

Abbreviations and Measurement Units in File *Saginaw Bay data*.

Cruise:	yy.n format (two digit year and cruise number)
Date:	dd-mmm-yy format
Time:	local time in 24-h format
STA:	Station ID
Latitude, N:	dd mm.m format
Longitude, W:	dd mm.m format
Cloud Cover:	percent of sky obscured by clouds
Zmax:	maximum station depth, m
Secchi:	Secchi transparency depth, m
Depth:	Sample depth, m
Temp:	Temperature, C
D.O.:	Dissolved oxygen, mg/L
PAR dw:	Downwelling PAR, $\mu\text{mol}/\text{m}^2/\text{s}$
PAR uw:	Upwelling PAR, $\mu\text{mol}/\text{m}^2/\text{s}$
Ksp-25	Specific conductance at 25C
Chl a:	Chlorophyll a, $\mu\text{g}/\text{L}$
ANC:	Acid neutralizing capacity, mEq
Ca:	Calcium, mM
NO ₃ +NO ₂ :	nitrate + nitrite, μM
SRP:	Soluble reactive phosphorus, μM
DP:	Total dissolved phosphorus, μM
TP:	Total phosphorus, μM
SRSi:	Soluble reactive silicon, μM
SO ₄ :	Sulfate, μM
DN:	Total dissolved nitrogen, μM
TN:	Total nitrogen, μM
na	Data not available

Sampling Methods

Water samples from discrete depths were obtained by Niskin Go-Flo bottle samplers. Filtrate was prepared either dock-side or in the Lehman laboratory in Ann Arbor, MI using GF/F glass fiber filters. GF/F filters for pigment analyses were prepared in triplicate and the filters were enclosed in aluminum foil envelopes then held frozen over silica gel desiccant until extraction and analysis. Filtrate and raw water samples were transported in coolers over ice.

Temperature, Oxygen, PAR, and Secchi Transparency

Temperature and dissolved oxygen were measured at discrete depths using a YSI Model 55 calibrated by Winkler titration. Photosynthetically Active Radiation (PAR: 400-700 nm) was measured with LiCor cosine-corrected quantum sensors, one facing upward to measure downwelling light and another facing downward to measure

upwelling light. Secchi disk transparency depth was measured using a plain white disk 20-cm in diameter.

Specific Conductance and pH

40-ml samples of raw water from each sampling depth were placed in borosilicate tubes in a 25 C water-bath. Specific conductance corrected to 25 C was measured by a Corning Model 441 conductivity meter. The conductivity meter was calibrated for each use against air (zero conductance) and commercial 1409 $\mu\text{S}/\text{cm}$ KCl standard. The water samples were then measured for pH (Orion pH meter model 420A or Beckman pH meter model 34) using a combination electrode and two-point buffer calibration.

Phosphorus

Soluble Reactive Phosphorus (SRP): 40-ml Samples

1. Prepare all standards (triplicate) and samples (quaduplicate) as 40-ml volumes measured by graduated cylinder and dispensed into borosilicate tubes.
2. Pour DI water blanks in triplicate.
3. Pour 1.61 μM P standards in triplicate.
4. Pour filtrate samples in duplicate.
5. Calculate required volume of mixed reagent: 4 ml per sample tube.
6. Prepare required volume of mixed reagent by combining, in order, 2 parts (a) 3% ammonium molybdate, 5 parts (b) SRP sulfuric acid, 2 parts (c) ascorbic acid, 1 part (d) potassium antimonyl tartrate.
7. Set spectrophotometer wavelength to 885 nm; use 10-cm cylindrical cells and zero with DI water.
8. Dispense 4-ml mixed reagent to samples. Make additions only to enough samples at a time that you can read within 30 minutes.
9. Wait 5 minutes for color development, then record absorbance values.

SRP Color Blanks: 40-ml Samples

1. Prepare all samples as 40-ml volumes in duplicate, measured by graduated cylinder, and dispensed into borosilicate tubes.
2. Prepare mock mixed reagent by combining equal volumes of SRP sulfuric acid solution and DI water.
3. Dispense 4-ml mock mixed reagent to samples. Samples are ready to read immediately and are stable indefinitely.

4. Set spectrophotometer wavelength to 885 nm; use 10-cm cylindrical cells and zero with DI water.
5. Record absorbance values using DI water as the instrument blank.

Dissolved Phosphorus (DP)/Total Phosphorus (TP): 40-ml Samples

1. Prepare all standards (quadruplicate) and samples (duplicate for DP or triplicate for TP) as 40-ml volumes measured by graduated cylinder and dispensed into borosilicate tubes.
2. Pour DI water blanks in quadruplicate.
3. Pour 1.61 μM P standard in quadruplicate.
4. DP = filtrate; TP = raw water.
5. Pour DP (duplicate) and TP (triplicate) samples.
6. Add 0.4 g of potassium persulfate to each tube using a calibrated scoop.
7. Oxidize in dry block heater at 105 C for 2 hours; cool to room temperature.
8. Calculate required volume of mixed reagent: 4-ml per tube.
9. Prepare required volume of mixed reagent by combining, in order, 2 parts (a) 3% ammonium molybdate, 5 parts (b) SRP sulfuric acid, 2 parts (c) ascorbic acid, 1 part (d) potassium antimonyl tartrate.
10. Set spectrophotometer wavelength to 885 nm; use 10-cm cylindrical cells and zero with DI water.
11. Dispense 4-ml mixed reagent to samples. Make additions only to enough samples at a time that you can read within 30 minutes.
12. Wait 5 minutes for color development, then record absorbance values.

SRP/DP/TP Reagents:

- (a) Ammonium Molybdate: 15 g to 500-ml DI water.
- (b) SRP Sulfuric Acid: 140-ml concentrated sulfuric acid to 900-ml DI water.
- (c) Ascorbic Acid: 27 g to 500-ml DI water.
- (d) Potassium Antimonyl Tartrate: 0.34 g to 250-ml DI water.

Mix in order and in volume ratio: 2(a) : 5(b) : 2(c) : 1(d)

SRP/DP/TP Working Standard = 1.61 μM P:

Dilute 1.0-ml P Stock (1-ml = 0.050 mg P) to 1000-ml with DI water.

Chlorophyll a

1. Freeze filters over silica gel desiccant until extraction. For fluorometric assays, use 100-mL samples.
2. Macerate filters in ice-cold 90% v/v acetone by tissue grinder.
3. Filter resulting slurry through a Whatman GF/D filter.
4. Record extract volume.
5. Chlorophyll is measured fluorometrically using a Turner Designs TD700 fluorometer with 436 nm excitation filter and 680 nm emission filter; fluorescence is calibrated against commercial standard solutions of Chl *a*.

Soluble Reactive Silicon (SRSi)

1. Place 5-ml of 50 μ M standard in polyethylene vessels in triplicate; use 5-ml of DI water in triplicate for blanks.
2. Place 5-ml of sample filtrate in polyethylene vessels in duplicate.
3. If sample dilution is necessary, place 1-ml sample plus 4-ml DI water in polyethylene vessels (N.B. this is a dilution to 20% full strength)
4. Add 0.3 ml of mixed reagent (3); swirl to mix and wait for 15 minutes.
5. Add 0.2 ml of Tartaric acid (4); swirl to mix and wait 2 minutes
6. Add 0.1 ml reductant (6); swirl to mix.
7. Read at 815 or 660 nm in 1-cm cell after 5 minutes.

SRSi Reagents:

1. Ammonium molybdate, 10% w/v.
2. HCl, 1N: Dilute 83 ml HCl to 1 L.
3. SRSi Mixed reagent: mix 1 part (1) with 1 part DI water and 3 parts (2); prepare fresh daily.
4. Tartaric Acid, 10% w/v.
5. Stannous Chloride stock, 3.5 N: dissolve 40 g $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ in 100-ml HCl.
6. Working Stannous Chloride, 0.05 N: dilute 1-ml (5) to 70-ml with DI water; prepare fresh daily.

SRSi Standards:

Stock standard: 10 mM Si (1 ml = 10 μ mol Si)

50 μ M Si: dilute 0.5 ml Stock to 100 ml.

Sulfate

Sulfate was measured by ion chromatography (Dionex) calibrated with commercial standards.

Nitrogen

Total nitrogen (TN), NO_2^- and NO_3^- were measured spectrophotometrically by flow injection analysis (FIA) using a Tector FIAstar 5032 Detector. For TN, 10 ml of raw water was treated with 15 ml of alkaline persulfate oxidant (D'Elia et al. 1977: <https://doi.org/10.4319/lo.1977.22.4.0760>), and then heated to 105 C for 2 h. After the samples were cooled, 0.2 ml 30% HCl and 2.0 ml of borate buffer were added. Both treated TN samples, and filtered water samples for NO_2^- and NO_3^- were then poured into 10 ml sample cups that were used by the flow-injection sampler (Tector 5007 Sampler). The samples were then passed through a copperized cadmium column that reduced all NO_3^- to NO_2^- . Samples testing for NO_2^- bypassed the cadmium column so that dissolved NO_3^- was not converted into more NO_2^- . The Tector FIAstar 5020 Analyzer then added 2 additional reagents to the samples. In the first reagent, sulphanilamide ($\text{C}_6\text{H}_8\text{N}_2\text{O}_2\text{S}$) was added along with concentrated HCl. Together, the reagent formed a diazo compound with NO_2^- . The second reagent, ethylenediamine dihydrochloride, complexed with the diazo compound to form a purple azodye that absorbs light at 540 nm. The amount of light absorbed corresponded directly with the concentration of NO_2^- and was recorded as a peak height absorbance. Absolute concentrations were determined by comparing sample peak heights to deionized water (DI) blanks and standards of TN, NO_2^- , and NO_3^- .

Electrochemical Gran Titration for Acid Neutralizing Capacity

Analytical Theory

Acid neutralizing capacity (ANC), also known as titration alkalinity (ALK), is determined by electrochemical titration using a method invented by G. Gran in 1952. The Gran Titration is a precise method for determining concentrations by use of specific ion electrodes. Specific ion electrodes respond to ion activities by generating electrical potentials at their electrochemical half-cells. The magnitude of the electrical potential is measured with respect to a reference half-cell immersed in the same solution.

Gran Titrations use a simple principle of logic. In pure solution, addition of an ion such as H^+ must result in accumulation of the ion. If you add 1 mmol, there should be 1 mmol present. However, if the ion can enter into chemical reactions (e.g., buffer reactions), the ion will not accumulate until the reacting conditions are saturated. Thus, you can add strong acid to lake water, but H^+ will not begin to accumulate in proportion to the addition until all of the bicarbonate has been neutralized.

Protocol

1. Use 50-mL lake water filtrate samples.
2. Titrations for bicarbonate use a pH electrode calibrated with phosphate buffer solutions.
3. Titrant solutions are 0.1 N H₂SO₄ for bicarbonate.
4. Repetitive automatic pipettes calibrated in multiples of 0.1 ml are used as micro-burettes.
5. Pour the 50-ml lake water sample into a clean 125-ml beaker.
6. Clean the electrodes and immerse them in the lake water.
7. Record initial pH.
8. Add titrant solutions in 0.1 ml increments, mixing and recording stable pH or mv values after each addition.
9. Titrate to pH 3 for bicarbonate.
10. Calculate the total amount of H⁺ in the beaker during titration as:
$$\text{Gran-H} = (\text{ml sample} + \text{ml added}) \times 10^{-\text{pH}}$$
14. Plot Gran-H versus ml added. The data will exhibit a linear region including the last several additions.
15. Fit a straight line to the linear data region. The intercept point on the X-axis is the end-point, or equivalence point of the titration.
16. Calculate the ANC from the endpoint as:
$$(\text{endpoint ml}) \times (\text{titrant normality}) / (\text{sample volume})$$

Determination of Calcium and Magnesium in Lake Water

Determination of Calcium by EDTA titration to a colorimetric endpoint

This method relies on the fact that the dye Eriochrome Blue-Black forms a wine-red complex with calcium ion and with magnesium ion at basic pH. If the divalent ions are chelated by the ligand EDTA, the dye changes to a pure blue color. There are two potential problems that must be overcome. First, if the sample contains bicarbonate, when the pH is made more basic, the calcium may form calcium carbonate and precipitate from solution, causing erroneous results. Second, there needs to be a way to eliminate the problem with interference by magnesium.

We overcome the carbonate problem by acidifying the sample to pH < 5, driving the inorganic carbon out of solution in the form of carbon dioxide. We overcome the magnesium problem by subsequently raising the pH to higher than 12, which precipitates the magnesium as hydroxide.

Protocol:

1. Obtain a 50 ml volumetric lake water sample
2. Refer to the results of alkalinity titration and acidify the sample to pH < 5 by addition of 0.1 N H₂SO₄ (typically about 2 ml or less)
3. Place the acidified sample on a stirring plate and allow it to degas for 1 minute
4. Add 0.5-ml of 5 M NaOH to elevate the pH > 12
5. Add about 0.2 g of indicator dye powder
6. Titrate the sample with 0.1 M EDTA using 0.05 ml increments near the endpoint

7. The solution turns from rose pink to pure blue through a purple intermediate. Record the volume required to first achieve the pure blue color.
8. Calculate Ca concentration: one mole of EDTA chelates one mole of calcium.

Determination of Calcium + Magnesium by EDTA titration

Follow the protocol for Calcium determination but omit step 4 (addition of NaOH).