

ALBERTA LAKE MANAGEMENT SOCIETY'S LAKEKEEPERS PROGRAM

Welcome to Winter LakeKeepers!

Thank you for expressing an interest in Alberta's aquatic environments and for participating in the Winter LakeKeepers program. You have proven that ecological apathy can be overcome and give us hope that our water resources will not be the limiting factor in the health of our environment. Throughout this process, you will be involved in the collection and preparation of scientific data important to assessing the health of your lake of interest. This manual is meant to be a reference for Winter LakeKeepers sampling protocol.

LakeKeepers has several important objectives, one of which is to address the gap of winter lake water quality data for lakes in Alberta. At ALMS, our mission is to promote the understanding and comprehensive management of lakes and reservoirs and their watersheds. With Winter LakeKeepers, we hope to expand the breadth of lake monitoring, education, and management in Alberta.

For field sheets, safety training, and the safety quiz, visit:

https://alms.ca/winter-lakekeepers/

This manual was prepared by Bradley Peter and Kirsten Letendre. For more information, please contact lakekeepers@alms.ca

Manual last updated: December 4, 2023





Sampling Protocol Overview

AT HOME

- 1. Complete the online safety quiz and informed consent form at www.alms.ca/winter-lakekeepers/
- 2. Make sure your probe is charged (see battery on top right of probe screen).
- 3. Fill your hot water bottle and place it in your YSI kit.

AT THE LAKE

- 1. Calibrate your probe in your vehicle to avoid freezing. Record the barometer value on your field sheet.
- 2. Using the ice measuring stick, measure how much snow/slush there is before clearing the area.
- 3. Auger two holes near each other (~2m apart) for your sampling.
 - a. One hole will be used to take bottom depth and probe readings (water temperature and dissolved oxygen).
 - b. The second hole will be used to fill the bottle set.
- 4. Using the ice measuring stick (Figure 2 on page 8), measure the total ice thickness and white ice thickness (page 15). Record these on your field sheet.
 - a. The ice measuring tool is measured in centimeters (cm).
- 5. Fill in all the environmental observations on page 1 of your field sheet (type of auger, water colour, water clarity, GPS coordinates).
- 6. Take the bottom depth measurement with the tape and weight in one of the augured holes, then take the probe readings from the same hole.
 - a. Record this on the field sheet.
- 7. Put on the green or yellow gloves provided. Using the other augured hole, rinse each bottle 3 times, then fill each bottle and preserve the phytoplankton with Lugol's solution (P1&P2) and the G2-Preserved bottle with sulfuric acid (P1&P2).

AFTER SAMPLING

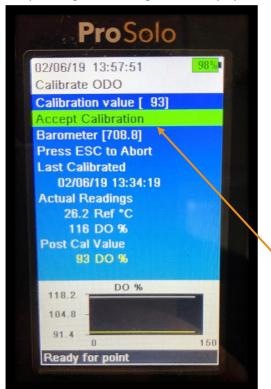
- 1. If you are doing the P2+Chl-a protocol, go to step 10 on page 9 to follow the filtering process
 - 1. Make sure to freeze your filter papers once complete.
- 2. See page 9 for instruction on returning your samples and kit, in person or via mail.

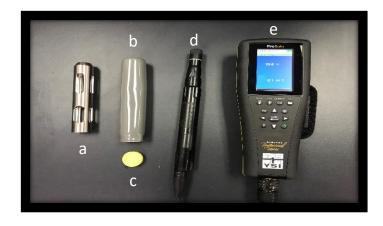




1) BEFORE YOU HEAD OUT:

- a) Complete the online safety quiz and informed consent form at www.alms.ca/winter-lakekeepers/
- Fill your hot water bottle and place it in your YSI kit.
- c) Make sure your probe is charged (see battery on top right of probe screen).
- d) Confirm with ALMS (<u>lakekeepers@alms.ca</u>) whether you're following P1 or P2 protocols; look for P1 or P2 in the step name to know which steps to follow. Check the materials list on page 11 (Appendix section A2) to make sure you have all materials.
- e) If you need help finding your GPS coordinates, follow the steps on page 9 (Appendix section A1). If you are sampling the same location at a lake more than once, refer to your initial GPS in **Table 2** on page 10 (Appendix section A1) to aid in navigating to the same site location.
- f) Plan a timeline for sample return with ALMS, depending on filtering for chlorophyll-a.





2) CALIBRATE PROBE AT THE LAKE (P1 & P2):

- a) Calibrate your probe in your vehicle to avoid freezing.
- b) Remove the **grey sleeve** (b) from your **probe** (d).
- c) Remove the **metal probe guard** (a) and gently dab any water droplets from the probe with a Kimwipe (supplied tissue).
- d) Carefully place the metal guard back over your probe.
- e) There is a yellow sponge inside the grey calibration sleeve.
 Using water from the calibration bottle, wet the **yellow sponge**(c) with a few drops of water.
- f) Place the grey sleeve (with yellow sponge inside) over the metal guard.
- g) Wait **five minutes** to allow the air in the probe to become saturated with moisture from the sponge.
- h) Connect your probe to your **handheld unit** (e).
- i) Press the green power button on your handheld unit.
- j) Press Cal.
- k) Choose ODO or DO by pressing Enter.
- I) Choose DO % by pressing Enter.
- m) Wait one minute.
- n) Record the Barometer value on the front of your field sheet.
- o) Choose 'Accept Calibration' by pressing Enter.
- p) Press escape until you see the 'log one sample ' screen.
- q) Keep the probe in its grey sleeve and in the sampling kit until you are ready to collect data.





3) RECORD BOTTOM DEPTH AND MEASUREMENTS (P1 & P2):

- a) Fill in the Environmental Observations portion of your field sheet. See Appendix section A5 on page 14 for help with Total Ice & White Ice Thickness measurements.
- b) Use the 'tape and weight' to determine the bottom depth and record the depth in the 'Approximate Bottom Depth' box on the back of the field sheet.
- c) Remove the grey sleeve. With your probe turned on to the 'Log One Sample' screen, lower the probe until the 0.1 m marker is at the surface of the water.
- d) If your backlight turns off during sampling, press any key to reactivate it.
- e) Write the temperature and dissolved oxygen measurements on your field sheet following the depths indicated in the 'Depth (m)' row (see Appendix section A3 on page 11 for guide on cord depth markings).
- f) You may need to wait 30-60 seconds for your dissolved oxygen readings to stabilize at each depth.
- g) Continue this process until you have hit the bottom of the lake.
- h) If not done already, record the bottom depth in the 'Approximate Bottom Depth' box on your field sheet.
- i) Hold the Power Button to turn off your probe.

j) Place the grey sleeve with wet sponge inside back over the metal guard. Return the probe to the warm sampling kit.



Preservative MSDS information can be found on the ALMS website at: https://alms.ca/winter-lakekeepers/

ProSolo 02/06/19 14:21:35 Run Log One Sample 26.2 °C 9.3 Do ₹

4) COLLECT WATER SAMPLE WITH G2-Preserved BOTTLE (P1 & P2):

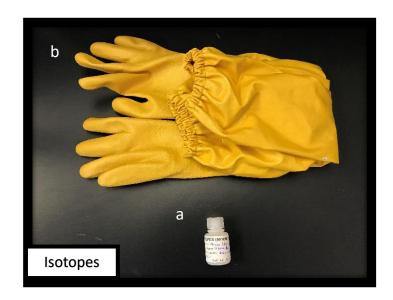
- a) Using a Sharpie, label your **G2-Preserved Bottle** (a) with the Lake Name, Location Name, Date, and Time.
- b) Wearing the **sampling glove** (b), rinse the bottle three times with water from the hole. Dump the rinse water onto the ice away from the hole.
- c) After rinsing, fill your **G2-Preserved Bottle** with water from below the surface, as deep as you can reach down.
- Add the **yellow capped preservative** (c) to your **G2-Preserved Bottle**. Wear gloves and safety glasses as this preservative contains sulfuric acid.
- e) Place the bottle into your cooler.





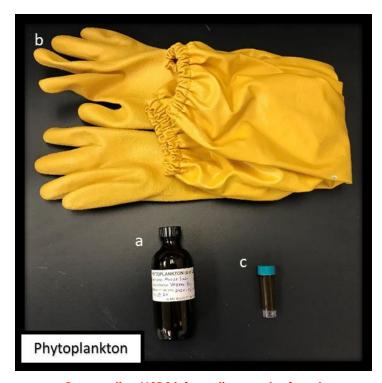
5) COLLECT WATER SAMPLE WITH ISOTOPES BOTTLE (P1 & P2):

- a) Using a Sharpie, label your **Isotopes Bottle** (a) with the Lake Name, Location Name, Date, and Time.
- b) Wearing the **sampling glove** (b), rinse the bottle three times with water from the hole. Dump the rinse water onto the ice away from the hole.
- c) After rinsing, fill your **Isotopes Bottle** with water from below the surface, as deep as you can reach down.
- d) Place the sample into your cooler.



6) COLLECT WATER SAMPLE WITH PHYTOPLANKTON BOTTLE (P1 & P2):

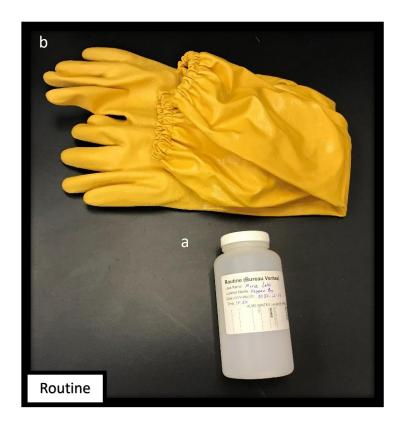
- a) Using a Sharpie, label your Phytoplankton Bottle
 (a) with the Lake Name, Location Name, Date,
 and Time.
- b) Wearing the sampling glove (b), rinse the bottle three times with water from the hole. Dump the rinse water onto the ice away from the hole.
- c) After rinsing, fill your Phytoplankton Bottle with water from below the surface, as deep as you can reach down.
- d) Add the green capped preservative (c) to your **Phytoplankton Bottle**. Wear gloves and safety glasses as this preservative contains iodine and glacial acetic acid, and easily stains.
- e) Place the sample into your cooler.
- f) IF FOLLOWING P1, SKIP AHEAD TO STEP 11.
- g) IF FOLLOWING P2, PROCEED TO STEP 7.



Preservative MSDS information can be found on the ALMS website at: https://alms.ca/winter-lakekeepers/







7) COLLECT WATER SAMPLE WITH ROUTINE BOTTLE (P2):

- a) Using a Sharpie, label your **Routine Bottle** (a) with the Lake Name, Location Name, Date, and Time.
- b) Wearing the **sampling glove** (b), rinse the bottle three times with water from the hole. Dump the rinse water onto the ice away from the hole.
- c) After rinsing, fill your Routine Bottle with water from below the surface, as deep as you can reach down.
- d) Place the sample into your cooler.



Preservative MSDS information can be found on the ALMS website at: https://alms.ca/winter-lakekeepers/

8) COLLECT WATER SAMPLE WITH <u>G2-F</u> BOTTLE (P2):

- a) Using a Sharpie, label your **G2-F Bottle** (a) with the Lake Name, Location Name, Date, and Time.
- b) Wearing the sampling glove (b), rinse the bottle three times with water from the hole. Dump the rinse water onto the ice away from the hole.
- c) After rinsing, fill your **G2-F Bottle** with water from below the surface, as deep as you can reach down.
- d) Place the sample into your cooler.





9) COLLECT WATER SAMPLE WITH CHLOROPHYLL-a BOTTLE (P2):

- a) Using a Sharpie, label your **Chlorophyll-A Bottle** (a) with the Lake Name, Location Name, Date, and Time.
- b) Wearing the **sampling glove** (b), rinse the bottle three times with water from the hole. Dump the rinse water onto the ice away from the hole.
- c) After rinsing, fill your **Chlorophyll-***a* **Bottle** with water from below the surface, as deep as you can reach down.
- d) Place the sample into your cooler.
- e) IF FILTERING WATER FROM CHLOROPHYLL-a BOTTLE, PROCEED TO STEP 10. IF NOT, PROCEED TO STEP 11.







10) FILTER WATER FROM CHLOROPHYLL-α BOTTLES (P2 + ChIA):

- a) Filtering must be done away from direct light and j) on a level surface within 24hrs of collecting water.
 If delaying filtering because location is too cold, keep sample refrigerated at home until filtering but do not let the sample freeze.
- b) Put on disposable gloves to avoid contamination. Set up the chlorophyll filtering apparatus as shown in Figure 1 on page 7. Make sure the tubing is connected tightly. You will either have the filter flask kit or the screw on filter kit. Additionally, you will need a hand pump, filter paper, graduated cylinder, pure water poured into your squirt bottle, and tweezers.
- c) Use the tweezers to place one filter paper on the n) funnel covering all the holes. Make sure the rubber stopper is secure. *Follow same protocol with screw-on filter set cover all holes, and secure screw-on funnel.*
- d) Wet the filter paper with **pure water**, provided in the kit.
- e) Use the hand pump to gently increase pressure and allow water to filter through to the flask p) underneath. Try not to exceed 20 psi on the dial.
- f) Shake the **Chlorophyll-a bottle**, measure 100 mL q) of lake water using the graduated cylinder and pour onto filter paper. **Maintain pressure while pouring lake water onto the filter paper.** Pump until all the water has drained.
- g) Repeat step **f** until there is only a SLIGHT green or brown colour visible on the filter paper. **Do not** s) **filter more than 300 mL per filter** (there is only 1L of water in the bottle). If you can't tell if the paper is green, pick it up with tweezers and look at it.
- h) Once enough lake water has been filtered, rinse the graduated cylinder 3x with **pure water** onto the filter paper. Pump as you go.
- i) Once you have rinsed the graduated cylinder 3x, rinse the filter apparatus 3x with **pure water** onto the filter paper. Pump as you go.

- If the filter flask becomes full, remove the rubber stopper, or unscrew the filter apparatus, and discard the lake water – NOT down the drain – and continue filtering. Make sure the flask does not get full enough to reach the pump tubing.
- k) Once you are done filtering and rinsing, fill in the total volume of filtered sample water on the field sheet along with colour of each filter (green, brown, etc).
- I) Add three drops of magnesium carbonate onto the filter paper, pumping as you go.
- m) Remove the top portion of the filter apparatus.
- n) Using tweezers, fold the filter paper in half, then half again (fold into a quarter). Avoid touching any portion of the paper that has colour.
- o) Finally, place the folded filter paper into a petri dish using tweezers. Using a sharpie and the provided filter labels, label the dish with the lake name, location, date, and total volume of lake water filtered.
- p) Wrap the petri dish in aluminum foil to protect it from light.
- q) Place a new filter paper on the apparatus and repeat this procedure two more times to obtain three filter papers per lake.
- Excess water from the bottle and filter flask can be discarded back into the lake, or outside – NOT down the drain.
- s) Keep chlorophyll filters with other samples until returned home, then keep them in a baggie in the **freezer.**
- t) If sampling later in the season at the same site, rinse out the **Chlorophyll-a Bottle** with tap water, and allow to dry. Re-label with additional Chlorophyll-a bottle labels provided in your kit.

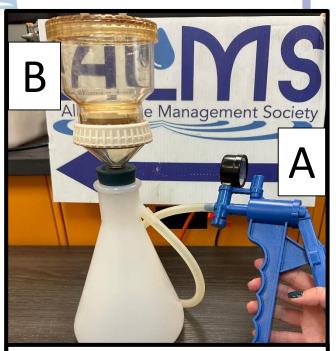


Figure 1a: Filter flask set up - with pump

- A. Hand pump connected with rubber tubing to filtration system
- B. Filtration system



Figure 1b: Screw on filter set up - with pump

- A. Hand pump connected with rubber tubing to filtration system
- B. Filtration system

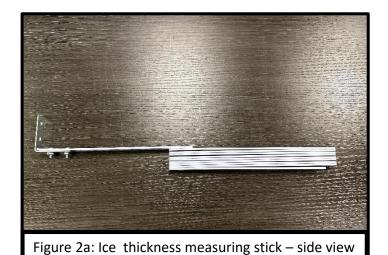




Figure 2b: Ice thickness measuring stick - top view





11) WHAT TO DO AFTER SAMPLING:

Please follow the table below to know how quickly samples need to be returned to ALMS, and how to ship / deliver them, depending on your protocol steps.

Table 1. Shipment timing & process for Winter LakeKeepers 2023-2024

Protocol	Return Within:	How to ship:
P1	2 weeks	Keep G2-Preserved , Phytoplankton , and Isotopes bottles cold.
P2	24hrs	Keep G2-Preserved , G2-F , Isotopes , Routine , Phytoplankton , and Chlorophyll- <i>A</i> bottles cold.
P2 + Chlorophyll-A Filtering	3 days	Keep G2-Preserved, G2-F, Isotopes, Routine, and Phytoplankton bottles cold. Keep Chlorophyll-A filters FROZEN.

FILL IN YOUR VOLUNTEER HOURS AT THE TOP OF THE FRONTSIDE OF THE FIELD SHEET

RETURNING YOUR SAMPLES & KIT IN PERSON:

- If you are returning your sampling kit in person to the ALMS office please contact ALMS at 780-702-2567 or lakekeepers@alms.ca to arrange delivery timing.
- The ALMS office is located at 4816-89 St. Edmonton, AB. T6E-5K1.
- When returning the kit, park anywhere you like in the parking lot and buzz at the front door when you arrive.

RETURNING YOUR SAMPLES & KIT IN THE MAIL:

- Please use Purolator to ship your kit back to ALMS. Ensure the contents of the kit are secure, and that the pelican case and cooler have been taped closed with packing tape.
- To ship, use ALMS Purolator Account Number <u>8734863</u> and choose Receiver Pays.
- If shipping, arrange with ALMS prior to sampling so we can provide coolers for shipping samples.
- For information on how to fill out your Shipping Form, see ALMS Winter LakeKeepers page at www.alms.ca/winter-lakekeepers/





KEEPING YOUR KIT FOR FURTHER SAMPLING:

- If you plan on using the probe in another waterbody, ensure it has been cleaned with tap water. It is best if the tap water used to clean the probe is discarded outside and not down your drain.
- **Do not** use any cleaners on your probe. When storing the probe, ensure the yellow sponge is damp and the grey sleeve is over the probe.
- If you plan on using this probe again in the same waterbody, no cleaning is required.
- Even though you plan to continue sampling, your samples should be sent back to ALMS (see the 'in person' or 'in mail' options on page 8). Ensure you process and ship your samples according to the timeline and processes outlined in Table 1 on page 8.

APPENDIX

A1) GPS Coordinates Instructions & Documentation

- 1. Go to https://www.googlemaps.com/maps, and find your lake (search its name).
- 2. Using your mouse, right click on the location of the lake where you collected your sample.
- 3. Choose "What's Here?"
- 4. The GPS coordinates will appear at the bottom of your screen in the format of: 55.217876, -113.252806. Record these coordinates on your field sheet.

IF YOU PLAN ON SAMPLING THE SAME SITE MORE THAN ONCE IN THE WINTER, USE THE TABLE BELOW TO RECORD YOUR SITE GPS FROM THE FIRST SAMPLING EVENT TO BE USED FOR THE NEXT SAMPLING EVENTS. USE BOTTOM DEPTH AS ANOTHER REFERENCE FOR LOCATING THE SAME APPROXIMATE SITE LOCATION.

Table 2. Site GPS log (reference for subsequent sampling events)

SITE (Lake, Location Name) Eg. Moose Lake, Vezeau Bay	Latitude	Longitude	Bottom Depth (m)

¹Degree Minutes Seconds example: 53°29'06.5"N 113°27'54.6"W

²Decimal Degrees example: 53.485127, -113.465178

³Degree Decimal Minutes example: 53°29.1076'N, 113°27.9107'W





A2) USE THIS TABLE TO MAKE SURE YOU HAVE EVERYTHING YOU NEED FOR SAMPLING

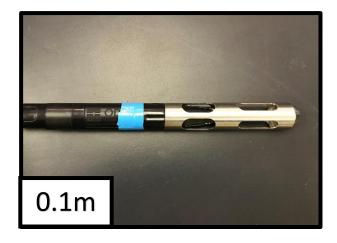
Table 3. Equipment & Material List

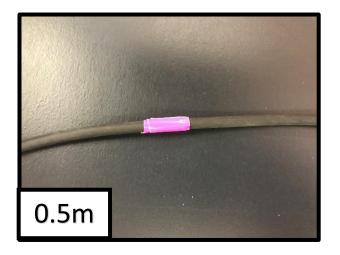
P1	P2	P2 + Chlorophyll-a Filtering	
YSI ProODO or YSI ProSOLO Probe	*Same as P1	*Same as P1 & P2	
Long yellow or green gloves	G2-F Bottle	Filter flask set up OR screw on filter set up	
G2-Preserved Bottle + yellow cap preservative (2mL sulfuric acid)	Routine Bottle	Hand pump & tube	
Isotope Bottle	One 1L Chlorophyll-a bottle	Graduated cylinder	
Phytoplankton Bottle + green cap Lugol's solution	Phytoplankton Bottle + green cap preservative (3mL Lugol's Solution)	Squirt bottle & pure water	
Hot water bottle		Filter papers	
Ice Measuring Tool		Tweezers	
Tape and Weight		Magnesium Carbonate	
Clipboard		Aluminum foil	
Field Sheets		Petri dishes	
Extra disposable gloves		Ziploc baggies	
Kimwipes (tissues)		Chlorophyll- <i>a</i> filter labels	
Extra batteries, or charger, for probe		Disposable gloves	
Calibration bottle			
Sharpies			

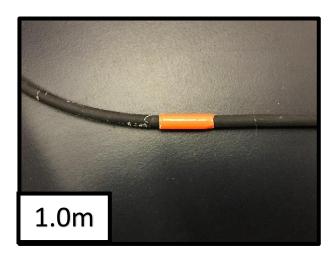


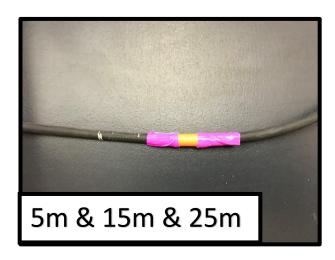


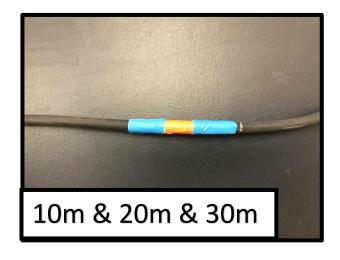
A3) YSI PROBE DEPTH MEASUREMENT MARKING GUIDE















A4) DATA COLLECTION BACKGROUND

Below are descriptions of what the data and samples collected through Winter LakeKeepers will be used for, and how they relate to better understanding lakes in the winter. Also provided is where the data will eventually be used and reported.

- Environmental Observations: 'Total Ice Thickness,' White Ice Thickness,' 'Snow Coverage,' 'Snow and/or Slush Thickness,' 'Air Temperature,' 'Water Colour,' 'Odour Present,' and 'Water Clarity' are reported so that we can understand the data collected in context of the winter environment field conditions that were present at the time of sampling. Ice thickness and snow thickness (if present) can be used to understand how much light may be penetrating the ice. See Appendix section A5 on page 14 for a further description about white ice and clear ice. Recording water colour and the general water clarity can identify algae or cyanobacteria growth, and even the type of algae or cyanobacteria. Seeing how these parameters change may also help contextualize trends in other data collected through Winter LakeKeepers. These data are reported in the ALMS Winter LakeKeepers reports.
- **GPS Coordinates:** Very important to include, since the particular location on the lake where the sample is collected is used to contextualize all other data collected. Used to make maps for presentations and reporting about Winter LakeKeepers.
- Probe Calibration: Used to ensure probes are reading accurately given local environmental conditions.
- Lake Measurements: Temperature readings from the top to bottom of the lake (lake profile) are used to understand lake mixing, dissolved oxygen levels, and for evaluating habitat for plants and animals. Dissolved oxygen readings are also taken through the lake profile to understand fish habitat. Winter can be a stressful time for fish, as low oxygen levels often present at the end of winter can cause die-offs of certain species of fish. Determining the rate at which oxygen decreases through the winter season can also be used to understand the impact of summer algae and cyanobacteria growth, as greater growth will cause oxygen to be depleted more quickly as the algae and cyanobacteria decompose. Low oxygen levels can impact nutrient levels, as lake sediments will release phosphorus into the lake if oxygen is absent seasonal oxygen levels may inform seasonal nutrient changes. These data are reported in the ALMS Winter LakeKeepers reports.
- **G2-Preserved:** Collected to measure total phosphorus and total nitrogen levels, which are important nutrients for algae, cyanobacteria, and aquatic plant growth. High levels of these nutrients may indicate pollution, and inform the amount and type of algae and cyanobacteria present. These data are reported in the ALMS Winter LakeKeepers reports.





- Isotopes: Isotopes of hydrogen and oxygen are used to understand lake-groundwater connectivity. Groundwater connectivity can contextualize lake water chemistry, and overall lake water quality or quantity. Samples will be sent to Alberta Innotech, where the isotope data will be used in their ongoing research about groundwater in Alberta.
- Phytoplankton: This bottle preserves the cyanobacteria and algae species that are present just below the ice, where their levels will be the highest. Knowing which species are present, and in what amounts, will help us to understand the biodiversity of algae and cyanobacteria, and their contribution to the winter lake food web. Information will also be used to understand how nutrient levels impact algae and cyanobacteria in the winter. These samples will be archived and may be analyzed at a later date if chlorophyll-a levels are high.
- G2-F: Water from this bottle is used to determine total dissolved phosphorus and dissolved organic carbon levels, which are important nutrients for algae, cyanobacteria, and aquatic plant growth. High levels of these nutrients may indicate pollution, and contextualize the amount and type of algae and cyanobacteria present. Reported in the ALMS Winter LakeKeepers reports.
- Routine: Water from this bottle is used to determine pH, a parameter that is used to understand the acidity of water, and is important for evaluating fish habitat and general lake water chemistry. Conductivity and chloride are also determined from the Routine sample bottle, and are parameters that help understand the levels of salts in lake water. As ice forms, salts may not be incorporated into the ice, leading to elevated levels of salts in the winter. Salt levels are an important aspect of habitat for algae, cyanobacteria, aquatic invertebrates, and fish. Levels can indicate groundwater connectivity, road salt pollution, and may also increase in lakes with large surface areas during times of low rainfall and snowmelt. Reported in the ALMS Winter LakeKeepers reports, as well as other provincial research on the impact of road salts.
- Chlorophyll-a: Collected to determine the levels of chlorophyll-a in lake water. Chlorophyll-a is a green pigment found in all algae and cyanobacteria, and is used in photosynthesis. Chlorophyll-a levels are used to understand the amount of algae and cyanobacteria in lake water. Higher levels, in conjunction with high nutrient levels, may indicate nutrient pollution, or reflect the lake's natural ability to support high levels of algal and cyanobacterial growth. Chlorophyll-a levels compared with ice conditions will also improve the understanding of what influences algae and cyanobacteria growth in Alberta Lakes in the winter. Reported in the ALMS Winter LakeKeepers reports.

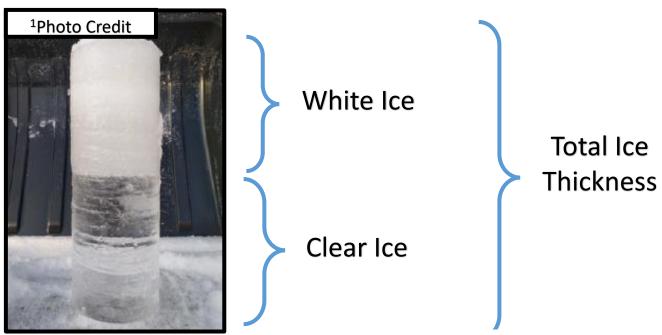




A5) SNOW, SLUSH, WHITE ICE, AND CLEAR ICE

The quality, or characteristics, of the snow and ice that covers lakes in the winter can be extremely variable. One of the major ways that snow and ice can vary on a lake is vertically, where snow, slush, white ice and clear ice can be identified. Snow and slush will be loose, while both white ice and clear ice will be hard. After auguring the hole in the ice, looking down the hole you should see up to two distinct layers of ice. On the top will be opaque or 'white' ice, and below will be clear, transparent 'clear' ice (also known as black ice). If you are sampling early in the season, there is a good chance that there will be little or no white ice, but later in the season, the layer of white ice may grow substantially. White ice is formed when snow melts and refreezes, which can happen during warm spells, rain events, or if the snow layer is heavy enough to force water up through cracks in the ice.¹

How to measure snow, slush, white ice, and clear ice: Before you clear your auger site, measure the snow and/or slush depth, or nearby the auger hole where you have not altered the snow. Next, clear the site where you will auger your hole. Make sure you clear all the way down to the hard layer of ice, if possible. After you auger your hole, measure the total ice thickness, using the ice measuring tool provided (Figure 2a/b on Page 7). To use the tool, unfold the device and loop the rope around your wrist – this will prevent you from dropping the tool through the hole. Next, measure the thickness of the white ice layer, which will be from the surface of the ice down to the line where white ice transitions into clear ice. The thickness of clear ice is the difference of total ice thickness and white ice, which is why only total and white ice thickness are required on the field sheet.



¹Weyhenmeyer, G.A., Obertegger, U., Rudebeck, H. et al. Towards critical white ice conditions in lakes under global warming. Nat Commun 13, 4974 (2022). https://doi.org/10.1038/s41467-022-32633-1