIT OF QUANTITATION (LOQ) -- The minimum levels, concentrations, or quantities of a target variable (e.g., target analyte) that can be reported with a specified degree of confidence.

METHOD DETECTION LIMIT (MDL) -- The lowest level at which an analyte can be detected with 99 percent confidence that the analyte concentration is greater than zero.

MilliQ WATER - Reagent water that has been treated to remove impurities through reverse osmosis and filtration and has a resistivity above 18.0.

QUALITY CONTROL CHECK SAMPLE (QCCS) -- A sample containing analytes of interest at known concentrations (true values) of analytes. The QCS is obtained for a source external to the laboratory or is prepared from standards obtained from a different source than the calibration standards. The purpose is to check laboratory performance using test materials that have been prepared independently from the normal preparation process.

Quality control measures:

All laboratory personnel using this SOP will also comply with all general laboratory QA and QC measures as stated elsewhere in this QAM. QA and QC procedures specific to this protocol are documented in this SOP.

Initial Demonstration of performance:

- Method Detection Limit (MDL) - The minimum detection limit will be determined by analyzing blank variability and by spiking a blank with analyte at a concentration from 2 to 10 times the estimated MDL. Seven blanks and seven MDL spikes will be carried through all aspects of preparation and analysis in a minimum of three separate sample batches on three separate calendar dates. The reported MDL will be the larger of the MDL calculated from the blanks and the MDL calculated from the spiked matrix samples.
    - To determine the MDL of the spiked matrix samples:
        - Calculate the standard deviation of the 7 spikes.
        - Multiply the standard deviation of the spiked matrix by 3.143 (for seven replicates).
    - To determine the MDL of the blanks:
        - Calculate the mean of the method blanks.
        - If the mean is negative equate it to zero.
        - Multiply the standard deviation of the blanks by 3.143 (for seven replicates) and add the result to the mean.
- Limit of Quantitation - Obtain the limit of quantitation by calculating the mean and standard deviation of replicate standards between 2 - 10 times the estimated or known LOD. Multiply the standard deviation by the t value obtained from the t test table for the replicate degrees of freedom and the desired cumulative probability based on a two tailed test. For instance, the 99% cumulative probability.
- Quality Control Check Sample (QCCS) - Semi-annually, a QCCS will be ordered from an EPA recommended provider lab. The sample will be analyzed, and results sent to the provider lab. If results are not within the acceptable limits of stated values, the source of the problem will be identified and corrected.

- Demonstration of Linearity - A standard curve over the working range (0.05 - 0.40 mg/l) of the method will be run once per year to demonstrate linearity.

Ongoing quality control measures:

- Duplicate Laboratory Blanks - Duplicate lab reagent blanks will be analyzed with each batch of samples.
- Laboratory Control Standard - Duplicate LCSs are analyzed with each batch of samples, no less than one for every ten samples to determine the accuracy of the analytical protocol. If the measured analyte concentration varies by more than 10% from the

certified concentration, the analysis will stop, and the source of error will be determined before sample analysis resumes.

- Calibration Standards - A set of CAL standards, covering 4 concentrations that span the expected sample concentrations, will be analyzed with each batch of samples.
- A CAL standard at or below the RL will be analyzed at minimum once per month. The concentration obtained should be within 40% of the true concentration.
- Laboratory Fortified Sample Matrix - 10% of the analyzed samples will be spiked with analyte to give a concentration at least four times the MDL prior to digestion. The percent recovery will be determined from these samples. If the percent recovery falls outside to the 80%-120% range, analysis will stop, and the source of the error will be determined and resolved before continuing analysis.

- Laboratory Duplicates - A set of LDs will be analyzed in each batch of samples. Duplicates should not vary more than 10%.

Summary of Method:

Application:

This method is used to obtain the total concentration of nitrate plus nitrite (NOx), from filtered freshwater and seawater samples. The method is appropriate for samples with total concentration up to 0.4 mg/l NOx - N or 400 µg/l NOx - N. Samples with higher concentrations of NOx -N may be diluted and the diluted sample analyzed using this protocol.

Summary of Method:

A filtered sample is mixed with a pH 8.5 buffer. The mixture of sample and buffer passes through a column containing reduced granulated copper-cadmium which provides hydrogen for the reduction of nitrate to nitrite. The nitrite (consisting of nitrite that was originally present plus reduced nitrate) is determined by diazotizing nitrite with sulfanilamide and coupling with N-(1-napthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye which is absorbs light at a wavelength of 540 nm. The dye is detected spectrophotometrically and is proportional to the total nitrite concentration in the sample. Though the method is designed for seawater and brackish water, it is also applicable to freshwater sample matrices. The method is calibrated using standards prepared in deionized water. Once calibrated, samples of varying

salinities (0 to 35 ppt) may be analyzed. The determination of background is necessary only for samples which have color absorbing at 540 nm

Interferences:

- Particulate matter can clog the cadmium reduction column restricting flow and reducing sensitivity. To avoid this issue, samples are filtered before analysis.
- Samples containing high levels of metals such as copper and iron, may have reduced response. Increasing the EDTA concentration in the buffer can mitigate this problem.
- Significant amounts of oil and grease in samples, interfere with the reduction process and can damage the cadmium reduction column. Samples with visible oil and grease will not be analyzed.
- Samples that absorb significantly at 540 nm, can be analyzed with and without the color reagent to determine the background absorbance.
    - In our experience samples with significant absorbance at 540 nm are rare in environmental water samples.
    - If samples are colored this interference can be determined and subtracted.
        - First, calibrate in the standard fashion.
        - Next, replace the color reagent with a solution containing 100 ml/L H_3PO_4.
        - Finally, reanalyze the samples.
        - The color interference signal can then be subtracted from the sample signal.
- All plastic and glassware must be thoroughly cleaned to avoid contamination.

Safety:

Hazards: This protocol requires the use of hazardous chemicals. All laboratory personnel using this protocol must be current with all University of Maine safety training requirements.

Hazardous chemicals: Read SDS sheet for all chemicals before use. Wear appropriate personal protective equipment including appropriate gloves, lab coat and safety glasses. The following list highlights some of the hazards of the listed chemicals but IS NOT intended to replace thorough reading of the SDS sheets by laboratory personnel.

Phosphoric Acid (CAS#: 7664-38-2). Corrosive.

- **Sodium nitrite (CAS#: 7664-38-2). Oxidizer. Toxic.**
- **Sodium hydroxide (CAS#:1310-73-2). Toxic. Corrosive.**
- **Ammonium Chloride (CAS#:1310-73-2). Irritant.**
- **Cadmium in reduction column 9CAS#: See SDS from Hach. Toxic.**

Hazardous Liquids Under Pressure: Liquids being pumped through the Lachat manifold are under pressure and can release in s spray if a leak develops.

Equipment and Supplies:

- 50 mL conical tubes (50 ml conical screw cap HDPE centrifuge tubes) for sample storage, one for each sample.
- Bottles -
    - 1 - 1 liter bottle for Buffer.
    - 1 - 1 liter bottle for MilliQ carrier.
    - 1 - 1 liter bottle for MilliQ Rinse water.
    - 1 - 1-liter dark bottle for Sulfanilamide Solution.
- Sample collection tubes (100 ml or larger) or bottles (acid washed), one for each sample.
- 1 - Milli-Q squirt bottle.
- Analytical Balance, accuracy to 0.1 mg.
- Stir plate
- Ring stand and clamp.
- Lachat QuickChem flow injection analyzer with autosampler and NOx manifold.
- Stir plate
- 0.45-micron disposable filter cartridges for filtering samples. Note: disposable or reusable cartridges can be used. Reusable cartridges are acid washed and loaded with 0.45 um membrane filters before use.
- Several 50 ml syringes for filtering samples.
- 1- 10 ml pipette with tips.
- 1 - 1 ml pipette with tips.
- 1 - 1 0.1 ml pipette with tips.
- Volumetric flasks:
    - 1 - 1-liter volumetric flask for buffer solution.
    - 1 - 1-liter volumetric flask for sulfanilamide solution.
    - 1 - 250 ml volumetric flask for primary standard.
    - 7 - 50 ml volumetric flasks for secondary and working standards.
    - 1 - 50 ml flask for the LCS working solution.
- pH meter and probe with calibration buffers, milliQ squirt bottle, rinse beaker.
- Stir bars.
- Sulfuric acid solution for preserving samples.
- Sodium hydroxide solution for adjusting sample pH.

Glass and plasticware preparation:

All glassware and plasticware in contact with samples will be:

- Washed with Sparkleen detergent and rinsed 5 times with milliQ water.
- Acid rinsed using 0.5 M - 1.5 M hydrochloric acid solution and rinsed thoroughly with milliQ water.
- This includes bottles, tubes, syringes filter cartridges.

Sample collection and preservation:

Sample Collection:

- Samples (100 ml) are collected in clean, acid rinsed bottles or tubes (see above).
- Samplers must wear gloves and be careful not to touch any unclean surfaces with gloves before or during sampling.
- Samples may be collected from Niskin bottles or directly from river, stream, estuary, or other water source.
- Collection of sample:
    - Rinse collection bottle with sample.
        - Fill the bottle with a volume of sample sufficient to rinse the interior of the bottle (for a 100 ml bottle, 10 ml is adequate).
        - Cap bottle, invert, and swirl sample in the bottle to rinse the entire inner surface of the bottle.
        - Dispose of rinse water downstream of the sampling site.
        - Repeat 2 times.
    - Fill the bottle and cap.

Sample filtration:

- Samples are filtered through a 0.45-micron syringe cartridge filter.
    - Disposable or reusable filter cartridges may be used.
        - If using disposable filters, keep them in a clean Ziploc bag before use and dispose of used filters in a separate garbage bag.
        - If using reusable cartridge filters:
            - Acid rinse cartridge top and bottom and Teflon O-ring.
            - Dry in the drying oven.
            - Add a clean 0.45-micron membrane filter and assemble cartridge.
            - Store cartridges in a clean Ziploc bag until use.
    - The syringe, plunger and filter must be rinsed with the sample before use.
        - Remove filter cartridge from syringe and pull plunger out of syringe being careful not to let the syringe, syringe plunger or cartridge touch any unclean surfaces.
        - Reattach filter to syringe.
    - Pour about 5 ml of sample from the collection bottle into the top of the syringe.
    - Reinsert plunger into the syringe and push the sample through the syringe to waste.

        - Be sure to dispose of the waste rinse sample downstream of the sampling location.
    - Repeat two more times to adequately rinse syringe, plunger, cartridge, and filter.
    - Remove cartridge from syringe and remove plunger from syringe.
    - Reattach cartridge to syringe.
    - Fill the syringe with the sample.
    - Insert plunger into syringe,
    - If the tube for filtered sample does not contain preservative:
        - Push about 5 ml of samples through the filter and into the collection tubes,
        - Cap tube, shake to rinse.
        - Dispose of sample.
        - Repeat two more times.
        - It may be necessary to refill syringe with sample to provide adequate filtered sample.
        - Then filter a total of 30 ml of sample into the collection tube,
    - If the collection tube for filtered samples does contain preservative, it is not possible to rinse the tube with filtered sample. Use the following protocol:
        - Push 10-15 ml of samples through the filter to waste.
        - Fill collection tubes with 30 ml of the sample.

Sample Storage:

- Store samples in the cooler with ice packs immediately after filtration.
- If samples are not preserved in the field and are not to be analyzed immediately, they are kept in the dark on ice until preservation in the laboratory.
- Samples must be preserved within 8 hours of collection.
- Samples are preserved by adding sufficient 0.5 M sulfuric acid to lower the pH to < 2.0 within 8 hours of collection or by adding sufficient acid to the collection tube prior to filing with the filtered sample.
- Preserved samples are stored at < 6 degrees C in a refrigerator for up to 28 days.
- Before analysis, the pH of the samples must be raised to between 5 and 9 with the addition of 50% sodium hydroxide. See detailed method below.

Reagents and Standards:

Reagents should be made up the day before analysis at the latest. Reagents made on the same day as analysis, can give variable results.

1. **MilliQ water -**
    a. Resistivity greater than 18.0.

b. Water is pretreated with particulate and carbon filters, passed through a reverse osmosis membrane, filtered through a Millipore milliQ clean water system, and filtered through a final particulate filter before use.

2. Ammonium chloride buffer (Three methods):

a. Method 1:

i. Add 800 ml of milliQ water to a 1-liter flask or a 1-liter LDPE or HDPE bottle with a 100 ml mark.

ii. Add 85 g of ammonium chloride.

iii. Add 1.0 g of disodium ethylenediamine tetraacetic acid dihydrate (disodium EDTA, dihydrate).

iv. Add a stir bar to the flask or bottle.

v. Put the flask on the stir plate, turn on the stir plate to mix the reagent until the components are dissolved.

vi. Add milliQ to a volume of about 950 ml.

vii. Adjust the pH to 8.5 with 15 N sodium hydroxide.

1. If using a flask to make solution, transfer solution to reagent bottle for pH adjustment.
2. Calibrate the pH meter using instructions included with the meter.
3. Set a stir plate next to the ring stand.
4. Put the bottle on a stir plate.
5. Drop a clean stir bar into the bottle.
6. Thoroughly rinse pH probe with MilliQ water to minimize contamination of buffer reagent.
7. Secure pH probe in ring stand clamp with tip at least 1/2” into the reagent.
8. Turn on the stir plate to mix the buffer.
9. Using the 1 ml or 2.5 ml pipette, slowly add sodium hydroxide until the pH reaches 8.5.
10. Once reagent is at pH 8.5, cap bottle immediately. Note as pH nears 8.5, solution will give off ammonia fumes.
11. Take the bottle to hood and fill to the mark on the bottle using a squirt bottle containing milliQ water from the same batch.
12. Alternatively, if the bottle does not have a marked fill line:
    a. In the fume hood, return reagent to 1 liter flask.
    b. Fill the flask to the mark with milliQ from squirt bottle.
    c. Transfer buffer from flask back to bottle.
13. Cap bottle.
14. Store in MWQL laboratory refrigerator.

b. Method 2:

i. Add 500 ml of milliQ water to a 1-liter flask.

ii. In the fume hood, add105 ml of concentrated hydrochloric acid. Caution: Solution will be hot!!
iii. In the fume hood add 95 ml of ammonium hydroxide.
iv. Add 1.0 g of disodium ethylenediamine tetraacetic acid dihydrate (disodium EDTA, dihydrate).
v. Stir until dissolved.
vi. In the fume hood, adjust pH to 8.5 with either hydrochloric acid or sodium hydroxide following instructions above. Caution: fumes!

c. Method 3:
i. Purchase the Lachat Pre-made reagent: Part# 52003.

3. **Sulfanilamide Color Reagent: (Note: Store in dark bottle and refrigerate.)**
a. Method 1:
i. Add 600 ml of milliQ water to a 1-liter flask.
ii. In the fume hood, add 100 ml of 85% phosphoric acid.
iii. Add 40 g of sulfanilamide
iv. Add 2 g of N-(naphthyl)ethylenediamine dihydrochloride (NED).
v. Shake to wet.
vi. Add stir bar to flask.
vii. Stir to dissolve for 30 minutes.
viii. Dilute to mark, invert to mix.
ix. Store in a dark bottle.
x. Solution is stable for one month or until it turns pink.

b. Method 2:
i. Purchase Hach Part # 52022: sulfanilamide and 52033: NED.

4. Standards
a. Primary stock (1000 mg/l nitrate-N: Potassium nitrate -
i. Add 200 ml of milliQ water to an acid washed 250 ml flask.
ii. Add 1.8047 g of potassium nitrate.
iii. Swirl to dissolve.
iv. Fill to the 250 ml mark with milliQ water.
v. Store in the refrigerator. Make fresh monthly.

b. Working secondary stock (10 mg/l of nitrate-N) -
i. Add 0.5 ml of primary stock to a 50 ml flask.
ii. Fill to the 50 ml mark with milliQ water. Make fresh daily.

c. Working Standards: See Table 1.

Table 1.

Nitrate - N	Vol NOx secondary	Total volume

(mg/l)	stock (ml)	(ml)
0.25	1.25	50
0.10	0.5	50
0.05	0.25	50
0.025	0.125	50
0.005	0.025	50
0	0	50

Table 1. Working calibration standards.

5. **LCS**: Use the Hach (Part # 1279249) premade nitrate standard to verify the laboratory standards.
    a. Make a 1:10 dilution of the 1000 mg-N/l Hach standard in a volumetric flask (5 ml of Hach #1279249 in a total of 50 ml). Other concentration may be used but must be between 2 - 10 times the MDL.
    b. If the measured concentrations are not between 90% and 110% of the certified concentration, the results will be considered unacceptable, and the source of error will then be determined and fixed before samples are analyzed.

Calibration and standardization:

- The instrument is calibrated before analyzing samples.
    - Analyze at least two samples of milliQ water to flush and equilibrate the system before analyzing standards.
    - Analyze The working (CAL) standard series.
    - Analyze two laboratory blanks.
    - Analyze the LCS after the CAL standards to confirm the accuracy of the CAL standards.
    - Then analyze at least two of the CAL standards a second time to ensure that the readings are stable.
    - If the LCS is within 10% of certified concentration and the second set of CAL standards is within 10% of the first set, then samples may be run.

- If LCS is not within 10% of certified value:
  - Compare CAL standard peak areas between the first and second set.
    - If CAL standard sets vary by more than 10%, find source of error and mitigate.
    - If CAL standards vary by less than 10%, rerun LCS.
      - If LCS variability remains at more than 10%, remake LCS and rerun.
- Analyze a set of LB and LCS standards for every ten samples in a batch of samples. If the LCS samples vary more than 10% from the mean values, then a new set of CAL standards should be analyzed.
- The lowest CAL standard should be measured to within 40% of the true value.
  - If the lowest concentration CAL standard is not within 40% of the true value, stop the run and start the troubleshooting process.
- If the cadmium column has been used to reduce more than 100 samples or if the instrument response is less than expected for a given concentration of analyte, then a 250 microgram/l sodium nitrite standard should be analyzed to verify the efficiency of the cadmium column. More detail can be found in the "Quality Control" section of this SOP.

NOx Colorimetric Analytical Procedure:

The NOx manifold is installed on Channel 4 on the MWQL QuikChem.

- If samples have been preserved with sulfuric acid, add NaOH to bring them to a pH of between 5-9. This process is time consuming. Be sure to allow adequate time to adjust pH before the sample analysis run.
  - Calibrate pH meter with pH 4, pH 7, and 10 buffers according to meter directions.
  - Using an acid cleaned 10 ml pipet tip, take a 10 -20 ml subsample of the preserved sample, and put into a clean small beaker
  - Add a small stir bar to the beaker
  - Put the beaker on the stir plate.
  - Turn on the stir plate.
  - Thoroughly rinse pH probe.
  - Put the pH probe in the clamp of a ring stand and adjust height so that the pipet tip is below the surface of the sample.
  - Measure and record the pH, being careful that the spinning stir bar doesn't hit the pH probe.
  - Add a small amount of 0.5- 2N sodium hydroxide to the subsample, measure the pH.
  - Continue until the pH of the subsample is between 5 and 9.

    - Record the normality of the NaOH and the total volume of NaOH required to bring the sample to the proper pH.
    - Calculate the proper amount of NaOH to add to the remaining sample.
        - VNaOH(ml)=Volume in ml of NaOH needed to bring a X ml volume of sample to pH between 5 and 9.
        - VS(ml) = volume of sample remaining after 10-20 ml removed for test.
        - Volume NaOH to add to remaining sample = (VNaOH(ml)*VS(ml))/volum of test sample.
    - Add the calculated amount of NaOH to the remaining sample.
- Check that the instrument NOx manifold detector filter is in place and the cell is properly seated.
    - If the filter is not in the slot, it will be found in the second Lachat instrument.
- Verify that the sample line is connected to the sample valve of the NOx channel.
- Verify that the reduction column valve is in the “off” position.
- Turn on power to autoanalyzer, autosampler, and pump.
- Turn on the computer.
- Open the Lachat software
- Open the NOx method or a run file from a previous NOx run.
- Enter the identifiers for the milliQ rinse, CAL standards, LCS and the LB in the sample run template.
- If pump tubing needs replacing, see chart in Appendix A for tubing reference.
- Put all reagent lines in a bottle of milliQ water.
- Put the channel effluent lines into the water collection bottle.
- Load the tubing into pump channels.
- Making sure that tabs on the pump cartridges are all the way to the left, push down on each cartridge to secure it to the pump. Each side of the cartridge will make a “click” as it locks in.
- Tighten pressure controls on pump cartridges by pushing tab all the way to the right and then back to the left until it clicks in place.
- Set the pump speed to 35.
- Start the pump.
- Check for leaks and other problems.
- Verify that water is flowing through all reagent tubing.
- Reduce pump speed to less than 5.
- Pump milliQ water through lines until baseline is stable, at least a half hour.
- Remove manifold waste lines from the water collection bottle and put them into the chemical waste carboy.
    - Make sure that the end of the waste tubing is above the waste level.
    - Cover opening with parafilm.
    - Set cap on top of carboy to prevent loss.
- Cut parafilm to cover all reagent bottles.
- Remove intakes from water and put them in corresponding reagent bottles, covering each with parafilm as you proceed. Note to switch lines to water following the diagram in Appendix A

starting at the bottom of the diagram and moving upward. When removing lines from reagent, start at the top of the diagram and go downward.

- Set up a run containing the following.
    - At least two samples of milliQ water which will be used to give the system a final rinse before analysis.
    - The CAL standards.
    - A LCS and a LB immediately following the CAL standards.
    - A second set of at least two of the CAL standards to verify instrument stability.
    - Put rinse tubes, blanks, and standards in the autosampler rack.
- Let reagents run through manifolds for at least 10 minutes.
- After 10 minutes turn the cadmium reduction column to the "on" position.
- Use the "preview" function of the software to monitor the baseline.
- When the baseline appears flat and stable, start the standard run.
- Graph the response for CAL standards to check linearity of instrument response.
- If the r squared value is not above 0.98, the instrument may not have stabilized; rerun the standards.
- Use these curves to test instrument function and set the integration parameters for the analysis.
- Calculate the concentration of the LCS from the linear regression of the CAL standards.
- If the LCS recovery deviates more than 10% from certified value, resolve before proceeding sample run.
- Once standard curve, blanks and certified standards are finished and determined to be within QC parameters, samples can be analyzed.
    - The sample run must contain:
        - An LCS and a LB for every 10 samples.
        - One LFSM for every batch of 20 samples.
        - One replicate sample for every 10 samples.
- Once analysis is finished, close the valve to the cadmium column.
- Switch reagent lines to water starting with the sulfanilamide, then the buffer.
- Rinse lines with milliQ water for at least 15 minutes.
- When finished rinsing, stop pump and release tubing from pump cartridges.
- Remove ends of tubing from water.
- Remove waste lines from the hazardous waste container and put them into the water collection bottle.
- Cap waste container.
- Release trigger on pump manifolds.
- Remove pump tubing from pump cartridges.
- Turn off autosampler, flow injection analyzer, pump, and computer.

Data Analysis and Calculations:

The spectrophotometric detector for NOx forms peaks of voltage response which are integrated within the software to provide the peak area of the response. The parameters for integration are set for each

run based on the standard and blank analysis. This peak area is directly proportional to analyte concentration.

CAL - Standard concentration and peak areas are graphed with a scatter plot in Excel or another graphing program. A linear regression of the data is calculated. The r-squared value of the standard curve must be above .98 for use in this method. If the r-squared value falls below 0.98, then the instrument will be checked for malfunction and the standards will be run again. The linear regression is used to calculate the concentration of the analytes in the samples.

Low CAL Standard - The lowest CAL standard should be equal to or lower in concentration than the current RL. The measured value should be within 40% of the actual value.

Accuracy - The QCCS or LCS concentration is calculated using the linear regression obtained from the CAL standard regression. The accuracy of the CAL standards is calculated as follows:

(ABS(QCCS/LCS certified concentration - QCCS/LCS measured concentration)/QCCS/LCS certified concentration) * 100.

Precision - The percent error for replicate samples or standards (including FD, LD, QCCS, LCS or CAL standards) is calculated using the absolute value of the difference between each of the duplicates using the following equation:

$Variability_{Rep}$ =(Error/Sample Mean) x 100,

Error = (ABS(Rep1-Rep2))/2

Percent Recovery:

The percent recovery of the replicate spiked samples (LFSM) is calculated using the following equations:

% Spike Recovery = Mean($Recovery_1$, $Recovery_2$)

$Recovery_1$ = (($Analyte_{Spike1}$ - $Analyte_{S1}$)/Calculated concentration of spike) x 100.

$Recovery_1$ = (($Analyte_{Spike2}$ - $Analyte_{S2}$)/Calculated concentration of spike) x 100.

$Analyte_{S1}$ = Measured concentration of sample 1.

$Analyte_{Spike1}$ = Measured concentration of sample1 spike.

$Analyte_{S2}$ = Measured concentration of sample 2.

$Analyte_{Spike2}$= Measured concentration of sample 2 spike.

Minimum Detection Limit:

The minimum detection limit is calculated from the error of seven blanks or 7 low concentration standards, whichever is largest. See quality control section above for more details.

The minimum detection limit is calculated as follows:

1. Calculate the concentrations of the 7 blank replicates and the 7 spikes from the CAL standard curve.
2. Calculate the mean of the 7 blanks. If it is negative, equate it to zero.
3. Calculate the standard deviation of the 7 blanks.
4. Multiply the standard deviation of the 7 blanks by 3.143.
5. Add the mean of the 7 blanks to the result from line 4.
6. Calculate the standard deviation of the 7 spikes.
7. Multiply the standard deviation of the 7 spikes by 3.143.
8. The minimum detection limit is the greater of line 5 and line 7.

Method Performance:

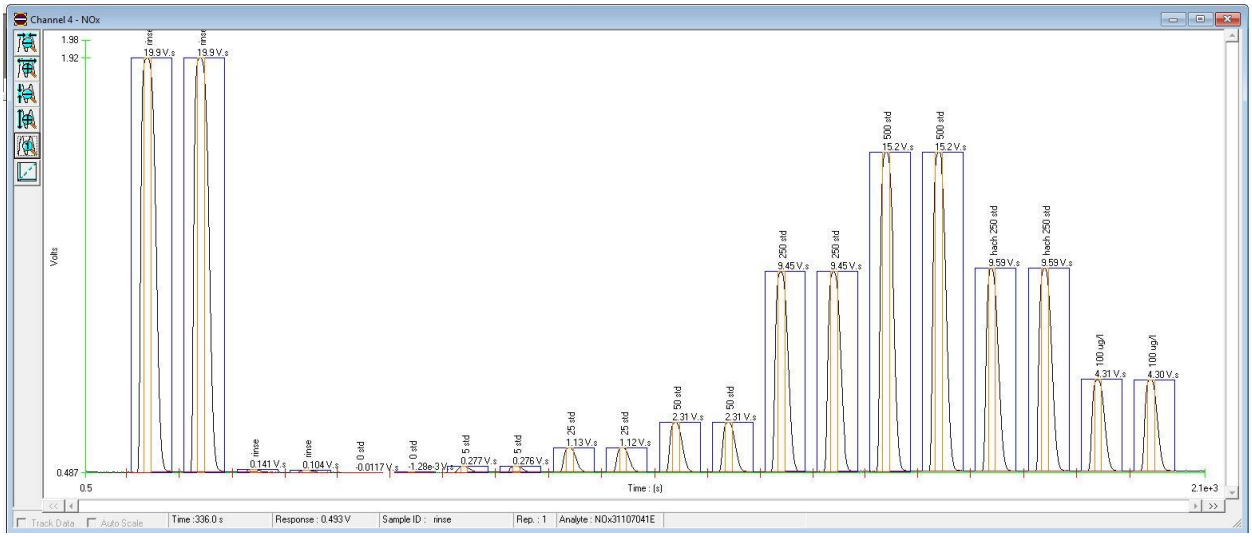


Figure 1. Lachat NOx detector output from NOx standard analysis (November 19, 2019).

CAL Standards	Mean	Range
NOx (mg/l)	Peak Area	Peak Area
0.250	17.3	0.000
0.100	7.61	0.010
0.050	3.91	0.010
0.025	1.99	0.010
0.005	0.42	0.006

0.000	-0.01	0.002

Table 2. 1/17/2020 NOx Standard Calibration Series using CAL standards.

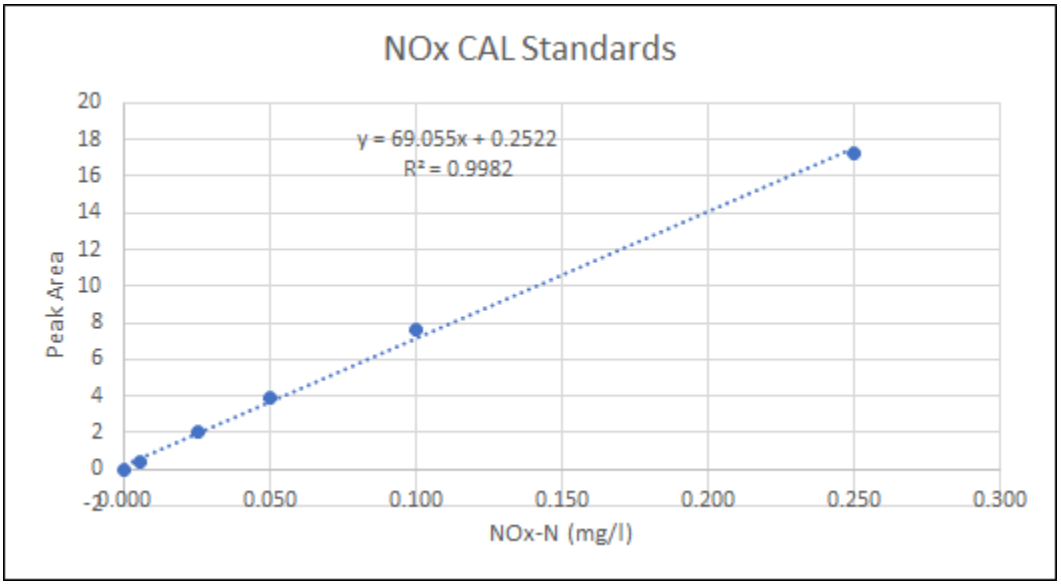


Figure 2. Graph of 1/17/2020 NOx CAL standards. Error bars are the range of duplicate standards.

CAL Standards			Mean
NOx (mg/l)	Peak Area	Peak Area	Peak Area
0.400	14.300	14.3	14.300
0.250	9.790	9.790	9.790
0.100	4.450	4.450	4.450
0.025	1.170	1.170	1.170
0.005	0.283	0.280	0.282
0.000	-0.005	-0.005	-0.005

Table3. NOx - Linear Range of CAL Standards -11/19/2019.

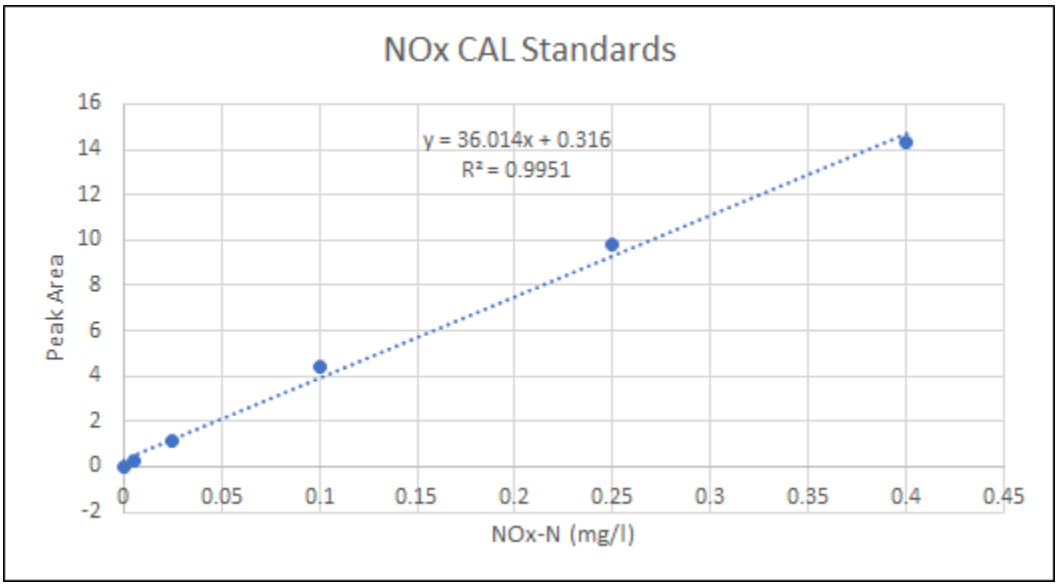


Figure 3. Linear range of NOx CAL standards 11/19/2019.

Corrective Actions:

General precision problems:

If baseline drifts, peaks are too wide, or peaks are atypical in shape (see Figure 1, for typical peak shape):

- Check that there is not an obstruction in the tubing that is causing pressure buildup and retarding the flow of the carrier.
    - The cadmium column is a common source of obstruction.
        - It is important to not let air get into the cadmium column.
            - When flushing column always keep buffer flowing through the column.
        - Check the cadmium column switching valve and the column fittings for obstructions or crimped tubing.
    - Check flow of reagents. 'If reagent is not flowing properly:
        - Replace pump tubing.
        - Check all fittings for obstructions or crimping tubing.
    - Check waste tubing for crimps or obstructions.
- Check sulfanilamide reagent for precipitate in the bottom of the bottle. If there is a precipitate, solution should be filtered into a clean dark bottle using a 0.22-micron filter.
- If the above actions do not alleviate the problem, clean the manifold by the following procedure:
    - Switch off the cadmium column valve.
    - Place transmission lines in water and pump to clear reagents (2-5 minutes).
    - Place reagent lines in 1 M hydrochloric acid (1 volume of HCl added

to 11 volumes of water) and pump for several minutes.

- Place all transmission lines in water and pump for several minutes.
- Resume pumping reagents.

Poor instrument response:

- Remake CAL standards.
- If sensitivity issues result from excessive bubbles forming in tubing:
    - Check that reagents are at room temperature. If not, warm reagents in tepid water bath and mix.
    - Degas reagents.
    - Check intake tubing in bottles for obstructions.
- If the peak heights are not within 25% of the heights achieved for the previous analytical run for similar concentrations:
    - The cadmium column is decreasing in sensitivity.
    - If the CAL standard variability is less than 10% and the LCS accuracy is within the 10% margin of error, you may continue the run.
        - Analyze a set of at least 3 LB and 3 replicates of a low CAL standard (within 2-10 times the previous MDL) to ensure that the sensitivity has not decreased.
        - Analyze two concentrations of sodium nitrite that are within the range of the CAL standards to characterize the reduction efficiency of the cadmium column.
        - If sensitivity has decreased, report new MDL with batch results.
        - Replace cadmium column before the next analytical run.
        - As noted above, the cadmium column can become plugged.
            - If this happens, remove the downstream fitting, and run reagent through into a beaker to flush the column.
            - If the column is still plugged, reverse the column flow and flush a second time.
            - If the calibration fails consistently, replace the column.

Poor precision:

- If replicate digested standards or sample analytical replicates vary by more than 10% from mean values:
        - Check pump tubing.
        - Check back pressure tubing for leaks.
        - Check for bubbles.
        - Let the instrument equilibrate longer (inadequate equilibration can cause variability).

        - Check for blockages in lines.
        - If precision of analytical replicates is still unacceptable, after implementing the above remedies, replace the cadmium column.
        - If cadmium column replacement does not improve signal, remake reagents.
    - If precision of analytical replicates of CAL standards are within 10% of the mean, but LCS values vary more than 10% from certified values:
        - Remake CAL standards.
        - Remake LCS diluted standards.
        - Rerun both CAL and LCS.
    - If analytical replicates vary by more than 10% but not by more than 20%, continue run, recording out of limit QC parameters.
        - If replicates vary by more than 20% stop run.
        - Remedy problems before running samples.

Pollution Prevention and waste management:

All wastes will be handled in accordance with University of Maine Safety and Environmental Management Guidelines. Waste will be stored in properly labeled containers with secondary containment. Waste will be located in approved areas and will be inspected weekly. When waste containers are full, they will be transported by the Darling Marine Center Safety Officer to a secure dedicated waste storage facility on the DMC campus. At the point of pick up and transport to the DMC waste storage facility, the responsibility for monitoring of the waste, is transferred to the DMC safety officer. Waste will be stored until pickup by a University of Maine approved waste disposal company. All MWQL staff who handle waste will be required to complete the Hazardous Waste training provided by the University of Maine.

The MWQL is committed to reducing chemical waste and will maintain the lowest volume of chemical stocks that is practical while maintaining adequate stock to guard against shortages and back orders. All expired chemicals will be disposed of as chemical waste and transferred to the DMC waste storage facility. The lab does not dispose of any chemicals down the drain. The spent cadmium columns are treated as hazardous waste and are disposed of as such when no longer useful.

Data Assessment and Acceptance Criteria:

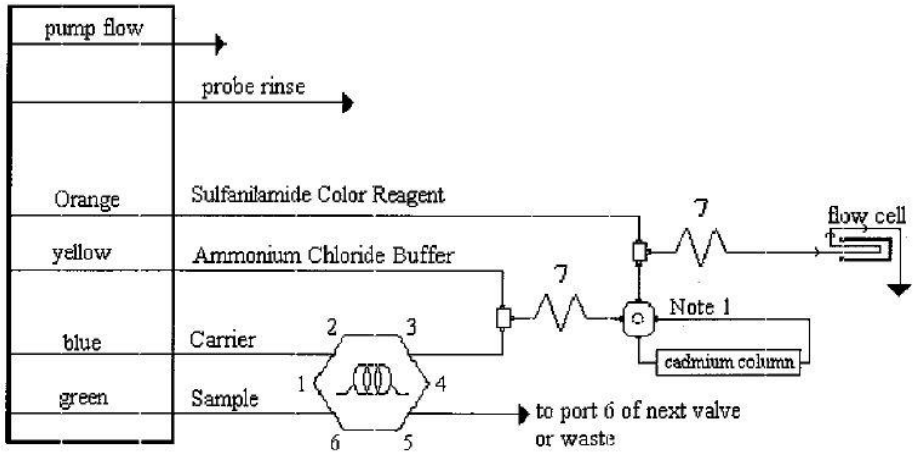
Data Acceptance Specifications:

- **LCS - 90% - 110% of certified value.**
- **LB - Below MDL.**
- **LD - 90% - 110% of mean value.**
- **LFSM - Spike Recovery - 80% - 120%.**

- **Linearity - Linear regression of CAL standards - > 0.98.**
- **Low CAL standard at or below the RL - Must be within 40% of actual value.**
- **Reproducibility of CAL standards over the course of a batch of samples - 90% - 110%. If CAL standard values are outside of this limit, a new set of CAL standards must be analyzed.**

Data Assessment Check List:

- **Were the samples analyzed within the required holding time (28 days)?**
- **Were the samples preserved properly?**
- **Was the pH raised to between 5 and 9 before analysis?**
- **Were the samples properly stored between sampling and analysis?**
- **Was this protocol followed as written?**
    - **If not, is there a record of variance from the method and documentation explaining the variance?**
- **Are there at least 4 concentrations of CAL standards for each sample batch?**
- **Were at least 2 CAL standards re-analyzed immediately after the initial CAL standard series to document method stability?**
- **Is each standard concentration analyzed in duplicate?**
- **Is the r squared value of the linear regression of the mean CAL standard response versus concentration greater than 0.98?**
- **Are the peak heights within 25% of the heights achieved for the previous analytical run for similar concentrations?**
    - **If not, were further LB and CAL standards analyzed to reassess sensitivity?**
- **Is the measured concentration of the LCS within 10% of the certified value?**
- **Is the LB less than the MDL?**
- **Was an LB and LCS analyzed for every 10 samples?**
- **Are all of the LB and LCS samples within acceptance criteria?**
    - **If not, is there documentation to explain?**
- **Was a duplicate sample analyzed for every 10 samples?**
- **Was the precision of the duplicate samples within 90% - 110%?**
    - **If not, is there documentation to explain?**
- **Was one LFSM analyzed for every 10 samples?**
    - **If not, is there documentation to explain?**
- **Is there a properly documented NOx analysis log (NOXAL)?**
- **Is there a proper chain of custody?**

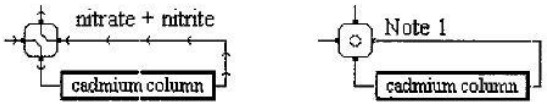


Carrier: DI water
Manifold Tubing: 0.8 mm (0.032 in) i.d. This is 5.2 µL/cm.
AE Sample Loop: 150 cm x 0.042 in i.d.
QC8000 Sample Loop: 150 cm x 0.042 in. i.d
Interference Filter: 540 nm

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required.

7: 135 cm of tubing on a 7 cm coil support

Note 1: This is a 2 state switching valve used to place the cadmium column in-line with the manifold.



Appendix C

MWQL Total Kjeldahl Nitrogen (TKN) and Total Phosphorus (TP) Standard Operating Procedure (SOP)

Document Code: MWQLSOPTKNTP Rev. 5, 2/18/21

EPA Reference: TKN: EPA 351. 2 /TP: EPA 365.4

Table of Contents

SPECIFICATIONS

This method is applicable to marine and freshwater samples.

Applicable concentration ranges:

TKN working range: RL - 5.0 mg N/l

TP working range: RL - 5.0 mg N/l

DEFINITIONS

The definitions and purposes below are specific to this method but have been conformed to common usage as much as possible.

ANALYST -- The designated individual who performs the "hands-on" analytical methods and associated techniques and who is the one responsible for applying required laboratory practices and other pertinent quality controls to meet the required level of quality.

ANALYTICAL BATCH -- An analytical batch is a group of environmental samples that are prepared and/or analyzed together with the same process and personnel, using the same lot(s) of reagents. A preparation batch is composed of at least one and no more than 20 environmental sample(s) of the same quality systems matrix, meeting the above-mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 24 hours. An analytical batch is composed of prepared environmental samples (extracts, digestates or concentrates) which are analyzed together as a group. An analytical batch can include prepared samples originating from various quality system matrices and can exceed 20 samples, unless the method requirements are more stringent.

BIAS -- The systematic or persistent distortion of a measurement process, which causes errors in one direction (e.g., the expected sample measurement is different from the sample's true value).

CALIBRATION -- A set of operations that establish, under specified conditions, the relationship between values of quantities indicated by a measuring instrument or measuring system, or values represented by a material measure or a reference material, and the corresponding values

realized by standards. In calibration of support equipment, the values realized by standards are established through the use of reference standards that are traceable to the International System of Units (SI).

In calibration according to methods, the values realized by standards are typically established through the use of reference materials that are either purchased by the laboratory with a certificate of analysis or purity or prepared by the laboratory using support equipment that has been calibrated or verified to meet specifications.

CALIBRATION BLANK (CB) -- A volume of reagent water in the same matrix as the calibration standards, but without the analyte.

CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

CERTIFIED REFERENCE MATERIAL -- (CRM) means reference material, accompanied by a certificate, having a value, measurement of uncertainty and stated metrological traceability chain to a national metrology institute

CHAIN OF CUSTODY FORM (COC) -- A record, either paper-based or electronic, that documents the possession of the samples from the time of collection to receipt in the laboratory in accordance with chain-of-custody protocol. This record, at a minimum, must include the sample location, the number and types of containers, the mode of collection, the collector, the date and time of collection, preservation, and requested analyses. See also Legal Chain of Custody Protocols.

FIELD BLANK (FB) -- An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, including exposure to a sample bottle holding time, preservatives, and all pre-analysis treatments. The purpose is to determine if the field or sample transporting procedures and environments have contaminated the sample.

FIELD DUPLICATE (FD) -- Two samples taken at the same time and place under identical circumstances which are treated identically throughout field and laboratory procedures. Analysis of field duplicates indicates the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

INSTRUMENT BLANK -- A clean sample processed through the instrumental steps of the measurement process and used to determine instrument contamination.

LABORATORY BLANK (LB) -- An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, except that it is not taken to the sampling site. The purpose is to determine if the if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

LABORATORY CONTROL STANDARD (LCS) -- A solution prepared in the

laboratory by dissolving a known amount of one or more pure compounds in a known amount of reagent water. Its purpose is to assure that the results produced by the laboratory remain within the acceptable limits for precision and accuracy. (This should not be confused with a calibration standard).

LABORATORY DUPLICATE (LD) -- Two aliquots of the same environmental sample treated identically throughout a laboratory analytical procedure. Analysis of laboratory duplicates indicates precision associated with laboratory procedures but not with sample collection, preservation, or storage procedures.

LABORATORY FORTIFIED SAMPLE MATRIX (LFSM)-- An environmental sample containing an analyte spike of known concentration at least 4 times the MDL. Used to evaluate the effect of the sample matrix on the analytical results.

LIMIT OF QUANTITATION (LOQ) -- The minimum levels, concentrations, or quantities of a target variable (e.g., target analyte) that can be reported with a specified degree of confidence.

MINIMUM DETECTION LIMIT (MDL), also known as the Limit of Detection -- The lowest level at which an analyte can be detected with 99 percent confidence that the analyte concentration is greater than zero.

MilliQ WATER - Reagent water that has been treated to remove impurities through reverse osmosis and filtration and has a resistivity above 18.0 .

QUALITY CONTROL CHECK SAMPLE (QCCS) – Also known as a proficiency sample. A sample containing analytes of interest at known concentrations (true values) of analytes. The QCS is obtained for a source external to the laboratory or is prepared from standards obtained from a different source than the calibration standards. The purpose is to check laboratory performance using test materials that have been prepared independently from the normal preparation process.

SENSITIVITY -- The capability of a method or instrument to discriminate between measurement responses representing different levels (e.g., concentrations) of a variable of interest.

QUALITY CONTROL MEASURES:

All laboratory personnel using this SOP will also comply with all general laboratory QA and QC measures as stated elsewhere in this QAM. QA and QC procedures specific to this protocol are documented in this SOP.

Initial Demonstration of performance:

- Method Detection Limit (MDL) - The minimum detection limit will be determined by analyzing blank variability and by spiking a blank with analyte at a concentration from 2 to 10 times the estimated MDL. Seven blanks and seven MDL spikes will be carried through all aspects of preparation and analysis in a minimum of three separate sample batches on three separate calendar dates. The reported MDL will be the larger of the MDL calculated from the blanks and the MDL calculated from the spiked matrix samples.
    - To determine the MDL of the spiked matrix samples:
        - Calculate the standard deviation of the 7 spikes.
        - Multiply the standard deviation of the spiked matrix by 3.143 (for seven replicates).
    - To determine the MDL of the blanks:
        - Calculate the mean of the method blanks.
        - If the mean is negative equate it to zero.
        - Multiply the standard deviation of the blanks by 3.143 (for seven replicates) and add the result to the mean.

- Limit of Quantitation – Also known as the Reporting Limit (RL). Obtain the limit of quantitation by calculating the mean and standard deviation of replicate standards between 2 - 10 times the estimated or known MDL. Multiply the standard deviation by the t value obtained from the t test table for the replicate degrees of freedom and the desired cumulative probability based on a two tailed test. For instance, the 99% cumulative probability.

- Quality Control Check Sample - Semi-annually, a QCCS will be ordered from an EPA recommended provider lab. The sample will be analyzed, and results sent to the provider

lab. If results are not within the acceptable limits of stated values, the source of the problem will be identified and corrected.

- Demonstration of Linearity - A standard curve over the working range of the method will be run at least once per year to demonstrate linearity.

Ongoing quality control measures:

- Duplicate Lab Reagent Blanks - Duplicate lab reagent blanks will be analyzed with each batch of samples.

- Laboratory Control Standard - Duplicate LCSs from a second source, different from the CAL standards, are analyzed with each batch of samples, no less than one for every ten samples to determine the accuracy of the analytical protocol. If the measured analyte concentration varies by more than 10% from the certified concentration, the analysis will stop, and the source of error will be determined before sample analysis resumes.

- Calibration Standards - A set of CAL, covering a minimum of 4 concentrations that span the expected sample concentrations, will be analyzed with each batch of samples

- Laboratory Fortified Sample Matrix - 10% of the analyzed samples will be spiked with analyte to give a concentration at least four times the MDL prior to digestion. The percent recovery will be determined from these samples. If the percent recovery falls outside to the 80%-120% range, analysis will stop, and the source of the error will be determined and resolved before continuing analysis.

- Laboratory Duplicates - A set of LDs will be analyzed in each batch of samples. Duplicates should not vary more than 10%.

- Minimum Detection Limit must be recalculated every 6 months.

SUMMARY OF METHOD

Suitability of method and application:

This method is used to obtain TKN, which is composed of the ammonium in the sample plus organic nitrogen and to obtain the TP of the sample, from unfiltered liquid samples. The method is appropriate for fresh and seawater samples with a TP or TKN concentration up to 5 mg/l. Samples with higher concentrations of TP or TKN may be diluted, and the diluted sample analyzed using this protocol. The method consists of a digestion and subsequent analytical steps.

Digestion:

Using copper sulfate as a catalyst, liquid samples are heated in glass digestion tubes in the presence of concentrated sulfuric acid. Potassium sulfate is also incorporated to raise the boiling temperature of the digestion and therefore, to lessen the time required. Hangar crystals are added to each digestion tube to prevent splattering and boiling over of samples. During the digestion, the samples' phosphorus is converted to the orthophosphate (PO_4^{3-}) anion. Organic nitrogen compounds are converted to ammonium sulfate $(NH_4)_2SO_4$, under the conditions of the digestion described. After the digestion period, the digestion tubes are removed from the digester and the residue is cooled. The digest is then diluted to its original volume with DI water and the sample is mixed to dissolve any precipitate.

TKN colorimetric analysis:

The TKN is analyzed using a Lachat Quickchem 8500 autoanalyzer. The digested sample is injected onto the chemistry manifold where its pH is raised to a known, basic pH, by in-line neutralization and maintained with a concentrated buffer. This inline neutralization converts the ammonium cation to ammonia, and also prevents undue influence of the sulfuric acid matrix on the pH-sensitive color reaction which follows. The ammonia thus produced is heated with salicylate and hypochlorite to produce blue color which is proportional to the ammonia concentration. The color is intensified by adding sodium nitroprusside. Ethylenediaminetetraacetic acid (EDTA) is added to the buffer to prevent

precipitation of calcium and magnesium in the lines of the instrument. The absorbance is measured at 660 nm and is proportional to the TKN in the sample.

TKN is the sum of the ammonium and the organic nitrogen in the sample.

Organic nitrogen concentration is obtained by subtracting the free-ammonia concentration from the TKN concentration. The total nitrogen in the samples can be obtained by adding the TKN and the nitrate plus nitrite (NOx) concentrations.

TP colorimetric analysis:

The total phosphorus is analyzed using the Lachat 8500 Quickchem autoanalyzer. The orthophosphate produced in the digestion reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is subsequently reduced with ascorbic acid to form a blue complex. The absorbance of this complex is measured at 880 nm and is proportional to the orthophosphate in the sample.

INTERFERENCES

- Samples must not consume more than 10% of the sulfuric acid during the digestion. The buffer will accommodate a small range of H_2SO_4 concentrations in the digested, diluted samples with no change in signal intensity.
- Digests must be free of turbidity. Digested samples may be filtered if turbid.
- Some boiling stones have been shown to crumble upon vigorous vortexing. Care must be taken to avoid collecting boiling stone pieces when pouring samples into 50 ml tubes.

SAFETY

Hazards:

Hazards include hazardous chemicals, high temperature surfaces, boiling acidic solutions. All laboratory personnel using this protocol must be current with all University of Maine safety training requirements.

Hazardous chemicals: Read SDS sheet for all chemicals before use. Wear appropriate personal protective equipment including appropriate gloves, lab coat and safety glasses. The following list highlights some of the hazards of the listed chemicals but IS NOT intended to replace thorough reading of the SDS sheets by laboratory personnel.

- **Sodium nitroprusside (CAS#: 13755-38-9). Warning! Toxic if swallowed, inhaled, or absorbed through the skin. Contact with acids liberates hydrogen cyanide, a very toxic, flammable gas or liquid. May cause eye and skin irritation. May cause respiratory tract irritation. Hygroscopic (absorbs moisture from the air).**
- **Sulfuric acid (CAS#: 7664-93-9) - Warning! Toxic if swallowed, inhaled, or absorbed through the skin. Caustic! May cause severe burns and eye injury.**
- **Sodium hypochlorite (CAS#: 7681-52-9).**
- **Sodium hydroxide (CAS#: 1310-73-2). Harmful in contact with skin. Causes severe skin burns and serious eye damage. May be corrosive to metals.**
- **Copper sulfate (CAS#: 7758-99-8). Toxic.**
- **Ammonium molybdate (CAS#: 7758-99-8). Irritant.**
- **Antimony potassium tartrate (CAS#: 331753-56-1). Toxic.**

Splash hazard - digestor. Wear splash and impact protective goggles, lab coat and appropriate gloves when using the digester.

High temperature hazard - digestor. Do not touch digester block when in use. Use caution removing tube tray from the digester block.

Possibility of breaking glass - digestor. Wear splash and impact protective goggles, lab coat and appropriate gloves when using the digester.

Hazardous solutions under pressure - Lachat autoanalyzer. Solutions pumping through Lachat tubing are under pressure and may spray reagent if a leak forms. Wear safety glasses and a lab coat.

EQUIPMENT AND SUPPLIES

40 - 50 ml conical tubes (50 ml conical screw cap HDPE centrifuge tubes)

40 - Digest tubes (Lachat instruments 50 ml open-top digestion tubes)

40 - Cold fingers (Lachat instruments)

20 - Acid cleaned sample collection bottles.

2 - Milli-Q squirt bottle

250 ml graduated cylinder

500 ml graduated cylinder

100 ml graduated cylinder

10 ml pipette and tip

1-liter volumetric flasks

250 ml volumetric flasks for standards

Stir plate

Analytical Balance

Lachat QuickChem flow injection analyzer with autosampler and heaters.

Lachat Block Digestor

Vortex Mixer

Glass and plasticware preparation:

All glassware and plasticware in contact with samples will be:

- Washed with Sparkleen detergent and rinsed 5 times with milliQ water.
- Acid rinsed using 0.5 M - 1.5 M hydrochloric acid solution and rinsed thoroughly with milliQ water.

SAMPLE COLLECTION AND PRESERVATION:

See QAM for sample acceptance criteria. Samples (100 – 250 ml) are collected in clean, acid rinsed plastic bottles or tubes (see above). Samplers must wear gloves and be careful not to touch any unclean surfaces with gloves before or during sampling. Samples may be collected from Niskin bottles or may be directly collected from river, stream, estuary, or other water source. If possible, rinse bottle with sample:

- Bottles must be filled with a volume of sample sufficient to rinse the bottle (for a 100 ml bottle 10-20 ml is adequate}.
- Collect a small amount of sample, cap bottle, invert, and swirl sample in bottle to rinse the entire inner surface of the bottle.
- Dispose of rinse water downstream of the sampling site.
- Rinse 3 times, then fill the bottle and cap.

If acid preservative has been added to bottles prior to sampling, do not rise bottle.

Fill bottle Immediately store samples in the cooler.

Collected samples must be kept in the dark on ice until delivery to the laboratory.

If samples are not preserved in the field, they must be preserved upon arrival at the laboratory.

Samples are preserved by adding sufficient sulfuric acid to lower the pH to < 2.0 within 8 hours of collection (about 0.4 ml per 100 ml of sample).

Check the pH of at least 2 samples per batch to determine that pH is lower than 2 after acid addition.

Sample Storage:

Samples should be analyzed as soon as possible after collection. Preserved samples may be stored at <6ºC in a refrigerator for up to 28 days.

REAGENTS AND STANDARDS

1. **Digestion solution (We use the copper-based digestion solution in order to avoid the use of highly toxic mercuric chloride):**
    - **a.** Add 800 ml of ammonium-free milliQ (resistivity greater than 18.0) water to a 1-liter volumetric flask.
    - b. Add 134 g of potassium sulfate (K_2SO_4).
    - c. Add 7.3 g copper sulfate ($CuSO_4$).
    - d. In hood, measure 134 ml of concentrated sulfuric acid using a graduated cylinder.
    - e. Add sulfuric acid to flask. Caution! Solution will get hot!

f. Loosely, place the stopper on the flask and stir on the stir plate until the solution cools to room temperature.
g. Do not invert the flask to mix.
h. When cool dilute to the 1-liter mark.

2. **Hypochlorite solution** (0.315%): For TKN analysis.
    a. Use Lachat premade sodium hypochlorite solution (0.315%, part # 52011) or dilute Hach sodium hypochlorite (5%, part # 52007) 15 ml in total of 250 ml. Alternatively, a solution can be made from any pure hypochlorite solution of greater concentration.
3. **Salicylate nitroprusside reagent:** For TKN Analysis.
    a. Add 800 ml of milliQ water to a 1-liter flask. Add 150 g of sodium salicylate (Salicylic acid, sodium salt-$C_6H_4(OH)(COO)Na$).
    b. Add 1 g of sodium nitroprusside dihydrate ($Na_2Fe(CN)_5NO.2H_2O$).
    c. Invert to mix.
    d. Store in a dark bottle.
    e. Prepare fresh monthly.
4. **Buffer:** For TKN Analysis.
    a. Add 900 ml of milliQ water to a 1-liter flask. Add 35 g of sodium phosphate dibasic heptahydrate ($Na_2HPO_4.7H_2O$) to the flask and stir to dissolve.
    b. Add 20 g disodium EDTA. This will not dissolve until the sodium hydroxide is added.
    c. Add 50 g sodium hydroxide.
    d. Stir or shake until dissolved. Solution will be hot!
    e. Dilute to 1 liter.
    f. Degas weekly and prepare fresh monthly.

5. **Sodium hydroxide (0.8M).**
    a. Use Hach solution #52008 (0.8 M NaOH) or to make this solution in the laboratory:
        i. Add 800 ml of milliQ water to a 1-liter flask.
        ii. Add 32 g of sodium hydroxide.
        iii. Stir to mix.
        iv. Dilute to 1 liter mark.
6. **Digestion diluent:** Carrier for TKN and used for non-digested standards**–** same acidity as digested sample.
    a. Add 400 ml of digestion solution (Solution #1) to a 1-liter flask.
    b. Bring to 1 liter with milliQ water.
7. **Stock ammonium molybdate solution: May be stored in the refrigerator for 2 months.** For TP Analysis.
    a. Add 800 ml of milliQ water to a 1-liter flask.
    b. Add 40 g of ammonium molybdate tetrahydrate.
    c. Stir for four hours.

d. Dilute to 1 liter
e. Solution is table for 2 months

8. **Stock Antimony Potassium Tartrate Solution: May be stored in refrigerator for 2 months.**
    a. Add 800 ml of milliQ water to a 1-liter flask.
    b. Add 3.0 g of antimony potassium tartrate hemihydrate
    c. Dilute to the mark and stir with a magnetic stirrer.
    d. Store in a dark bottle and refrigerate.
    e. Solution is stable for 2 months.
9. **Molybdate Color Reagent: Discard if turns blue or a yellow precipitate forms.** For TP Analysis
    a. Add 500 ml of milliQ water to a 1-liter flask.
    b. Add 35 ml of concentrated sulfuric acid. (Caution solution will be hot!)
    c. Add 213 ml of the stock ammonium molybdate solution.
    d. Add 72 ml of the antimony potassium tartrate solution.
    e. Dilute to the mark and stir to mix.
    f. Degas with helium or let sit for at least 24 hours.
    g. Prepare fresh weekly or when reagent turns blue.
10. **Ascorbic Acid Reducing Solution (0.33 M). Prepare fresh weekly Discard if solution turns yellow. Do not degas.** For TP Analysis.
    a. Add 700 ml of milliQ water to a 1-liter volumetric flask.
    b. Add 60 g of ascorbic acid to flask.
    c. Mix.
    d. 1.0 g of sodium dodecyl sulfate (SDS).
    e. Dilute to the 1-liter mark with milliQ water.
11. **1 M Hydrochloric Acid - Used to clean tubing when needed.**
    a. In fume hood, add 200 ml of milliQ water to a 250 ml glass bottle.
    b. Add 21 ml of concentrated hydrochloric acid.
    c. Fill to the 250 ml mark with milliQ water.
12. **50% Sulfuric Acid for preserving samples:**
    a. Add 40 ml of milliQ water to a 100 ml reagent bottle.
    b. Add 50 ml of concentrated sulfuric acid.
    c. Allow to cool to the touch.
    d. Pour into Class A 100 ml volumetric flask.
    e. Bring to 100 ml with milliQ water.
    f. Pour back into reagent bottle and cap.
13. **Sulfuric Acid (11 N):** For TP Analysis.
    a. Add 600 ml of milliQ water to a 1-liter volumetric flasks.
    b. Add 305 ml of concentrated sulfuric acid.
    c. Mix by swirling and allow to cool.
    d. Bring to 1 liter.
14. **Sulfuric Acid (0.231 M):** For TP Analysis.

a. Add 500 ml of milliQ water to a 1-liter volumetric flask.
b. Add 42 ml of 11 N sulfuric acid solution.
c. Bring to 1 liter and invert to mix.
d. Prepare fresh weekly.

15. **Primary Standards: Note that standards must be acidified in the same manner as preserved samples before digestion.**
    a. TKN: Primary stock (0.1909 g/liter ammonium chloride = 50 mg/l ammonium - N). Store in the fridge, make fresh monthly.
    b. TKP: Primary stock (1.099 g/l anhydrous potassium dihydrogen phosphate (KH_2PO_4) in 1 liter = 250 mg/l P). Store in the fridge, make fresh monthly.

NH_4-N (mg/l) or PO_4-P (mg/l)	Vol. PO_4 primary stock (ml)	Vol NH_4 stock primary (ml)	Total volume (ml)
2	2	10	250
1	1.0	5	250
0.5	0.5	2.5	250
0.25	0.25	1.25	250
0.05(TP),0.1(TKN)	0.05	0.5	250
0	0	0	250

Table 1. Working TKN and TP Standards for digestion.

CALIBRATION AND STANDARDIZATION

Instrument function is tested before analyzing digested samples and standards, with blank reagent water and at least 2 concentrations of undigested CAL for each analyte. An undigested QCCS is also analyzed to confirm the accuracy of the laboratory made standards. This ensures the proper functioning of the instrument before the digested samples are analyzed. Each digestion and therefore, each analytical batch, contains a standard series, duplicate LCS and blanks. These are used to calibrate the instrument for the digested samples. More detail can be found in the " Quality Control" section of this SOP.

Procedure:

Digestion Procedure:

- Note: The following samples must be included in each digestion run:
    - Duplicate spiked (0.5 mg/l) samples.
    - Duplicate blanks (0 std).
    - One replicate sample for every 10 samples.
    - Standards in duplicate: 0 (blank), 0.05 (TP) or 0.1(TKN), 0.25, 0.5, 1, 2.0 mg/l standards (mixed N and P). Note: higher standards only need to be prepared if a sample is known to have higher than typical N or P. Samples with expected TKN or TP concentrations higher than 5 mg/l will require dilution.
    - Duplicate QCCS.
- **The following samples are periodically required (see Quality Control).**
    - Proof of capability, spike 0.5 mg/l to milliQ water for 4 reps.
    - Proficiency standards: Dilute 1:1000. Use this dilution as a sample. (100 microliters in 100 ml). Or follow other specific instructions from PT provider.
- Make up all reagents listed in the "Reagents" section of the SOP and the primary standards, no later than the day before analysis. If solutions will not be used the next day, store in the MWQL chemical refrigerator. If they will be used the next day, they may be stored at room temperature.
- Prepare all glassware, plasticware and check the status of all required equipment.
    - Change any pump tubing that looks worn or misshapen. Refer to the diagrams in Appendix A.
- On the day of the digestion, make up working standards listed in Table 1.

- Make a certified standard mix using the ICPMS standards which are 1000 mg/l ammonium - N (Sigma ICPMS std #89503) and (1000 mg/l PO_4-P (Sigma ICPMS std # 38338).
- Note: Some primary ICPMS stocks are 1000 mg/l - P and some are 1000 mg/l PO_4. See note below for volumes of each.
    - Make a stock standard mix (62.5 mg/l):
        - Using a 10 ml volumetric add the following volumes of stock:
            - 0.625 ml of the NH_4 stock.
            - 0.625 ml of the PO_4 stock if using primary with 1000 mg/l P. If using stock with 1000 mg/l PO_4, Add 1.917 ml of stock.
            - Bring to 10 ml with milliQ.
    - Add 200 microliters (0.2 ml) to each 25 ml sample for spike (500 ug/l).
    - Add 200 microliters (0.2 ml) to 25 ml milliQ water for QCCS.
- Note: Undigested CAL standards and LCS must be made up on the day of the analysis to verify that the instrument is in working order before analyzing the digested standards.
    - To make up the undigested LCS in diluent on the day of analysis:
        - Make up the secondary stock of ICPMS standards using the instructions above.
        - To make a 0.5 mg/l working standard, add 0.8 ml of the secondary stock to a 100 ml flask.
        - Fill the flask to the mark with diluent.
    - To make up the undigested CAL standards on the day of analysis:
        - To make 2 mg/l CAL Standard:
            - Add 4 ml of the ammonium primary stock to a 100 ml flask.
            - Add 0.8 ml of the phosphate primary stock to the flask.
            - Dilute to the make with diluent solution.
- Turn on hood ventilation.
- Verify sample preservation by measuring pH of at least one of the samples in the batch. pH should be 2 plus or minus 0.2 units.
- Use TKN/ TP digestion template (see appendix) to record the rack position of samples and standards in the digestor rack.
- Pour or pipet, 25 mL of standard or sample into respective glass digestion tube (each tube has a fill line at the 25 ml mark).
- Place digestion tubes, uncovered, in the digest rack in the hood.
- Add 2 Hangar boiling chips to each tube.
- Once this is complete and all tubes have sample or standard and boiling chips, turn on the digester and start the program called TKN (H). It will take approximately 20 to 25 minutes for the block to reach 200 degrees C.
- Add 10 mL of Digest Reagent (Reagent #1) to each tube using 10 ml pipette (this will take less than 20 minutes).
- Once the block reaches 200 degrees C lower the rack with tubes into the heating block, the program will maintain that temperature for 70 minutes and then ramp up to 380 degrees C over the course of an hour.

- Place cold fingers on the tubes after the digest has been at 200 degrees C for 70 minutes. Don't wait too long as temperature ramps up to 380 degrees C. The digest will incubate for 60 minutes at 380 degrees C.
- Label acid cleaned 50 mL conical tubes with proper identification including batch #, date and sample location and sample ID.
- Once the digest is complete, carefully lift the rack of tubes out of the heating block and allow it to cool for approximately 5-8 minutes.
- Once cooled, using a squirt bottle, add Milli-Q water to the tube until the level is back 25mL. Vortex sample for at least 20 seconds. Note: the longer the samples sit after digestion, the more difficult it is to suspend the precipitate in the bottom of the tube. It is important that all precipitate is mixed into solution. It may take longer to vortex the last samples than the first samples to be removed from the rack.
    - It is important to start processing samples containing saltwater first as they tend to cool and form a precipitate that may require minutes of vortexing.
    - Work with one tube at a time by first taking off the cold finger and immediately bringing volume back to 25mL with a Milli-Q squirt bottle. Once the sample is vortexed and transferred to the appropriate tube, move on to the next sample.
- Pour the suspended sample into an acid-cleaned and labeled 50 ml disposable centrifuge tube.
- Note: if the digested sample is turbid, filter through a 0.2-micron syringe filter.
    - Filtering procedure for turbid samples.
        - Filter at least one ml of sample to waste to rinse the filter before collecting filtered digested sample.

TOTAL KJELDAHL NITROGEN AND TOTAL PHOSPHORUS ANALYSIS PROCEDURE

Analysis of TKN and TP digested samples.

TKN is Channel 2 on the MWQL Lachat QuikChem 8500 #1.

TKP is Channel 3 on the MWQL Lachat QuikChem 8500 #1.

Analytical steps:

- Turn on power to autoanalyzer, autosampler, and pump.
- Turn on the computer.
- Open the Lachat software
- Open the TKN/TP method or a run file from a previous TKN/TP run.
- Enter the identifiers for the standard curve and certified standards in the sample run template.
- Put all reagent intake lines in the dedicated bottle of milliQ water.
- Note: If pump tubing needs to be changed, refer to diagram in Appendix A.
- Put channel effluent lines into the water collection bottle.
- Make sure that the sample line is attached to the TKN sample valve.
- Load tubing into pump channels.
- Making sure that tabs on pump cartridges are all the way to the left, push down on the cartridge to secure. Each side of the cartridge will make a “click” as it locks in.
- Tighten pressure controls on pump cartridges by pushing tab all the way to the right and then back to the left until it clicks in place.
- Turn on the pump and set the speed to 35.
- Check for leaks and other problems.
- Reduce pump speed to less than 5.
- Pump milliQ water through lines until baseline is stable, at least a half hour.
- Remove manifold waste lines from the water collection bottle and put them into the chemical waste carboy, making sure that the end of the tubing is above the waste level. Cover opening tightly with parafilm. Set cap on top of carboy to prevent loss.
- Cut parafilm to cover all reagent bottles.
- Be sure to save subsampled a 50 ml Falcon tube of TKN diluent carrier to run as an analytical blank before putting reagent line in the carrier bottle.
- Starting with the line lowest on the diagram in Appendix A, remove intake lines from water and put them in corresponding reagent bottles, covering each with parafilm as you proceed. Do this for both TKN reagents and TKP reagents. Note: carrier for TKN is diluent solution, for TP it is milliQ water.
- Set up a run containing the following.

  - Two samples of milliQ water which will be used to give the system a final rinse before analysis.
  - At least 2 mixed calibration standards of 0.5 mg/l and 2 mg/l of both Ammonium -N and phosphate-P in diluent to test the method and ensure that the instrument is working properly.

  - A diluent blank.
  - A QCCS containing 0.5 mg/l of both Ammonium - N and phosphate - P made up in diluent.
- Put rinse tubes, blanks, and standards in the autosampler rack.
- Turn the speed back to 35.
- Start the “Preview” to look at the baseline.
- Once the baseline is stable, end the preview and start the sample run.
- Graph the response for the blank (0 mg/l), 0.5 mg/l and 2 mg/l of analyte to check linearity of instrument response.
- Use these curves to test instrument function and set the integration parameters for the analysis.
- Calculate the concentration of the laboratory control check standard from the equations produced.
- If standard curves are not linear or certified sample recovery deviates more than 10% from certified value, resolve before proceeding with digested sample run.
- Once standard curve, blanks and certified standards are finished and determined to be satisfactory, digested samples can be analyzed.
  - Note: Although we try to minimize contamination, the diluent does contain some P, this will show up in the blank for P in the digested blank.
- Analyze digested QC samples and standards first. If standards are not linear or digested QCCS measured concentration falls outside of accepted values, stop the analysis, and remedy the problem before analyzing environmental samples.
- If standards are satisfactory, analyze environmental samples.
- Once analysis is finished, return all lines to milliQ. Switch reagent lines to water starting with the reagent at the top of the Lachat manifold diagram and working downward.
- Rinse with milliQ water for at least 15 minutes.
- When finished rinsing, turn off the pump, release tubing from pump cartridges and remove tubing intakes from water.
- Remove waste lines from the hazardous waste container and put them into the water collection bottle.
- Cap waste container.

DATA ANALYSIS AND CALCULATION

The spectrophotometric detectors for TKN and TP form peaks of voltage response which are integrated within the software to provide the integrated peak area of the response. This peak area is directly proportional to analyte concentration. Note that all data calculations will consider any sample dilutions that may occur in the course of the analysis.

CAL - Standard concentration and peak areas are graphed with a scatter plot in Excel or another graphing program. A linear regression of the data is calculated. The r-squared value of the standard curve must be above .98 for use in this method. If the r-squared value falls below 0.98, then the instrument will be checked for malfunction and the standards will be run again. The linear regression is used to calculate the concentration of the analytes in the samples.

Accuracy - The QCCS or LCS concentration is calculated using the linear regression obtained from the CAL standard regression. The accuracy of the CAL standards is calculated as follows:

(ABS(QCCS/LCS certified concentration - QCCS/LCS measured concentration)/QCCS/LCS certified concentration) * 100

Precision - The percent error for replicate samples or standards (including FD, LD, QCCS, LCS or CAL standards) is calculated using the absolute value of the difference between each of the duplicate using the following equation:

$Variability_{Rep}$ =(Error/Sample Mean) x 100,

Error = (ABS(Rep1-Rep2))/2

Percent Recovery:

The percent recovery of the replicate spiked samples (LFSM) is calculated using the following equations:

% Spike Recovery = Mean($Recovery_1$, $Recovery_2$)

$Recovery_1$ = (($Analyte_{Spike1}$ - $Analyte_{S1}$)/Calculated concentration of spike) x 100.

$Recovery_1$ = (($Analyte_{Spike2}$ - $Analyte_{S2}$)/Calculated concentration of spike) x 100.

$Analyte_{S1}$ = Measured concentration of sample 1.

$Analyte_{Spike1}$ = Measured concentration of sample1 spike.

$Analyte_{S2}$ = Measured concentration of sample 2.

$Analyte_{Spike2}$= Measured concentration of sample 2 spike.

Minimum Detection Limit:

The minimum detection limit is calculated from the error of seven blanks or 7 low concentration standards, whichever is largest. See quality control section above for more details.

The minimum detection limit is calculated as follows:

1. Calculate the concentrations of the 7 blank replicates and the 7 spikes using the CAL standard curve.
2. Calculate the mean of the 7 blanks. If it is negative, equate it to zero.
3. Calculate the standard deviation of the 7 blanks.
4. Multiply the standard deviation of the 7 blanks by 3.143.
5. Add the mean of the 7 blanks to the result from line 4.
6. Calculate the standard deviation of the 7 spikes.
7. Multiply the standard deviation of the 7 spikes by 3.143.
8. The minimum detection limit is the greater of line 5 and line 7.

METHOD PERFORMANCE

TP

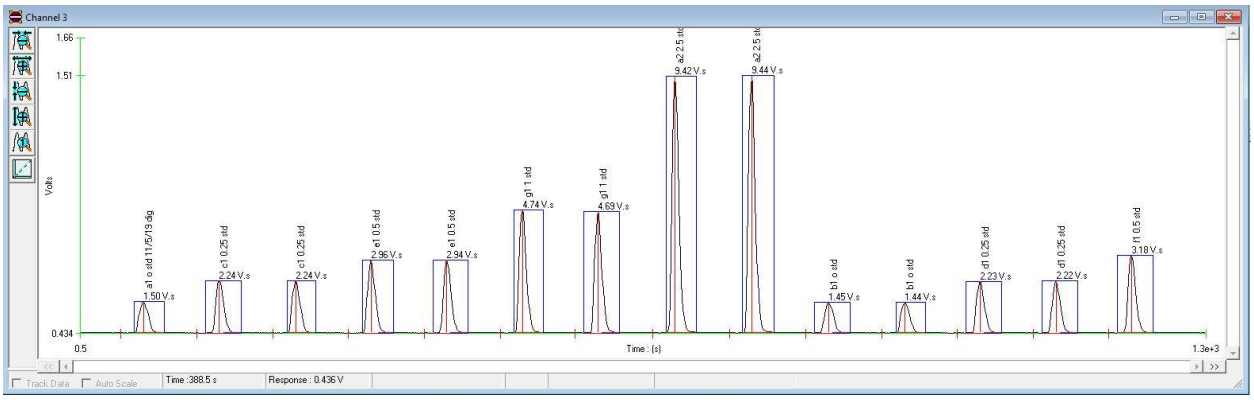


Figure1. TP CAL Standard Voltage Output from Lachat Quickchem Analytical Run.

Summary of digested TP Digested Standards Analyzed on 11/7/2019				
	Rep 1	Rep 2	Rep 3	Mean
TP (mg/l)	Peak Area	Peak Area	Peak Area	Peak Area
2.50	9.430	9.215		9.323
1.00	4.715	4.555		4.635
0.50	2.950	3.180	3.315	3.148
0.25	2.240	2.225		2.233
0.05	1.585	1.660		1.623
0	1.53	1.445		1.488
0	1.445			1.445

Table 2. Digested TP Standard Calibration Series using CAL standards.

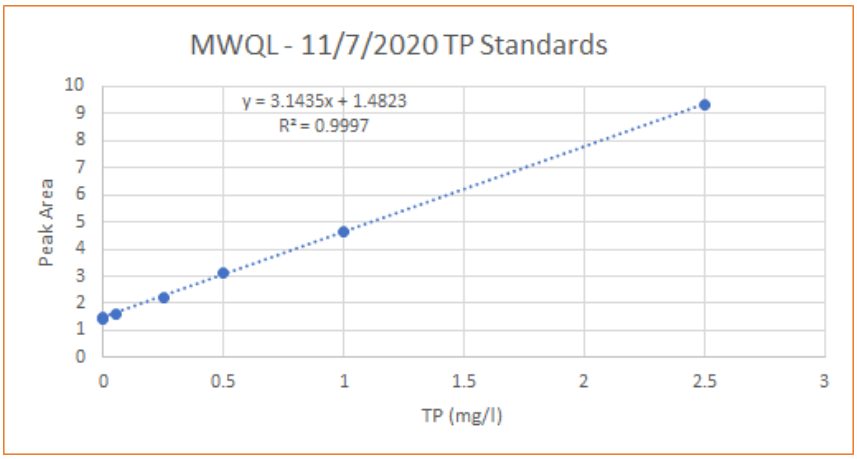


Figure 2. Graph of Digested TP CAL standards.

	Mean	Range
TP (mg/l)	Peak Area	Peak Area
5.00	18.650	0.050
2.50	10.200	0.000
1.00	4.805	0.005
0.50	3.145	0.015
0.25	2.285	0.085
0.05	1.650	0.015
0.00	1.450	0.000

Table 3. TP - Linear Range of Digested CAL Standards - 8/9/2019.

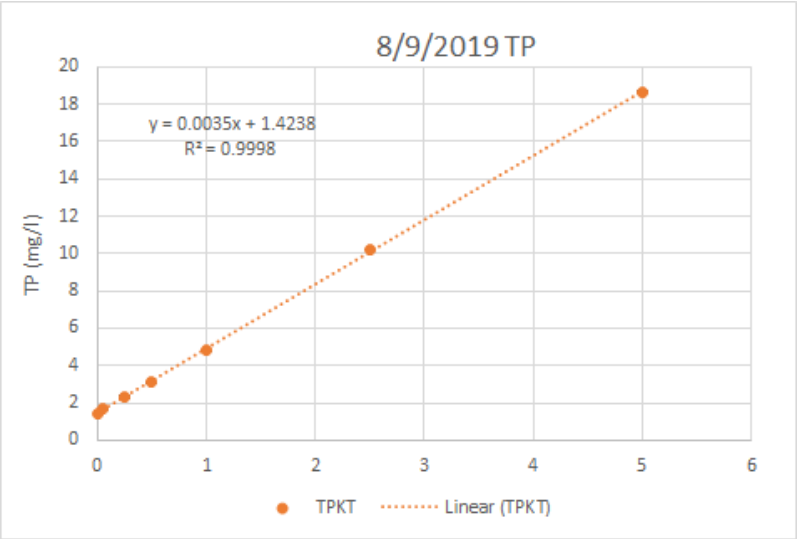


Figure 3. Graph of Linear Range of Digested TP CAL Standards - 8/9/2019.

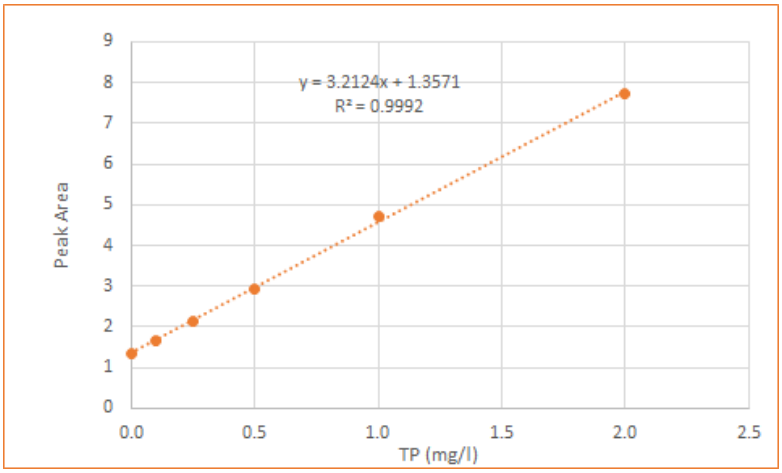


Figure 4. Undigested TP CAL Standards.

TKN

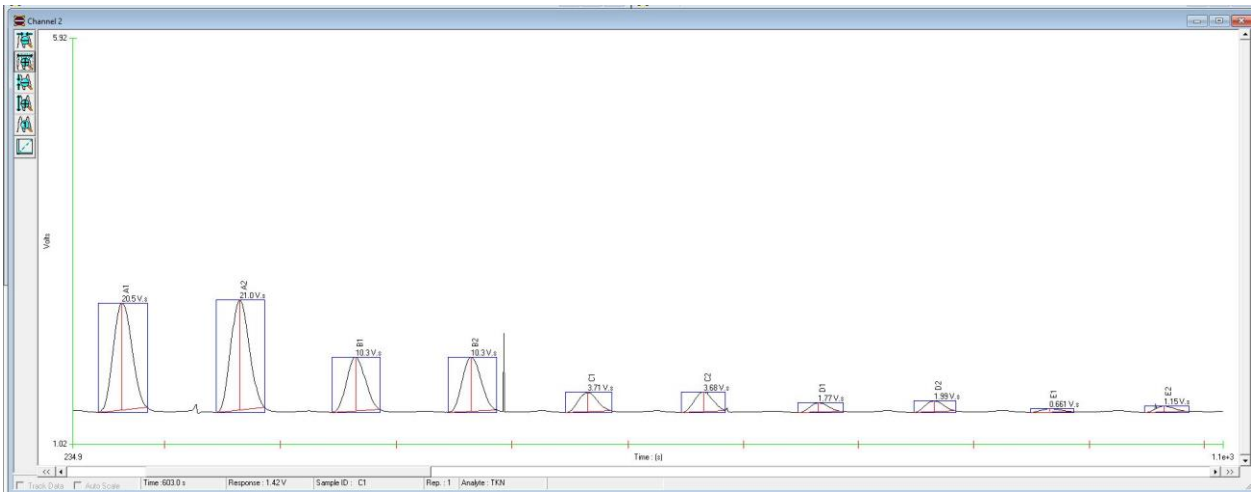


Figure 5. Example of TKN CAL Standard Voltage Output from Lachat Quickchem Analytical Run.

		Measured		Mean
TKN (mg/l)	Peak Area	TKN (mg/l)	% Residual	% Residual
5.0	14.73	5.058	1.17	3.43
2.5	7.01	2.399	4.05	
1.0	2.93	0.991	4.05	
0.5	1.61	0.535	0.89	
0.3	0.88	0.282	7.01	

Table 4. TKN Digested CAL Standard Series.

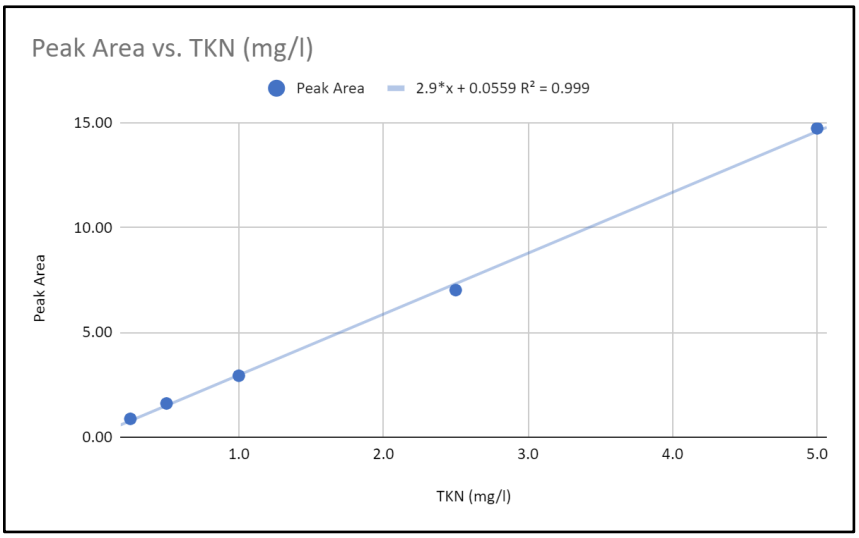


Figure 6. TKN Digested CAL Standard Regression.

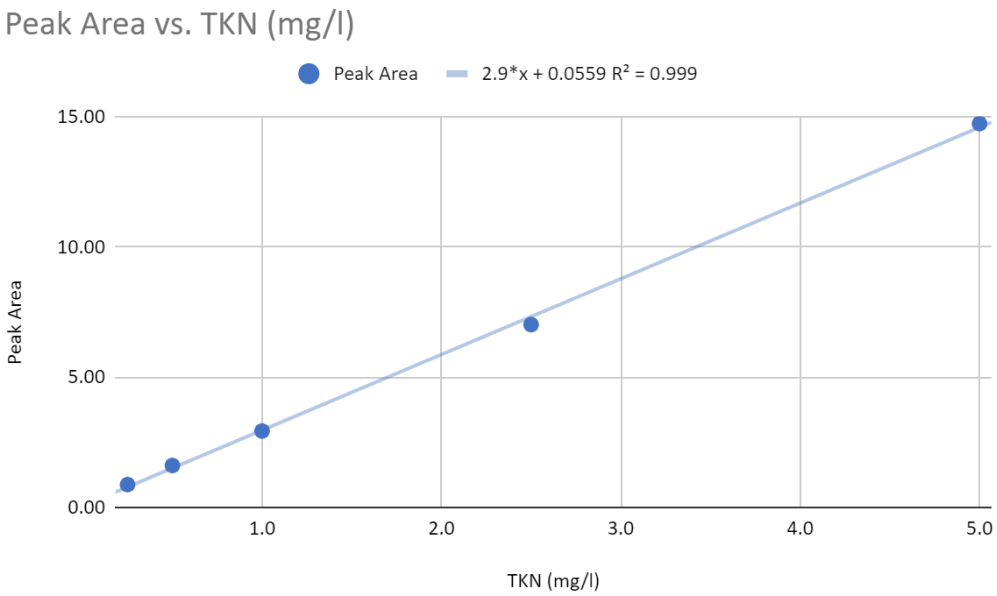


Figure 7. Linearity of TKN CAL Standard Working Range. Error bars are % Error of duplicate standards.

Corrective Actions:

General precision problems:

If baseline drifts, peaks are too wide, or other problems with precision arise:

- Check that there is not an obstruction in the tubing that is causing pressure buildup and retarding the flow of the carrier.
- Check flow of reagents. 'If reagent is not flowing properly:
    - Replace pump tubing.
    - Check all fittings for obstructions or crimping tubing.
- If the above actions do not alleviate the problem, clean the manifold by the following procedure:
    - Place transmission lines in water and pump to clear reagents (2-5 minutes).
    - Place reagent lines in 1 M hydrochloric acid (1 volume of HCl added to 11 volumes of water) and pump for several minutes.
    - Place all transmission lines in water and pump for several minutes.
    - Resume pumping reagents.

Turbidity:

Despite precautions taken to prevent this, Kjeldahl digests may contain some turbidity. To prevent the turbidity from interfering in the analysis, the following are recommended:

- Pour the diluted digests into the sample tubes, and allow them to settle prior to analysis.
- Set the probe height up from the bottom of the autosampler tube, so it does not hit the bottom to avoid aspirating the settled material.
- Let samples sit until the next day.
    - Pour them into bottles, cap tightly, and store in the refrigerator overnight. The turbidity will settle, so there is very little to none when you decant (carefully) the next day.
- If samples must be analyzed quickly, centrifugation of the sample tubes to speed the settling process can be used.
- To test whether turbidity is adding to the signal (by scattering light), replace the hypochlorite with water to prevent color formation (for TKN) or use DI water in place of the color reagent (TKP).

Weak or inconsistent signal:

- Reagents should be made up the day before. Freshly made reagents can give variable results.
- If excessive bubbles form:
    - Degas reagents.

- Check junctions for loose fittings.
- Check pump tubing. Replace worn tubing.
- Check intake tubing in bottles.

Poor precision:

- If replicate digested standards or samples vary by more than 10% from mean values:
  - Analyze undigested CAL standards and LCS to determine if the problem is with the analysis or the digestion step.
  - If problem is also present in undigested samples:
    - Check pump tubing.
    - Check back pressure tubing for leaks.
    - Check for bubbles.
    - Let the instrument equilibrate longer (inadequate equilibration can cause variability).
    - Check for blockages in lines.
  - If problem does not occur with undigested CAL standards and LCS:
    - Analyze other replicate standards and samples from digestion.
      - If other replicates or within the 10% variability, record all data and continue analytical run.
      - If all replicates vary by more than 10% but not by more than 20%, continue run, recording out of limit QC parameters.
      - If replicates vary by more than 20% stop run.

POLLUTION PREVENTION AND WASTE MANAGEMENT

- All wastes will be handled in accordance with University of Maine Safety and Environmental Management Guidelines.
- Waste will be stored in properly labeled containers with secondary containment.
- Waste will be located in approved areas and will be inspected weekly.
- When waste containers are full, they will be transported by the Darling Marine Center Safety Officer to a secure dedicated waste storage facility on the DMC campus.
- At the point of pick up and transport to the DMC waste storage facility, the responsibility for monitoring of the waste is transferred to the DMC safety officer.
- Waste will be stored until pickup by a University of Maine approved waste disposal company. All MWQL staff who handle waste will be required to complete the Hazardous Waste training provided by the University of Maine.

- The MWQL is committed to reducing chemical waste and will maintain the lowest volume of chemical stocks that is practical while maintaining adequate stock to guard against shortages and back orders.
- \All expired chemicals will be disposed of as chemical waste and transferred to the DMC waste storage facility.
- The lab does not dispose of any chemicals down the drain.

DATA ASSESSMENT AND ACCEPTANCE CRITERIA

Data Acceptance Specifications:

- **LCS - 90% - 110% of certified value.**
- **LB - Below MDL.**
- **LD - 90% - 110% of mean value.**
- **LFSM - Spike Recovery - 80% - 120%.**
- **Linearity - Linear regression of CAL standards - > 0.98.**
- **CAL standard at or below the RL will be analyzed at minimum once per month when instrument is in use and must be within 40% of true value.**
- **Reproducibility of CAL standards over the course of a batch of samples - 90% - 110%. If CAL standard values are outside of this limit, a new set of CAL standards must be analyzed.**

Data Assessment Checklist:

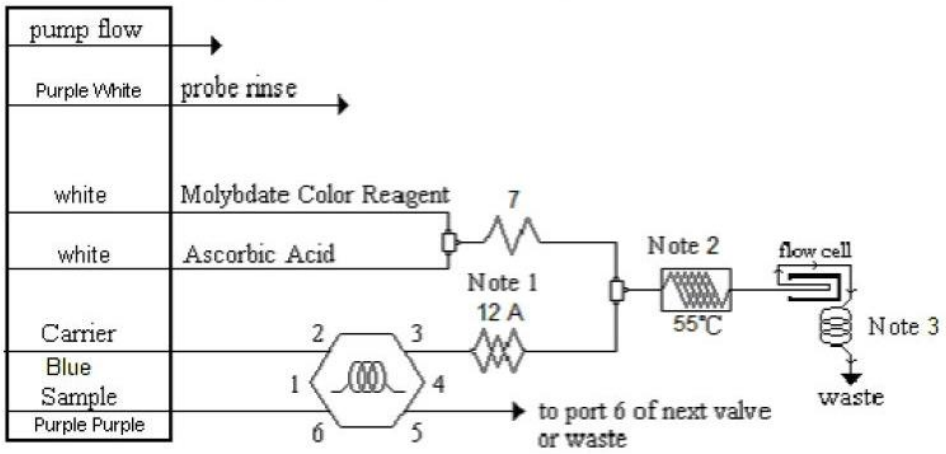
- **Were the samples analyzed within the required holding time (28 days)?**
- **Were the samples preserved properly?**
- **Were the samples properly stored between sampling and analysis?**
- **Was this protocol followed as written?**
    - **If not, is there a record of variance from the method and documentation explaining the variance?**

- **Are there at least 4 concentrations of CAL standards for each sample batch?**
- **Is there a CAL standard at or below the RL?**
- **Were at least 2 CAL standards re-analyzed immediately after the initial CAL standard series to document method stability?**
- **Is each standard concentration analyzed in duplicate?**
- **Is the r squared value of the linear regression of the mean CAL standard response versus concentration greater than 0.98?**
- **Are the peak heights within 25% of the heights achieved for the previous analytical run for similar concentrations?**
    - **If not, were further LB and CAL standards analyzed to reassess sensitivity?**
- **Is the measured concentration of the LCS within 10% of the certified value?**
- **Is the LB less than the MDL?**
- **Was an LB and LCS analyzed for every 10 samples?**
- **Are all of the LB and LCS samples within acceptance criteria?**
    - **If not, is there documentation to explain?**
- **Was a duplicate sample analyzed for every 10 samples?**
- **Was the precision of the duplicate samples within 90% - 110%?**
    - **If not, is there documentation to explain?**
- **Was one LFSM analyzed for every 10 samples?**
    - **If not, is there documentation to explain?**
- **Is there a properly documented Digestion Log (TKN/TPDIGT)?**
- **Is there a properly documented KTN/TP analysis log (TKN/TPAL)?**
- **Is there a proper Chain of Custody?**

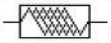
LIST OF FIGURES AND TABLES

Appendix A: Manifold and Valve Diagram

TP Manifold Diagram:



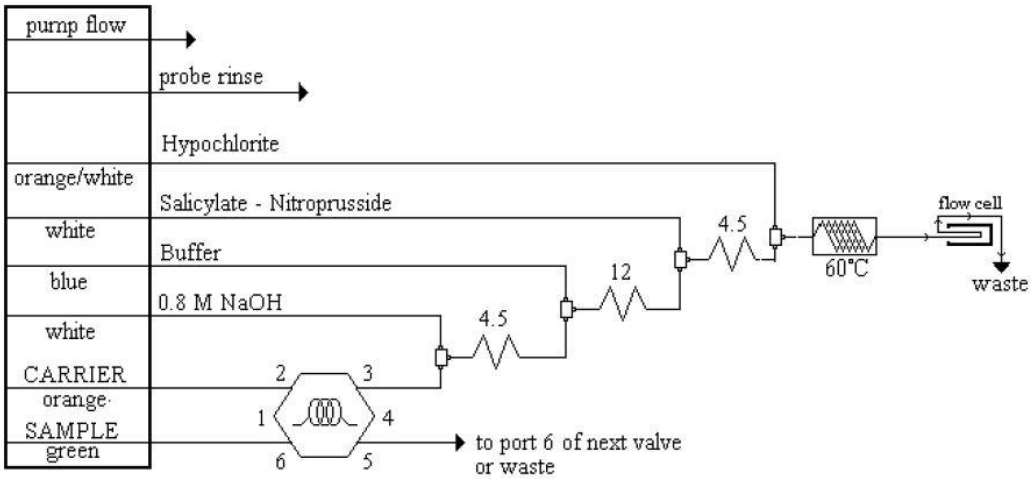
Carrier: **DI Water (See method notes)**
Manifold Tubing: **0.8 mm (0.032 in) i.d.** This is **5.2 µL/cm**.
Sample Loop: 14 cm 0.022" i.d.
Interference Filter: 880 nm

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required. The  shows **175 cm of tubing wrapped around the** heater block at the specified temperature.

7: **135** cm of tubing on a 7 cm coil support
12A **155** cm of tubing on a 12 cm alternating coil support

PVC PUMP TUBES MUST BE USED FOR THIS METHOD
Note 1 **155** cm of tubing on a 12 cm alternating coil support
Note 2: 175cm of tubing on the heater
Note 3: 200 cm x 0.022 in. i.d. backpressure loop

TKN Manifold Diagram:



Carrier: **Reagent 6 (Digestion Diluent)**
Manifold Tubing: **0.8 mm (0.032 in) i.d.** This is **5.2 µL/cm**.
AE Sample Loop: 25 cm
QC8000 Sample Loop: 30.5 cm
Interference Filter: 660 nm

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required. The  shows 650 cm of tubing wrapped around the heater block at the specified temperature.

4.5: **70** cm of tubing on a 4.5 cm coil support
12: **255** cm of tubing on a 12 cm coil support

Note 1: 650 cm of Teflon tubing is wrapped on the heater block.
Note 2: PVC PUMP TUBES MUST BE USED FOR THIS METHOD

Appendix D

Extracted Chlorophyll Analysis Standard Operating Procedure (SOP

Document Code: MWQLSOPCHL Rev.5

SM 10200 H

1. Specifications:

This method is applicable to marine, brackish and freshwater samples.

Applicable concentration ranges:

Chlorophyll a: 0.186 – 60 micrograms/l chlorophyll a. (Note: linearity exists between the LOQ and 200 micrograms/l)

2. Definitions

The definitions and purposes below are specific to this method but have been conformed to common usage as much as possible.

ANALYST - The designated individual who performs the “hands-on” analytical methods and associated techniques and who is the one responsible for applying required laboratory practices and other pertinent quality controls to meet the required level of quality.

ANALYTICAL BATCH -- An analytical batch is a group of environmental samples that are prepared and/or analyzed together with the same process and personnel, using the same lot(s) of reagents. A batch is composed of at least one and no more than 20 environmental sample(s) of the same quality systems matrix, meeting the above-mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 24 hours. An analytical batch is composed of prepared environmental samples (extracts, digestates or concentrates) which are analyzed together as a group. An analytical batch can include prepared samples originating from various quality system matrices and cannot exceed 20 samples.

CALIBRATION -- A set of operations that establish, under specified conditions, the relationship between values or quantities indicated by a measuring instrument or measuring system, or values represented by a material measure or a reference material, and the corresponding values realized by standards. In calibration of support equipment, the values realized by standards are established through the use of reference standards that are traceable to the International System of Units (SI).

CALIBRATION BLANK (CB) -- A volume of 90% acetone in the same matrix as the calibration standards, but without the analyte.

CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

CERTIFIED REFERENCE MATERIAL -- (CRM) means reference material, accompanied by a certificate, having a value, measurement of uncertainty and stated metrological traceability chain to a national metrology institute. This method uses a solid standard.

CHAIN OF CUSTODY FORM (COC) - A record, either paper-based or electronic, that documents the possession of the samples from the time of collection to receipt in the laboratory in accordance with chain-of-custody protocol. This record, at a minimum, must include the sample location, the number and types of containers, the mode of collection, the collector, the date and time of collection, preservation, and requested analyses.

FIELD BLANK (FB) -- An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, including exposure to a sample bottle holding time, preservatives, and

all pre-analysis treatments. The purpose is to determine if the field or sample transporting procedures and environments have contaminated the sample.

FIELD DUPLICATE (FD) -- Two samples taken at the same time and place under identical circumstances which are treated identically throughout field and laboratory procedures. Analysis of field duplicates indicates the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

LABORATORY BLANK (LB) -- An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, except that it is not taken to the sampling site. The purpose is to determine if the if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

LABORATORY CONTROL STANDARD (LCS) -- A solution prepared in the laboratory by dissolving a known amount of one or more pure compounds in a known amount of reagent water. Its purpose is to assure that the results produced by the laboratory remain within the acceptable limits for precision and accuracy. (This should not be confused with a calibration standard).

LABORATORY DUPLICATE (LD) -- Two aliquots of the same environmental sample treated identically throughout a laboratory analytical procedure.

LABORATORY FORTIFIED SAMPLE MATRIX (LFSM)-- An environmental sample containing an analyte spike of known concentration at least 4 times the MDL. Used to evaluate the effect of the sample matrix on the analytical results.

LIMIT OF QUANTITATION (LOQ) -- The minimum levels, concentrations, or quantities of a target variable (e.g., target analyte) that can be reported with a specified degree of confidence.

METHOD DETECTION LIMIT (MDL) aka Limit of Detection (LOD) aka Minimum Detection Limit -- The lowest level at which an analyte can be detected with 99 percent confidence that the analyte concentration is greater than zero.

MilliQ WATER - Reagent water that has been treated to remove impurities through reverse osmosis and filtration and has a resistivity above 18.0 .

QUALITY CONTROL CHECK SAMPLE (QCCS) -- A sample containing analytes of interest at known concentrations (true values) of analytes. The QCCS is obtained for a source external to the laboratory or is prepared from standards obtained from a different source than the calibration standards. The purpose is to check laboratory performance using test materials that have been prepared independently from the normal preparation process. Also known as the Proficiency Standard.

RELATIVE STANDARD DEVIATION (RSD) – The standard deviation expressed as a fraction of the mean.

3. Quality Control Measures:

All laboratory personnel using this SOP will also comply with all general laboratory QA and QC measures as stated elsewhere in this QAM. QA and QC procedures specific to this protocol are documented in this SOP.

3A. Initial Demonstration of performance:

- Minimum Detection Limit (MDL) – Note that this method follows the procedure documented in 40 CFR Part 136 and outlined in "Definition and Procedure for the Determination of the Minimum Detection Limit, Rev. 2 (EPA 821-R-16-006).
    - Estimate the initial MDL using the mean determined concentration plus three times the standard deviation of a set of method blanks.
    - To determine the ongoing MDL, choose a concentration between 2-10 times the estimated initial MDL.
    - Spike a minimum of 7 samples with the chosen concentration of standard.
    - In addition, analyze a minimum of 7 blanks.
    - The seven spiked samples and seven blanks must be analyzed on a minimum of 3 days.
    - Calculate the MDL of the spiked samples.
        - MDL (spiked samples) = the students t-value appropriate for a single - tailed 99th percentile t statistic and a standard deviation estimate with n-1 degrees of freedom, times the standard deviation of the replicate spike.
    - Calculate the MDL of the blanks.
        - MDL (blanks) = The mean of the method blanks times the student's t-value appropriate for the single-tailed 99th percentile t statistic and a standard deviation estimate with n-1 degrees of freedom times the standard deviation of the method blanks.
    - Select the greater of the spiked sample MDL and the blank MDL.
- Limit of Quantitation (LOQ) – The limit of quantitation will be determined by multiplying the standard deviation of the spiked samples by the student's t-value appropriate for a double-tailed 99th percentile t statistic and a standard deviation estimate with n-1 degrees of freedom. If the LOQ calculated by the above method is less than 2 times the MDL, then the LOQ = two times the MDL.
- Proficiency Standard (PT) - Annually, a PT will be ordered from an EPA recommended provider lab. The sample will be analyzed, and results sent to the provider lab. If results are not within the acceptable limits of stated values, the source of the problem will be identified and corrected. Also known as the QCCS.
- Demonstration of Linearity - A standard curve over the working range (0.186-60 micrograms/l) of the method will be analyzed annually to demonstrate linearity.
- Proof of Capability (POC): All staff performing SM10200 H, must demonstrate capability:
    - In a brown bottle, collect 1-liter clean matrix.

- Subsample 4 separate 100 ml samples using a Class A or lab calibrated graduated cylinder, being sure to mix the sample before taking each subsample.
    - Filter these samples, process, and analyze following the documented protocol.
- Subsample 4 additional 100 ml samples, being sure to mix the sample before taking each subsample.
    - Filter these samples following the documented protocol.
    - Spike these samples immediately following the addition of acetone solution, with a concentration of chlorophyll certified standard to give a final concentration in the extract of 10 - 20 µg/l.
- The % spike recovery and the standard deviation must be within 80-120% of the spiked concentration.

3B. Ongoing Quality Control Measures:

- Laboratory Blanks – One laboratory reagent blank consisting of milliQ water filtered and extracted in the same manner as the samples, will be analyzed for each batch of 10 samples. Blanks must be lower than the LOQ.
- Laboratory Control Standard – A solid chlorophyll a standard will be analyzed along with each batch of samples. If the LCS varies by more than 10% from the value recorded during the previous calibration, then a new calibration standard will be ordered, and the instrument will be recalibrated.
- Calibration Standards – Calibration standards will be ordered twice per year or when the LCS solid standard varies by more than 10% from the readings obtained during the previous calibration.
- Laboratory Duplicates - A set of LDs will be analyzed for every 20 samples. Duplicates should not vary more than 20% if the concentration is greater than 3 times the LOQ. Replicate samples whose concentration is below 3 times the LOQ, may have higher than 20% variability.
- Proof of Capability: Each analyst will perform a proof of capability once per year or when method changes are made or if problems arise with an analyst's work. See detailed procedure above.

4. Summary of Method:

A known volume of a water sample is filtered through a 25 mm glass fiber filter using low vacuum filtration. Filters are placed in a solution of 90% acetone, sonicated for 5 minutes and then extracted for greater than 4 and less than 20 hours at 4 degrees centigrade (4°C ±). Samples are centrifuged to clarify the extract. A subsample of the supernatant extract is transferred to a second tube for analysis. The tube is placed into the chamber of a Turner Trilogy fluorometer, and the raw fluorescence is recorded. The sample is then acidified, and a second fluorescence reading is taken. From these 2 readings, the corrected chlorophyll a and phaeophytin concentrations are derived.

5. Interferences:

- Filter residue and particulate matter can interfere with the fluorescence readings. Use caution not to disturb the sample while removing from centrifuge and pipetting off the supernatant. Do not pipette from the bottom of the extraction tube.
- Light can degrade chlorophyll. All steps of the process should be conducted with limited light, including during freezer storage, refrigerator storage, extraction, and analysis.
- Fluorescence readings change with the temperature of the sample. All extracted samples and liquid standards must be at room temperature when analyzed for fluorescence to avoid temperature derived variability or condensation on the fluorometer tubes.
- Dirty glass tubes can cause inaccurate readings. Glass chlorophyll tubes are either used straight from the box after each batch has been verified to be clean or washed thoroughly with laboratory detergent, rinsed with milliQ water, and rinsed with 90% acetone before use.

6. Safety:

Hazards:

This protocol requires the use of hazardous chemicals. All laboratory personnel using this protocol must be current with all University of Maine safety training requirements.

This protocol requires the use of a sonicator for which staff must receive specific instrument training. Improper use of the sonicator can damage hearing.

This protocol requires the use of a vacuum pump which if used improperly can cause vacuum carboys to implode. Read all instructions for use of pump.

Hazardous chemicals: Read SDS sheet for all chemicals before use. Wear appropriate personal protective equipment including appropriate gloves, lab coat and safety glasses. The following list highlights some of the hazards of the listed chemicals but IS NOT intended to replace thorough reading of the SDS sheets by laboratory personnel.

- **Hydrochloric Acid (CAS#: 7647-01-0). Corrosive. Toxic.**
- **Acetone (CAS#: 67-64-1). Flammable. Toxic.**

7. Equipment and Supplies:

- Brown plastic (HDPE or LDPE preferred) bottles for sample collection. 250 ml to 1 liter in size
- Niskin bottle if samples are to be collected at depth
- Vacuum pump, carboy and filtration rig with 25 mm diameter filter funnels and bases
- Glass fiber filters – 25 mm diameter
- 2 pairs of filter forceps
- Squirt bottle with milliQ water

- Test tubes with sealing caps for extraction and analysis (extraction tubes with Teflon screw caps and small fluorometer tubes with plastic caps for analysis). Note that the 7-ml tubes fit into the fluorometer cell and are used in place of cuvettes.
- Bottle top dispenser and amber glass bottle for 90% acetone
- Sonicator with water bath
- Ice pack for water bath
- Hearing protection appropriate for sonicator use
- Refrigerator for sample extraction
- Freezer for filter sample storage
- Aluminum foil, heavy duty
- Ziploc bags
- Centrifuge, bench-top swinging bucket
- Balance for equalizing tubes and centrifuge bucket weight in preparation for centrifugation
- Class A volumetric pipettes for extracted sample transfer
- Kimwipes
- Trilogy chlorophyll a and phaeophytin module - 7200-040-W Chlorophyll a Extracted – Blue excitation and red emission spectrum.
- Hydrochloric Acid, ACS grade or higher.
- Acetone, HPLC or Spectrophotometric grade
- Magnesium Carbonate – finely powdered
- Pipette for acid addition
- Glass test tubes with Teflon lined caps for making dilutions.
- 250 ml Pyrex bottle with Teflon or other acid-resistant cap for 0.1 N Hydrochloric Acid.
- Fume hood
- pH meter (for pH testing of samples suspected of having a pH lower than 6)

8. Labware Cleaning:

- Brown collection bottles are cleaned with laboratory detergent and rinsed with milliQ water.
- New glass tubes do not normally need to be washed before first use. Batches are initially checked for cleanliness by extracting and analyzing blanks using replicates from a batch of tubes.
- After use, tubes are washed with laboratory detergent, rinsed with milliQ, rinsed with 90% acetone, and left inverted in the fume hood to dry.
- Cuvettes are not required for this method because the Trilogy fluorometers are designed to read samples in the glass tubes.
- Labware must not be acid cleaned for this method.

9. Sample Collection and Preservation:

Sample Collection:

- A MWQL Chain of Custody form must be filled out for each batch of samples.
- Samples (250 - 1000 ml) are collected in laboratory detergent cleaned and milliQ rinsed, brown plastic bottles.
- Samplers must wear gloves and be careful not to touch any unclean surfaces with gloves before or during sampling.
- Samples may be collected from Niskin bottles or directly from river, stream, estuary, or other water source.
- Collection of the sample:
    - Rinse collection bottle with sample.
        - Fill the bottle with a volume of sample sufficient to rinse the interior of the bottle (for a 100 ml bottle, 10 ml is adequate).
        - Cap bottle, invert, and swirl sample in the bottle to rinse the entire inner surface of the bottle.
        - Dispose of rinse water downstream of the sampling site.
        - Repeat 2 times.
    - Fill the bottle to the neck and cap.
    - If sampling from a Niskin bottle, be sure to gently mix the water in the Niskin, before drawing the subsample.

Sample Storage:

- Store samples in the cooler, in the dark, with ice packs immediately after collection. Samples in collection bottles must remain below 4 degrees Centigrade, in the dark, until filtered.
- If water samples are collected from bodies of water that may have a pH lower than 6, for instance some freshwater bodies, the pH of the sample must be measured when samples arrive in the laboratory. If pH is less than 6, then samples must be extracted promptly after filtration.
- All samples must be filtered within 6 hours of collection.
- Except where noted above for low pH samples, filtered samples (contained on glass fiber filters) may be stored for up to 20 days after filtration at -20 degrees Centigrade.

10. Reagents and Standards:

6. **MilliQ water – Record water resistivity and temperature.**
    a. Resistivity greater than 18.0.
    b. Water is pretreated with particulate and carbon filters, passed through a reverse osmosis membrane, filtered through a Millipore milliQ or similar clean water system and filtered through a final particulate filter before use.
7. **90% Acetone: Studies have shown that excess magnesium carbonate can actually degrade chlorophyll by-products due to adsorption of the pigments to the magnesium carbonate (Aminot and Rey, 2001). Because seawater contains magnesium and carbonate, we do not make up the 90% acetone with magnesium carbonate or add magnesium carbonate to seawater samples. This is standard for oceanographic chlorophyll measurements.**
    a. Seawater Samples
        i. Make 1 liter of aqueous acetone solution (Note that if you are only analyzing a small amount of sample, make a lesser amount. Solution lasts for one month).
            1. Using the dedicated Class A 100 ml volumetric flask, measure 100 ml of milliQ water.
            2. Add milliQ water to the dedicated Class A 1-l flask.
            3. In the fume hood, fill the 1-liter flask to the mark with HPLC Grade Acetone.
            4. Cap and invert to mix.
            5. Transfer solution to 1-liter brown glass bottle with dispenser top.
            6. Make sure that cover is on dispenser tip when not in use.
            7. Log reagent in the MWQL Reagent Tracking Log.
            8. Use acetone in the hood.
    b. Fresh Water Samples:
        i. If samples are collected from fresh water and the pH of the water is less than 6, the 90% acetone should be made up as follows:
            1. Add 100 ml of the following $MgCO_3$ solution to a 1-liter flask.
                a. $MgCO_3$ solution:
                    i. Add 1 g of finely powdered magnesium carbonate to 100 ml of distilled or higher-grade water.
            2. In the fume hood, fill flask to the mark with HPLC Grade Acetone.
            3. Cap and invert to mix.
            4. Pour solution into 1-liter brown bottle with dispenser top or a brown glass bottle with Teflon cap.
            5. Make sure that cover is on dispenser tip when not in use.
            6. Record acetone solution in Reagent Tracking Log.
            7. Use acetone in the hood.

8. **Standards**.
    a. Certified liquid chlorophyll a standards are available for purchase. The Turner certified standard comes as two solutions, one with a high and one with a low concentration. For example, lot # CAS165-01 contained a high standard with a concentration of 240 micrograms/l, and a low concentration of 25.1 micrograms/l of chlorophyll a. These standards are diluted with 90% acetone solution in the lab to make a standard series from approximately 0.05 micrograms/liter to 60 micrograms/l or higher. Liquid standards are expensive and have a limited shelf life; solid standards are used for daily verification.

Standard Conc. (µg/l)	Standard Stock	Volume of Stock Added (ml)	Acetone Added (ml)
240.00	240	Straight Standard	
120.00	240	2.25	2.25
53.33	240	1.00	3.50
26.67	240	0.50	4.00
25.10	25.1	Straight Standard	
12.55	25.1	2.25	2.25
5.58	25.1	1.00	3.50
2.79	25.1	0.50	4.00
1.39	25.1	0.25	4.25
0.558	25.1	0.10	4.40
0.186	0.560	3	6
0.046	0.186	3	9

Table 1. Working standards from an August 2020 calibration.

    b. Solid Standard. Turner Designs provides a solid standard for the purpose of monitoring instrument drift. The solid standard is analyzed with every batch of samples. If a change in fluorescence readings occurs of 10% or greater from the reading at the last calibration, a new liquid certified standard instrument calibration is performed.

9. **0.1 N Hydrochloric Acid** (HCl):
    i. Add 90 ml of milliQ water to a clean 100 ml Class A flask.
    ii. Add 0.833 ml of concentrated HCl.
    iii. Bring to 100 ml with milliQ water.
    iv. Transfer to dedicated Pyrex bottle.

11. Calibration and standardization:

- The instrument is calibrated with a certified liquid standard twice per year, when problems arise, or if a change of greater than 10% in fluorescence readings of the solid chlorophyll standard occurs. Certified liquid chlorophyll standards are expensive and do have a limited shelf life, so it is important to prepare properly before ordering and diluting standards. To calibrate:
    - Turn on the spectrofluorometer and allow to warm up for at least 30 minutes.
    - Remove standards from freezer and allow to come to room temperature, in the dark.
    - In subdued lighting, the fume hood, carefully dilute the liquid standards with 90% acetone, roughly following the dilutions in Table 1, above. Note that each batch of certified standard is a different concentration, so dilutions will be slightly different for each batch.
    - Analyze a series of at least 10 acetone blanks. These blanks will be used as a baseline to verify instrument performance and for MDL determination.
    - Obtain a fluorescence reading from each standard concentration.
        - Dilution of the certified standard should continue until obtaining a fluorescence reading of approximately 3 times the blank average.
    - Acidify several of the samples to obtain the ratio of unacidified to acidified pure chlorophyll.
        - After obtaining an initial raw fluorescence reading, add 0.1 N Hydrochloric Acid to each sample to make an HCL concentration of 0.003 N in the final solution.
        - Mix sample gently.
        - Allow sample to sit for at least 30 seconds.
        - Wipe off outside of tube.
        - Obtain raw fluorescence of acidified sample.
    - Immediately before after liquid standard readings are taken, obtain a raw fluorescence reading of the solid chlorophyll standard, remove the standard from the cell holder. Repeat after 5 minutes. These readings will be used as a baseline to detect drift.
    - Record at least one solid standard reading in the Chlorophyll Solid Standard Log each time you analyze samples.

12. Chlorophyll Analytical Procedure

- Print out a “Chlorophyll Filtration Log” and “Chlorophyll Analysis Log” Fill out the relevant information as you proceed through the filtration.
- If samples will not be extracted immediately, prepare foil packets for chlorophyll filters:

- Cut a rectangular piece of foil large enough to enclose a folded 25 mm filter.
- Use a Sharpie to label outside of foil pouch with project name, date, station, depth, replicate and any other needed information.
- Turn down the lights in the laboratory.
- Prepare filter rig:
    - Check carboy to make sure it is not too full.
        - If carboy is ¾ full or more, empty the carboy.
    - If using a pump with an oil reservoir, check to make sure that vacuum pump glass oil receptacle has pump oil.
    - Check vacuum tubing to be sure all connections are tight.
    - Open channels on filter rig.
    - Rinse each filter cup and base with milliQ water from squirt bottle
    - Turn on pump to pull water through filter base.
    - Place a glass fiber filter on each filter base.
        - A drop of milliQ water placed on the base before adding the filter will help hold the filter in place while you attach the cup to the base.
    - Attach a filter cup to each base.
- **Filtration (in subdued light):**
    - Filter 100-250 ml of seawater through a glass fiber filter using the vacuum filtration rig. Note that sample volumes for samples with extremely high particulate concentrations may need to be less than 100 ml.
    - Remove samples from cooler.
    - Place each bottle next to the filter station through which it will be filtered.
    - Gently invert the bottle three or four times to mix sample. Do not shake, as you risk breaking cells.
    - Rinse the graduated cylinder with a few ml of sample.
    - Cap bottle and mix.
    - Repeat the rinse three times.
    - Mix sample and fill to the 100 ml mark of the gradated cylinder for most samples.
        - If water is exceptionally clear, you may need to filter more sample volume.
        - If phytoplankton are in high concentration, the volume filtered may need to be reduced.
        - You should be able to see the sample on the filter, if you can't, filter more sample. Keep a tally of the total volume filtered.
    - Make sure channel valve is open.
    - Pour sample into filtration cup.
    - Turn on pump and check vacuum.
        - Vacuum should not exceed 20 kPa.
    - Do not let filter completely dry out.
        - Turn off channel as sample level gets close to the level of the filter.
        - Turn off pump.

- Briefly open and close channel valve to remove any water that may remain above filter.
- Fresh water and low pH samples only:
    - Add 2 ml of $MgCO_3$ solution to each sample just before it is fully filtered through the filter.
        - To make magnesium carbonate solution, add 1 g of finely powdered magnesium carbonate to 100 ml of MilliQ water.

- Remove cup from filter station.
- Using forceps, carefully fold filter over onto itself so side with sample is on the inside.
- If not starting extraction immediately, place folded filter into foil pouch and fold over flaps of pouch to seal.
- Put pouch in Ziploc bag and store in freezer up to 20 days.
- If extracting immediately, put folded filter into the bottom of the extraction tube.
- Filter one blank of milliQ water for each batch of 10 samples.
- Rinse each filter base and cup with milliQ between samples.
- Store filters in foil pouches or tubes in subdued light while continuing to filter the batch.
- After final filtration, rinse filter cups with 90% acetone in the fume hood. Allow to dry in the hood, then rinse with milliQ water.

- **Extraction:**
    - Label 2 sets of chlorophyll tubes (extraction tubes with Teflon screw caps and small chlorophyll analysis tubes with plastic push-on caps) with electrical tape on the upper 1/3 of the tube. Use pencil as acetone will remove ink and Sharpie.
    - In subdued lighting and in the hood, place filter into an extraction tube, pushing filter to the bottom of tube.
    - Using the bottle-top pipette, add 7 ml of 90 % aqueous acetone being careful not to splash any of acetone outside of the tube.
    - Cap the tube and place in test tube rack.
    - When all filters have been placed in tubes and acetone has been added, cover entire rack with foil to block light.
    - Turn off lights in sonicator room.
    - Operator of sonicator must have received training in its use.
    - Take sample rack to sonicator and place rack in water bath.
    - Put a small ice pack in the water bath to prevent the water in the bath from heating up during sonication. Ice packs are in the freezer of the reagent refrigerator.
    - The water level in bath must cover the bottom inch of the tubes.
    - Insert small sonicator probe at least 1 " into the water making sure it does not touch rack or sides of bath.
    - Put on hearing protection.
    - Turn the intensity dial of the sonicator all the way down.

- Clear others from the area outside of the sonicator room or provide others with hearing protection.
- Turn on the power to the sonicator (red push button).
- Gradually turn up intensity to 30.
- Leave room and shut door.
- Either stand outside of room near door to prevent others from entering or place "Do Not Enter Sonicator in Use" sign on door. If standing next to the door, wear hearing protection.
- Sonicate for 5 minutes. For consistency, do not let samples sonicate for less than 4 ½ minutes or more than 5 ½ minutes.
- After 5 minutes, turn down sonicator intensity.
- Turn off power to sonicator.
- Remove rack from water bath, letting excess water drain into bath.
- Wrap rack in foil and place rack in MWQL sample refrigerator.
- Record temperature of refrigerator. There is a liquid bottle thermometer in each refrigerator.

- **Fluorometric Analysis:**
  - After greater than 4 and less than 20 hours of extraction, remove samples from refrigerator.
  - Allow them to come to room temperature, in a dark cabinet.
  - Turn on Turner Trilogy fluorometer one-half hour before the start of the analysis.
  - Centrifuge samples using the bench-top "swinging bucket' centrifuge.
    - Make sure that all buckets have small rubber inserts.
    - Equally balance the weight of the bucket collar and the buckets on opposite sides of the centrifuge rotor. The weights are embossed on the components.
    - Insert tubes into buckets in balanced pairs.
    - Check that the combination of collar, tubes and samples is balanced for opposite loads on the rotor.
      - Use the small swinging balance (with red and yellow trays).
      - The arrow should be touching the middle mark on the balance.
      - If it is off to one side too much, use a squirt bottle to add water to the bucket {tube holder} of the side that is too light to bring the arrow to the center mark. Note: DO NOT ADD WATER TO THE TUBE ITSELF!
    - Insert collars with buckets and tubes into the channel in the rotor.
    - Lock down centrifuge lid.
    - Centrifuge at 1400 rpm (500g) for 20 minutes.
    - The centrifuge motor will stop after 20 minutes but it will take longer for motion to cease. Do not use break but instead wait for centrifuge to stop.

- Remember that opening the lid does not stop the rotor. There is no safety to prevent the lid from opening when the centrifuge rotor is spinning. DO NOT OPEN THE LID UNTIL THE ROTOR HAS COMPLETELY STOPPED.
- Once motion has stopped, open lid and remove tubes making sure light is diminished in room.
- Cover rack with foil when not removing or placing tubes in rack.

- Using a Class A volumetric glass pipet and bulb, transfer 3 ml from the top of the sample extraction tube to a small clean chlorophyll tube.
- Samples must be at room temperature (they should not feel cold to touch and should have no condensation). Temperature variations will cause fluorescence variation, so it is important to make sure that the samples are at room temperature (21 degrees centigrade plus or minus 3 degrees centigrade) when they are analyzed.
- Wipe off outside of tubes with a Kimwipe.
- Place tube in fluorometer, close lid and read fluorescence.
- If sample is at or above 90% of the reported range (roughly 200,000 raw fluorescence units on the MWQL Trilogy), dilute and analyze again.
  - Dilute samples by adding sample and 90% acetone to a new tube in the proper proportions.
  - Do not bring the total volume to more than 3 ml.
  - Record dilution factor in the Chlorophyll Analysis Log.
- Remove tube from fluorometer.
- After all samples have been read, then acidify the samples and read again:
  - Add (0.1 ml for the 3 ml subsample) 0.1 N HCl to each sample.
  - Acidify samples in small batches which can be analyzed within the 3-minute time frame (see below).
  - Cap tube and gently invert to mix acid into sample.
  - Do not let samples sit less than 30 seconds or more than 3 minutes after acid addition before reading.

13. Data Analysis and Calculations:

Corrected Chlorophyll a and Phaeophytin calculations.

- The raw fluorescence reading per microgram of chlorophyll (response) is obtained from the slope of the linear regression obtained from analysis of liquid certified chlorophyll a standard.
- The ratio of the un-acidified raw fluorescence to the acidified raw fluorescence (f0max) of pure chlorophyll a is also determined by acidifying the pure chlorophyll standards.
- f0 is equal to the unacidified raw sample fluorescence.
- fa is equal to the acidified raw sample fluorescence.
- The chlorophyll a concentration is:

(response*(f0max/(f0max-1))*(f0-fa))*((extraction vol. (l)*dilution factor)/vol. filtered (l)

- The phaeophytin concentration is :

  (response*(f0max/(f0max-1))*(f0max*fa-f0))*((extraction vol. (l)*dilution factor)/ vol. filtered (l))

Accuracy – The accuracy of the chlorophyll a method is determined by yearly QCCS (PT) which are samples of know concentration provided by an outside entity which are analyzed blind by the laboratory.

Precision of Duplicate Samples- The percent error for replicate samples or is calculated using the absolute value of the difference between each of the duplicates using the following equation:

$Variability_{Rep}$ =(Error/Sample Mean) x 100,

Error = (ABS(Rep1-Rep2))/2

Instrument Drift:

The instrument drift is determined by analyzing the fluorescence signal of a solid standard with every batch of samples and comparing its raw fluorescence to that obtained with the same standard at the time of the most recent calibration. A running log of solid standard fluorescence is maintained in the Chlorophyll Solid Standard Fluorescence Log which is located next to the fluorometer.

Minimum Detection Limit:

The lowest level at which an analyte can be detected with 99 percent confidence that the analyte concentration is greater than zero. See “Initial Demonstration of Performance” above for details of calculations.

Limit of Quantitation:

The limit of quantitation is determined by one of the following methods.

- Determining the concentration at which the fluorescence signal from the certified liquid chlorophyll sample is equal to three times the blank value.
- Determine from the 2 tailed t-test for the 99% probability from a set of blanks and a set of spiked blanks and choose the larger of the 2 concentrations.
- Multiply the MDL by 2.

14.MethodPerformance:

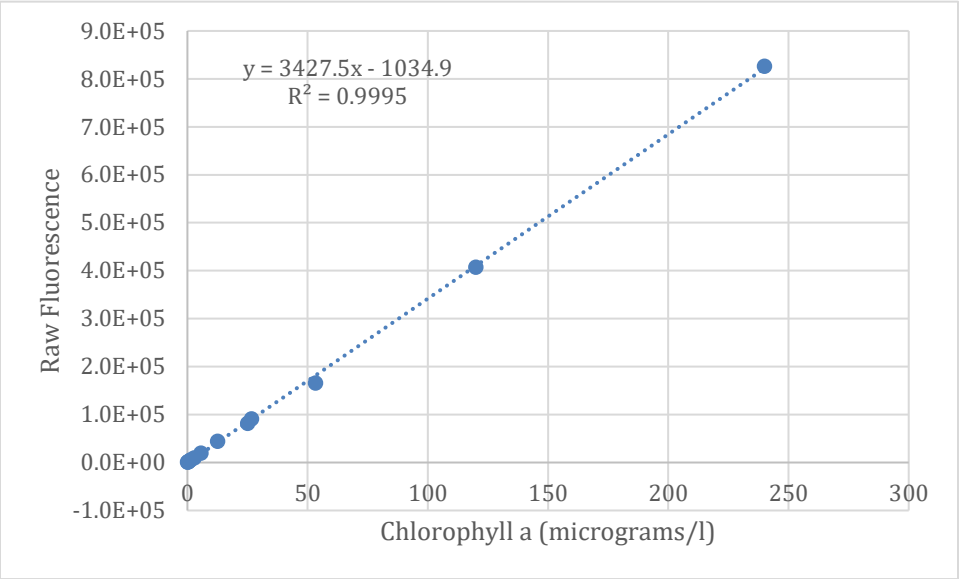


Figure 1. An example of linearity. Raw fluorescence of liquid chlorophyll standard, 8/13/2020.

15. Corrective Actions:

General precision problems:

1. If replicate variability is greater than acceptable limits (20% variability):
    a. Determine if the sample raw fluorescence is above or below 10 times the LOQ:
        i. If it is above 10 times the LOQ:
            1. Rerun samples making sure to mix sample well before subsampling.
            2. Verify that extracted samples are at room temperature before sampling.
            3. Allow 30 minutes for fluorometer to warm up before analyzing samples.
            4. Make sure that acidified samples are well mixed and are allowed to sit for 30 seconds after acid addition and mixing.
            5. Check the raw fluorescence of the solid standard to make sure that readings have not exceeded 10% of what they were at the time of the most recent calibration.
            6. If sample variability is still unacceptably high, replace lamp in fluorometer.
        ii. If the sample raw fluorescence is below 10 times the LOQ, variability may be higher than 20%.
2. If solid standard varies by more than 10% from the readings at the time of the last calibration:
    a. Check to make sure fluorometer was warmed up for at least one hour.
    b. Check to make sure that solid standard was properly seated and in the correct orientation. Improper seating of standard can cause large variations in reading.
    c. Notify lab manager who will clean the instrument chamber.

   d. If none of the above actions brings fluorescence readings to within 10% of last calibration: replace lamp.
3. Issues of unfiltered sample temperatures or other procedures being out of specification are dealt with in the QAM. If these issues occur with SM 10200 samples or procedures, the QAM protocols will be followed.

16. Pollution Prevention and waste management:

All wastes will be handled in accordance with University of Maine Safety and Environmental Management Guidelines. Waste will be stored in properly labeled containers with secondary containment. Waste will be located in approved areas and will be inspected weekly. When waste containers are full, they will be transported by the Darling Marine Center Safety Officer to a secure dedicated waste storage facility on the DMC campus. At the point of pick up and transport to the DMC waste storage facility, the responsibility for monitoring of the waste, is transferred to the DMC safety officer. Waste will be stored until pickup by a University of Maine approved waste disposal company. All MWQL staff who handle waste will be required to complete the Hazardous Waste training provided by the University of Maine.

The MWQL is committed to reducing chemical waste and will maintain the lowest volume of chemical stocks that is practical while maintaining adequate stock to guard against shortages and back orders. All MWQL expired chemicals will be disposed of as chemical waste and transferred to the DMC waste storage facility. The lab does not dispose of any chemicals down the drain.

17. Data Assessment and Acceptance Criteria:

Data Acceptance Specifications:

- **Solid standard - 90% - 110% of raw fluorescence at the time of the last calibration.**
- **LB - Below LOQ.**
- **Linearity - Linear regression of certified liquid standards - > 0.98.**
- **Proof of Capability replicates:**
    - **Standard deviation of spiked samples must be within 20%.**
    - **Spiked samples must be within 20% of certified concentration.**
- **Holding Time: Sample processing, preparation and analysis must be completed within 21 days of collection. If not, the variation from specifications must be recorded with the data.**

Data Assessment Check List:

- **Were the samples analyzed within the required holding time (21 days)?**
- **Were the samples stored in adequate refrigeration?**
- **Were the samples kept in the dark?**
- **Were the samples properly stored between sampling and analysis?**

- **Was this protocol followed as written?**
    - **If not, is there a record of variance from the method and documentation explaining the variance?**
- **Were the solid standard values recorded?**
- **Were the replicate sample values within 20%?**
- **Were the blanks less than the LOQ?**
- **Is the r squared value of the linear regression of the liquid certified standard response versus concentration greater than 0.98?**
- **Was an LB analyzed for every 10 samples?**
- **Are all of the LB replicate samples within acceptance criteria?**
    - **If not, is there documentation to explain?**

REFERENCES:

Aminot, A. and F. Rey. 2001. ICES Techniques in Marine Environmental Sciences #30: Chlorophyll a Determination by Spectroscopic Methods. International Council for Exploration of the Sea.

Appendix E

MWQL - Sampling, Sample Receipt and Handling Instructions

MWQSCI Rev.2

Purpose:

To outline the procedures for the receipt, handling, protection, storage, retention and/or disposal of test items.
To outline the procedures and appropriate facilities for avoiding deterioration, loss, or damage to the test or calibration item during storage, handling, preparation, and testing.

General Information:

- The MWQL will supply bottles for sampling.
- Use caution when collecting samples and follow instructions precisely.
    - Some bottles may contain corrosive preservatives.
        - These bottles will have a label indicating that the bottles contain hazardous material.
        - The instructions for filling bottles differ between those that contain preservative and those that do not contain preservative.

- Preservative MUST be added to bottles if samples will be shipped to the MWQL.

- If you must ship samples to us, please follow the instructions in the "Shipping" section below.

Sample Collection: Please follow the instructions below for specific sampling protocols. Any deviation from the protocols in this document must be noted and flagged.

Labels: Labels must include Project Code, Date of collection, Location ID, Time of collection, Initials of Sampler, Preservation method (if preserved), Sample Type and protocol.

Chain of Custody (COC): A MWQL Chain of Custody Form must be filled in for each sample batch. All samples must be recorded on the COC. All information must be completed. Every person handling or transporting samples must be listed on the MWQL COC.

Storage and Transport of Samples:

All samples must be stored in a cooler on ice (loose ice or a combination of loose ice cubes and ice packs). A tube with water is included and is labelled "Temperature Solution". This tube must be kept in the cooler with the samples until delivery to the MWQL.

Sampling for EPA 351.2/EPA 365.4: Total Kjeldahl Nitrogen (TKN)/Total Phosphorus (TP)/and EPA 350.1: Ammonia.

a) Collect samples in 250-1000 ml bottles provided by MWQL.
b) Check to make sure that all required information is on the label (see "Labelling" section above).
c) Wear gloves and safety glasses at all times when sampling.
   i) Samples contain a small amount of caustic sulfuric acid as a preservative.
   ii) Gloves also prevent material from your hands from contaminating the sample.
d) Inspect bottles to make sure they are clean and in good condition.
e) Collect the whole water sample. DO NOT FILTER.
f) Fill the bottle with sample to the top of the straight side of the bottle. Do not overfill.
g) Attach cap securely.
h) Immediately place bottle in cooler with ice.

Sampling for EPA 353.2: NOx (Nitrate plus Nitrite) samples.

a) These samples are filtered into 50 ml tubes (fill to the 40 ml mark).
b) Check to make sure that all required information is on the label (see "Labelling" section below).
c) Wear gloves and safety glasses at all times when sampling – the tubes contain a small amount of sulfuric acid.
d) Inspect bottles to make sure they are clean and in good condition.
e) Filtration:

i) Samples are filtered through a 0.45-micron syringe cartridge filter. Disposable or reusable filter cartridges may be used. If using disposable filter cartridges, keep them in a clean Ziploc bag before use and dispose of used filters in a separate garbage bag. Syringes can be used for multiple stations as long as they are thoroughly rinsed with sample first. Filters can also be used for more than one station – be sure to sufficiently flush with sample before collecting.
   (1) If using reusable filter cartridges:
      (a) Cartridge top and bottom and Teflon O-ring, and syringe must be acid-washed, rinsed, and dried prior to sampling.
      (b) Add a clean 0.45-micron membrane filter and assemble cartridge.
   (2) Store cartridges in a clean Ziploc bag until use.
   (3) The syringe, plunger and filter must be rinsed with the sample before use:
      (a) Remove filter cartridge from syringe and pull plunger out of syringe being careful not to let the syringe, syringe plunger or cartridge touch any unclean surfaces.
      (b) Reattach filter to syringe.
      (c) Pour about 5 ml of sample from the collection bottle into the top of the syringe.
      (d) Reinsert the plunger into the syringe and push the sample through the syringe to waste. Be sure to dispose of the rinse sample downstream of the sampling location.
      (e) Repeat two more times to adequately rinse syringe, plunger, cartridge, and filter.
      (f) Remove cartridge from syringe and remove plunger from syringe.
      (g) Reattach cartridge to syringe.
      (h) Fill the syringe with the sample.
      (i) Insert plunger into syringe,
      (j) The collection tube for filtered samples contains preservative.
      (k) Use the following protocol to fill the collection tube for the filtered sample:
         (i) Push 10-15 ml of samples through the filter to waste.
         (ii) Fill collection tubes with 50 ml of the sample and cap tightly.
      (l) Immediately place bottle in cooler with ice.
      (m) Samples must remain in the dark and on ice until arrival in the MWQL.

Sampling for SM 10200H: Extracted chlorophyll.

a) These samples are collected in 100 - 250 ml brown plastic bottles or tubes.
b) Check to make sure that all required information is on the label (see "Labelling" section below).
c) Wear gloves at all times when sampling.
d) Inspect bottles to make sure they are clean and in good condition.
e) These bottles do not contain preservative.
f) The collection bottles must be rinsed.
g) To rinse the collection bottle:
   ii) Collect a small amount (10-20 ml) of water in the bottle.
      (1) If you are sampling directly from the body of water or a bucket, immerse the bottle to fill and cap the bottle under water.
   iii) Cap the bottle and swirl to coat the inside of the bottle.

iv) Empty the contents downstream of where you are collecting sample.
v) Repeat 2 more times.

Shipping Samples

- The samples must arrive at the correct temperature which must be below 6º C.
- In order to ensure that samples stay cold, use loose ice or a combination of loose ice and ice packs placed in a garbage bag with the samples.
- Tie the garbage bag shut. Leaking water would be suspect and will result in a delay.
- Place all bottles back in the Ziploc bottles they came in and put those bags inside the garbage bag with ice.
- Fill the garbage bag with as much ice as possible. Tie bag tightly to prevent leaks.
- Attach custody sticker to cooler.
- Tape the cooler shut.
- If you would like us to return your ice pack/cooler, please request this on the chain of custody and write your name and address on the packs and cooler in a permanent way.
- Ship samples to arrive at the MWQL within 24 hours of shipping. We do not receive samples on weekend and it is best not to ship on Thursday in case the shipment is delayed, so please plan shipments accordingly.

Sample Receiving and Acceptance: Sample receiving (Log in) accepts samples and performs a series of checks and inspections to assure all necessary sampling and preservation requirements have been met, sample integrity has been maintained during transit, and that all necessary information has been supplied and that the samples meet the sample acceptance policy as listed below:
(Samples not meeting the following requirements will be brought to the attention of the customers prior to processing and may be rejected or will be appropriately qualified on the report of analysis).

1) Samples should be transported on ice/ice packs in a cooler, and the temperature will be recorded upon receipt.
2) Samples should have proper labeling and identification.
3) Samples should be taken in the appropriate sample containers as supplied by the laboratory.
4) Sampling instructions are to be followed as closely as possible.
5) Samples must have all required info about the customer, sample site, sample date & time, and sampler.
6) Samples must have adequate volume to perform the necessary tests.
7) All samples must be received with adequate time remaining to satisfy EPA holding time limits.
8) Any deviations from specified conditions will be noted and conversations with customers about those deviations area recorded.
9) When the customer desires to have samples analyzed, even though the samples are out of compliance with required specifications, a qualifier will be added to the data to indicate the deviation.
10) Preserved samples are stored in a temperature monitored refrigerator.

Appendix F

Marine Water Quality Laboratory
Cove Road Walpole, Maine 04573 207 563-8124

MWQLFDS, Rev. 1, 3/21/2022
Effective Date: ?

Date:			Project:	Kingfish Maine		Time of Low Tide:			
Sonde:				Niskin		Filter Batch:			
Sonde Calibration Date/Time:						Syringe Batch:			
Depth of Thermocline if Present (m)						Record depth range of salinity < 10 ppt):			
Field Staff:									
Secchi Depth (m):		Secchi Time:							
Secchi Notes:									

	Bottle Batch	Container Type	Container Volume (ml)	Preservative?
Chlorophyll				
TKN/TP				
NOx				

Site	Start Time	Finish Time	Collected by (initials)	Depth Code (S, B, T)*	Grab Sample Depth (m)	Total Site Depth (m)	NOx Filtered (Y/N)	NOx Filtered By (initials)	Preserved? (Y/N)
KF1				S					
KF1				B					
KF1				T					

* T is a sample taken just below the thermocline. A thermocline is present if there is a change of 1° C or greater with a 1 meter change in depth of the sonde profile. The thermocline (T) sample, should be taken just below the depth of the thermocline.

Wind Speed/Direction:	Sea Conditions/Current:	Weather/Cloud Cover:
Notes:		

Appendix G

Secchi Disk Standard Operating Procedure

Safety Information: Follow all shipboard safety protocols. Operate the disk from a safe and secure location

Protocol

1. Prior to using the secchi disk.
    a. Verify the markings on the line are accurate. Use a meter stick to verify the meter marks. Measure the distance from the secchi disk to the first mark and record (This distance will change if the line is shortened for any reason).
    b. Verify that the line is securely attached to the disk.
2. Work on shady side of boat, so that sun is not hitting water above the secchi disk and line. If necessary, use an umbrella or other sunshade.
3. Do not wear sunglasses or glasses that automatically darken in the sun.
4. Make sure that you are in a safe spot and can maintain your balance.
5. Before line goes overboard, have someone hold the end of the line or tie the line securely to the boat.
6. Lower disk into the water until white disk pattern disappears. Record the marking on the line for depth.
7. The line should be vertical while making measurements. Be mindful to avoid the line getting near the boat propellor in strong current. In strong currents, extra weights may be needed. If weighted secchi disk line is still not vertical note in the field notebook the estimated angle of the line.
8. Once you have recorded the first depth, lower the disk slightly further.
9. Raise disk until you can just see the white pattern again. Record the depth.
10. Average the two readings.
11. Once ashore, rinse the secchi disk and line in fresh water and hang on rack to dry.

Appendix H

Summary of Chlorophyll Tests	SM10200H		
4/27/22: Seawater samples collected from the DMC dock at 8:30 am on 4/27/22	Time Elapsed Betwwen Collection and Filtration (min.)	Chlor a (µg/l) In Sample	Phaeophytin (µg/l) In Sample
Blank	NA	0.004	0.0382
27APR22KT-1-1	343	2.203	0.671
27APR22KT-1 -2	346	2.671	1.642
27APR22KT-1- 3	347	2.910	1.540
27APR22KT-1 -4	483	3.393	0.810
27APR22KT-1 -5	486	3.030	1.226
27APR22KT-1 -6	488	3.192	1.089
27APR22KT-1- 7	489	2.413	1.754

5/24/22: Seawater samples collected from the DMC dock at 8:15 am on 5/24/22	Betwwen Collection and Filtration (min.)	Chlor a (µg/l) In Sample	Phaeophytin (µg/l) In Sample
24MAY22 Chlor Test 6 hr 1	375	3.446	-0.3910
24MAY22 Chlor Test 6 hr 2	376	1.986	0.5238
24MAY22 Chlor Test 6 hr 3	377	1.840	0.7392
24MAY22 Chlor Test 6 hr 4	378	2.701	1.2741
24MAY22 Chlor Test 8 hr 1	479	3.064	0.8692
24MAY22 Chlor Test 8 hr 2	481	2.324	1.8630
24MAY22 Chlor Test 8 hr 3	482	2.124	0.6118
24MAY22 Chlor Test 8 hr 4	484	3.178	0.8349
24MAY22 Chlor Test 6 hr Bk	NA	0.007	0.0244
24MAY22 Chlor Test 8 hr Bk	NA	0.000	0.0411

Data from two test of chlorophyll sample holding times. Sampled were processed and analyzed using the MWQL Chlorophyll SOP for SM10200H.

Megan Sorby
Kingfish Maine
LD 586

Senator Reny, Representative Hepler, and members of the Committee on Marine Resources, thank you for the opportunity to speak today.

I'm Megan Sorby, Operations Manager for Kingfish Maine. I've been raising multiple species of fish in several countries over the last 15 years. I graduated with honors from University of Miami Rosenstiel School of Marine, Atmospheric and Earth Science with a Bachelor of Science in Marine Biology and Chemistry. And I also graduated with honors from the University of Stirling with a Master of Science in Sustainable Aquaculture.

I am speaking in opposition of LD 586.

This bill is redundant of current state statues which manage land based aquaculture and attempts to usurp the authority of the Maine Department of Environmental Protection.

Let's look closely at each proposed standard:

#1- Degradation of water quality, air quality or increase overall carbon emissions.

Current state statute currently allocates oversight of these standards to the Maine DEP, specifically requiring that any type of development may not degrade water quality so as to change the classification of the water body.

By definition, this preserves water quality to the point that existing wildlife, fishing and recreational boating are all preserved.

With respect to carbon emissions, state statute and Maine DEP permitting specifies that operators must provide the best practical treatment options. These are analyzed as part of the permitting process and we must demonstrate through alternative analysis that adverse impacts are avoided, minimized and mitigated.

This standard, as outlined in this bill, is arbitrary and pernicious, as everyday human activity increases carbon emissions, including when you turned on your stove this morning or drove your car on your morning commute.

#2- Feed Sources

As an aquaculture company, we have a standard of care that ensures the fish are raised responsibly. It is our duty to provide complete nutrition to our fish that's adequate for their needs.

All ingredients used in fish feeds, including those used in RAS, are currently regulated by the FDA and are either derived from domestic fisheries or are subject to a variety of import controls.

It's important to note that marine-derived ingredients are in no way exclusive to recirculating aquaculture systems.

To single out a specific production system for its use of a legal and commonly used feed ingredient in this way calls into question the legality and sincerity of the proposed policy.

#3- Industrial recirculating aquaculture operations may not adversely impact native lobster, shellfish, seaweed or finfish operations.

This regulation falls under the current Maine DEP process for RAS and is also reviewed by many of these 30+ agencies listed as part of our extensive permitting process, including National Marine Fisheries Service, US Fish and Wildlife, Army Corp of Engineers, EPA as well as the regional authorities- New England Fisheries Management Council and Atlantic States Marine Fisheries Commission.

Again, aquaculture projects must demonstrate best practicable treatment technology and must demonstrate through alternative analysis that adverse impacts are avoided, minimized and mitigated.

This is redundant and repetitive and demonstrates that opponents of aquaculture didn't like the reviewing agencies decisions on their appeals to our permitting.

In their attempt to stop land based aquaculture at all costs, aquaculture opponents refuse to consider the synergies of what our industry can provide to existing fisheries and working waterfronts.

Working as collaborative industries, we can lessen the burden of associated costs and increase access to supply chain options in the marine ecosystem.

In Maine, we will support our local lobstermen and fisheries as we have in the Netherlands. One example, our heads, racks, and trimmings have been approved as bait for lobstermen and is currently used by some lobstermen in Jonesport.

Maine's labor department, community college and University of Maine system are putting precious resources into educating and training our young people for a future in the blossoming aquaculture industry.

We provide an alternative path and a collaborative resource to grow Maine's seafood brand, bringing economic development to regions who welcome our technology and are confident in the existing permitting process and oversight currently provided by numerous federal and state agencies, including the Maine DEP.

Kingfish Maine Reference Material

Kingfish Maine Presentation #1

Kingfish Maine Presentation #2