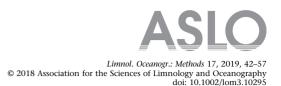
LIMNOLOGY and OCEANOGRAPHY: METHODS



The unique methodological challenges of winter limnology

Benjamin D. Block , 1* Blaize A. Denfeld , 2 Jason D. Stockwell, 1 Giovanna Flaim, 3 Hans-Peter F. Grossart , 4,5 Lesley B. Knoll , 6 Dominique B. Maier , 2 Rebecca L. North, Milla Rautio, 8,9,10 James A. Rusak , 11,12 Steve Sadro , 13 Gesa A. Weyhenmeyer , 14 Andrew J. Bramburger , 15 Donn K. Branstrator, 16 Kalevi Salonen, 17 Stephanie E. Hampton , 18

Abstract

Winter is an important season for many limnological processes, which can range from biogeochemical transformations to ecological interactions. Interest in the structure and function of lake ecosystems under ice is on the rise. Although limnologists working at polar latitudes have a long history of winter work, the required knowledge to successfully sample under winter conditions is not widely available and relatively few limnologists receive formal training. In particular, the deployment and operation of equipment in below 0°C temperatures pose considerable logistical and methodological challenges, as do the safety risks of sampling during the ice-covered period. Here, we consolidate information on winter lake sampling and describe effective methods to measure physical, chemical, and biological variables in and under ice. We describe variation in snow and ice conditions and discuss implications for sampling logistics and safety. We outline commonly encountered methodological challenges and make recommendations for best practices to maximize safety and efficiency when sampling through ice or deploying instruments in ice-covered lakes. Application of such practices over a broad range of ice-covered lakes will contribute to a better understanding of the factors that regulate lakes during winter and how winter conditions affect the subsequent ice-free period.

Of the world's 117 million lakes (Verpoorter et al. 2014), almost half periodically freeze (Weyhenmeyer et al. 2011; Denfeld et al. 2018). However, comparatively few ecological

studies have been carried out during winter (Hampton et al. 2015). Cold and dark winter periods have been assumed to be a time of high mortality, decomposition, and dormancy, and present more logistical difficulties than summer fieldwork (Sommer et al. 1986; Salonen et al. 2009). However, long-term patterns and drivers of ecosystem structure and function may

¹Rubenstein Ecosystem Science Laboratory, University of Vermont, Burlington, Vermont

²Department of Ecology and Environmental Science, Umeå University, Umeå, Sweden

³Department of Sustainable Agro-Ecosystems and Bioresources, Research and Innovation Centre, Fondazione Edmund Mach (FEM), San Michele all'Adige, Italy

⁴Department of Experimental Limnology, Leibniz Institute for Freshwater Ecology and Inland Fisheries, Stechlin, Germany

⁵Institute for Biochemistry and Biology, Potsdam University, Potsdam, Germany

⁶Itasca Biological Station and Laboratories, University of Minnesota Twin Cities, Lake Itasca, Minnesota

⁷School of Natural Resources, University of Missouri, Columbia, Missouri

⁸Département des Sciences Fondamentales, Université du Québec à Chicoutimi, Chicoutimi, Quebec, Canada

⁹Centre for Northern Studies (CEN), Université Laval, Québec City, Quebec, Canada

¹⁰Group for Interuniversity Research in Limnology and Aquatic Environment (GRIL), Université de Montréal, Montréal, Quebec, Canada

¹¹Dorset Environmental Science Centre, Ontario Ministry of the Environment and Climate Change, Dorset, Ontario, Canada

¹²Department of Biology, Queen's University, Kingston, Ontario, Canada

¹³Department of Environmental Science and Policy, Tahoe Environmental Research Center, University of California Davis, Davis, California

¹⁴Department of Ecology and Genetics/Limnology, Uppsala University, Uppsala, Sweden

¹⁵Natural Resources Research Institute, University of Minnesota Duluth, Duluth, Minnesota

¹⁶Department of Biology, University of Minnesota Duluth, Duluth, Minnesota

¹⁷Lammi Biological Station, University of Helsinki, Helsinki, Finland

¹⁸Center for Environmental Research, Education and Outreach, Washington State University, Pullman, Washington

^{*}Correspondence: ben.block@uvm.edu

be misunderstood if knowledge is derived primarily from sampling during the "growing season," hence winter work is needed (Bertilsson et al. 2013; Maier et al. 2018). We use the term "winter" and "ice-covered" synonymously in this article. However, the terms may not be synonymous in other applications as ice-cover varies by latitude and elevation.

Winter is an important period for limnological processes, which range from biogeochemistry to fish ecology. For example, even with some snow cover, light can still sufficiently transmit through the ice for photosynthesis (Cota 1985; Bolsenga and Vanderploeg 1992). Primary producers are present in winter, albeit at lower volumetric abundances than summer (Hampton et al. 2017), and thus provide food for primary consumers. Primary consumers may additionally fulfill their winter nutritional demands by storing prewinter diets rich in polyunsaturated fatty acids (Grosbois et al. 2017; Mariash et al. 2017). Fish must deal with low metabolisms (Fry 1971) and, sometimes, low-prey abundances and low concentrations of dissolved oxygen (Magnuson and Karlen 1970). Many generalist fish species reduce their forage niche width in winter and feed on whatever prey remains abundant (Eloranta et al. 2013; Hayden et al. 2013), while other species can increase body lipids during winter (Stockwell et al. 2014). From a food web perspective, winter can force actively overwintering organisms to obtain energy through new pathways.

Biogeochemical processes continually take place at the sediment-water interface and at the water-ice interface. New and accumulated organic matter is remineralized and affects a broad range of biogeochemical reactions that influence water quality and hence ecosystem function both in winter and subsequent seasons (Karlsson et al. 2008; Bertilsson et al. 2013; Powers et al. 2017). Moreover, the links between ice-cover dynamics, microbial ecology, and physical processes below ice have important implications for redox potential at the sediment-water boundary. Changes in redox have repercussions for under-ice internal loading of nutrients from the sediments (North et al. 2015; Joung et al. 2017; Orihel et al. 2017) and for the amount and type of greenhouse gases (GHGs; e.g., carbon dioxide [CO₂] and methane [CH₄]) emitted from lakes at ice melt (Denfeld et al. 2018). Therefore, biogeochemical processes that occur during the winter have the potential to affect spring and summer conditions (Bertilsson et al. 2013).

Winter research to date has largely been the purview of polar investigators (e.g., Greenbank 1945; Winslow et al. 2014), but interest is increasing. In 1996, a National Research Council report set forth two fundamental questions that have yet to be fully addressed (McKnight et al. 1996), and have grown in relevance as ice cover duration shortens worldwide (Magnuson et al. 2000): what are the critical events and conditions that control autotrophic and heterotrophic processes during winter, and what critical winter processes control the behavior of ecosystems in the subsequent spring and summer? More recently, international winter limnology

symposia have provided preliminary data and further enticed researchers to study winter dynamics (Salonen et al. 2009). The trajectory of winter limnology research activity—from scattered studies to symposia, reviews, and data syntheses—suggests the time is ripe for the limnology community to increase winter research. Most limnologists, however, are trained in the open-water season and are unfamiliar with detailed winter methodologies, which are widely scattered throughout the literature.

Researchers just starting limnological studies on ice-covered lakes may find numerous unfamiliar challenges compared to the open-water season. Certain aspects of winter sampling require additional equipment and some sampling protocols may require drastic alteration to function properly during winter. Given the growing interest in winter limnology and the unique considerations of winter field work, we take this opportunity to define and adopt standardized methods and to catalyze greater coordination among researchers worldwide. In addition, a detailed section on safety considerations for winter fieldwork is included.

Winter limnology equipment limitations and solutions

In this section, we discuss winter-specific sampling conditions and the strengths and weaknesses of method performance (Table 1). We recommend standardized winter protocols to increase prospects for data integration that enables comparative and synthetic analyses.

The challenge of cold conditions

Freezing is a persistent problem for most equipment and samples in winter. Water collection vessels (e.g., Van Dorn samplers), integrators (i.e., rubber/plastic hoses), peristaltic pump tubing, nets, and aquatic sensors (see "Power supply" section) have the capacity to fail or introduce bias when frozen. Most equipment freezes when water comes into contact with below-freezing air temperatures. Therefore, if possible, select field days with relatively warmer air temperatures. If equipment freezes, we recommend submerging the equipment to thaw in the relatively warmer lake water (compared to air). Otherwise, hot water or biodegradable antifreeze can be used to thaw small pieces of equipment. Insulated containers filled with heat packs are useful to ensure equipment and samples do not freeze; however, they do not present a long-term solution because heat generation ultimately ceases, and may increase the temperature of samples. Alternatively, within an insulated container, samples can be packed with slush and lake water to prevent freezing and immobilize samples during transport (Salmi et al. 2014). Once returned from the field, samples packed with slush and lake water will not need immediate refrigeration because they are properly insulated. In addition, sample bottles should be prearranged in ordered grids to improve efficiency during collection, particularly in unfavorable conditions.

Table 1. Summary of limnological techniques for general, physical, chemical, and biological variables and possible solutions for winter sampling limitations with examples of published literature on under-ice applications.

Manialala	Taskadana	Equipment	Caladana	- 1 6 1	
Variable	Technique	limitation	Solutions	Examples of relevant literature	
General	Matan callaction	Francisco compular to a	Keep equipment in water week in	D:*:41	
	Water collection (e.g., Van Dorn)	Freezing; sampler too large for ice hole	Keep equipment in water; work in shelter; use vertically oriented samplers	Bižić-lonescu et al. (2014) and Grosbois et al. (2017)	
	Water profilers (e.g., a sonde)	Low battery life; sensors freeze	Keep batteries warm; keep equipment in water	Denfeld et al. (2015)	
	Transportation on the ice	Ice thickness	Load capacity (see "Safety considerations" section)	Army Corps of Engineers (1996)	
	Water depth	Definition of surface depth (0-depth)	We propose that 0-depth is at the ice–water interface	_	
Sediment	Ekman/Ponar grab; Glew corer	Sampler too large	Use petite ponar; Glew corer works well in winter	Peter et al. (2016) and Glew et al. (2001)	
Physical				,	
Light (PAR)	Profiling instrument (e.g., Licor)	Temporal and spatial variation	PAR sensor with arm extension; in situ automated PAR recorder	Belzile et al. (2001), Rücker and Henschke (2004), and Wagner (2008)	
Convective mixing	Moored temperature logger chain	See automated samplers and loggers	See automated samplers and loggers	Kirillin et al. (2012), Cortés et al. (2017), and Pernica et al. (2017)	
	Automated samplers and loggers	Freezing into ice; ice damage; power issues	Anchor system or float freely; reduce frequency of data collection and transmission	Demarty et al. (2011), Marcé et al. (2016), and Obertegger et al. (2017)	
Chemical					
Oxygen	Winkler titrations; automated sensors	Samples freeze; sensors freeze	Collect water—bring back to lab; keep samples and sensor equipment in water	Terzhevik et al. (2010) and Domysheva et al. (2017)	
Gases	Headspace technique; automated sensors	Drilling disturbs surface-water gases; syringes freeze; samples freeze	Use hand drill/saw; collect water away from the hole; introduce headspace in lab	Michmerhuizen et al. (1996), Denfeld et al. (2015), and MacIntyre et al. (2018)	
Biological		sumples freeze			
Primary production	ΔDO; ¹⁴ C	Deployment limited by ice cover and temperature	¹⁴ C-spiked bottles; long-term DO sensors	Steemann Nielsen (1952) and Vollenweider et al. (1974)	
Plankton	Tow net; Van Dorn; or water pumps	Freezing equipment; sampler too large	Keep equipment in water; work in shelter; collapsible net or lead line on net	Gerten and Adrian (2001) and Grosbois et al. (2017)	
Fishes	Active and passive sampling techniques	Ice is a barrier to most sampling techniques	Use gill net with ice jigger	Eloranta et al. (2013) and Hayden et al. (2013)	
Organisms associated with ice	Melting or scraping of ice; under-ice cameras	Mixing of pelagic and on-ice communities	Partition ice core; use cameras focused on ice-water interface	Bondarenko et al. (2006) and Frenette et al. (2008)	

The challenge of varying snow and ice conditions

Lake ice is highly variable in structure and load capacity (Table 2). Ice phenology is largely dictated by regional variability in climate, lake morphometry (e.g., lake surface area, depth, and fetch), and water movements (e.g., inflows and

currents; wave action) (Kirillin et al. 2012; Leppäranta 2015). Interactions among these factors will ultimately determine the specific structure of ice on a given lake (Ashton 1986). The load capacity of ice has important implications for researchers' ability to conduct fieldwork and varies by the types of ice

Table 2. Ice classification and phenology. Adapted from Leppäranta (2015), Michel and Ramseier (1971), and Petrenko and Whitworth (1999).

Ice type	Category	Common name	Description	Relative strength
Primary	P1–P4 ice	Skim ice	First ice on lake surface. Ice category depends on air/water temperature gradient, calm/turbulent water conditions, and nucleation source.	Low
Secondary	S1–S5 ice	Black ice, clear ice	Forms beneath the primary ice layer, category depends on air temperature and turbulence.	High
Superimposed	T1–T2 ice	White ice, snow ice Forms on top of primary ice layer from precipitation (snow and rain). T1 is snow ice, T2 forms from refrozen drained snow.		Medium
Agglomerate	R ice	Frazil ice, pancake ice, candle ice	General term for any agglomeration of individual ice pieces which have refrozen. Rotten ice that develops in columns perpendicular to the lake surface.	Low

encountered. For example, black ice weakens as it thaws and "candles" at the end of ice cover, whereas snow ice is 50% weaker than black ice at all times (Leppäranta 2015).

Thickness, a proxy for load capacity, can be measured simply by drilling a hole through the ice. Ice thickness, however, can vary substantially even within a small area due to freezing history, snow cover, and water flow (e.g., Korhonen 2006). A popular ice coring system has been developed by the snow, ice, and permafrost research establishment (SIPRE) and is widely referenced in the polar literature as a "standard SIPRE corer." A SIPRE corer can be used to measure the thickness of discrete ice layers (e.g., white/black ice; Table 2). Several under-ice, automated techniques have been used to obtain more precise measurements of thickness, although they are much more complex and expensive than simply drilling a hole. Moored subsurface sonar sensors can measure ice thickness, but require temperature-dependent speed of sound corrections (Melling et al. 1995; Brown and Duguay 2011). Another technique is X- and Ku-band radar, which requires in situ information or assumptions about ice conditions (Gunn et al. 2015). A low-cost alternative is a soil water content reflectometer sensor, which detects phase changes of water, and can be repurposed to measure ice thickness (Whitaker et al. 2016).

Ice phenology

Historically, many communities have recorded ice-cover dates, but their methods differ. Scientific definitions of ice-on and ice-off dates are similarly variable (e.g., Magnuson et al. 2000; Hewitt et al. 2018) as are the methods that determine the dates. The methods include high-frequency water temperature data (Weyhenmeyer 2004; Pierson et al. 2011; Obertegger et al. 2017); direct visual observation of ice cover; satellite imagery (Wynne et al. 1996); and camera images (Obertegger et al. 2017). The ice-covered period can be simply defined as the time from the first complete freezing in fall, in which the ice remains frozen, until total clearing of ice in spring (Robertson et al. 1992). For large lakes, the ice-on and

ice-off dates are for the location of observation and not necessarily for the lake as a whole (Magnuson et al. 2000). Ultimately, the method of choice should reflect the objectives of the study and the size of the lake, but most importantly, whichever definition used should remain consistent to facilitate comparisons both within and among data sets.

Field site preparation

A well-prepared field site is needed for safe and effective work on ice-covered lakes. The efficiency of sample collection is paramount; all unnecessary steps will only complicate sampling excursions. For researchers who conduct winter limnology frequently, shelters are necessary when conditions become unfavorable, and can be purchased or constructed. Collapsible tents are easily moved and allow research teams to sample multiple sites quickly. However, if a single site is routinely sampled, a more permanent structure can be erected on the ice, if permissible under local regulations. Winds in winter can be severe across the open areas of lakes, so structures and equipment must be fastened with guy lines and pitons. Even with a shelter, freezing conditions can still affect equipment and individuals. A mobile heat source improves equipment functionality and increases sampling comfort and safety. However, mobile heat sources should have proper ventilation because they typically produce carbon monoxide gases.

Equipment needed to penetrate ice at a field site depends on ice thickness. Both powered and hand ice augers are limited by their overall length and thus may limit the thickness that can be penetrated. Most augers can penetrate ~ 110 cm of ice, but some polar lakes can produce much thicker ice (> 200 cm). In such cases, an auger with an extension is required. When a gasoline-powered auger is used to drill holes, take care to ensure that no contamination occurs when water chemistry samples are taken. Blades for the auger should be sharp before heading into the field and spare blades are recommended; dull blades can significantly impede the drilling speed. Although slower, ice saws and ice chisels can penetrate

ice and connect augered holes to enlarge sampling areas. Finally, ice fragments should be removed from the augered hole with a sieve to avoid interference with sampling equipment.

Transportation on ice

Winter limnology research programs use a variety of modes of transportation on the ice. Some researchers simply ski, walk, or snowshoe across the ice, pulling a sled or small rowboat filled with gear. Snowmobiles and all-terrain vehicles are rugged, motorized alternatives that offer a quicker mode of transportation. Automobiles are ideal to carry large amounts of equipment. However, vehicles can get stuck in snow and break through thin ice. Therefore, load calculations should be made to determine the minimum thickness of ice that can be safely driven upon (see "Safety considerations" section). In addition, institutions may not allow research vehicles to be driven on frozen lakes because of liability restrictions. Consequently, one should obtain any necessary approval prior to using a vehicle on ice. Hovercrafts, hydrocopters, and airboats can be safe alternatives to wheeled or tracked vehicles because they will float if the ice collapses, which is more likely during ice formation and spring thaw.

Under-ice water sampling

Once the ice hole is made and ice fragments are removed, samples can be collected. Tube samplers with open flow paths (e.g., Limnos samplers, Niskin samplers) that open and close vertically are preferred because they require a smaller ice hole than horizontal Van Dorn samplers. In addition, opaque samplers are preferred when conducting algal work to prevent light-shock to dark-adapted phytoplankton when brought above the ice. To ensure that discrete samples at the water/ice interface are minimally disturbed, sample collection should start directly below the underside of the ice and away from the drilled hole. After the ice hole has been drilled, ice thickness should be measured to determine at which depth the water sampler should be deployed such that water is collected below the ice bottom. A homemade device can be constructed to extract water from near the water/ice interface, horizontally away from the drilled hole (e.g., Ricão Canelhas et al. 2016). A siphon sampler for collecting water at various depths can be used without pumps or electrical power (Magnuson and Stuntz 1970); various designs have been used and even a plastic bottle large enough to hold a sample can be used with plastic tubing lowered to a sample depth.

Under-ice sediment sampling

Sediments are much easier to sample with coring devices on ice-covered lakes than during the open-water season because ice cover provides a more stable platform than boats. Tips on sediment core equipment, collection, extrusion, and the adaptation of methods to winter conditions can be found in several sources (Renberg 1981; Wright 1991; Nesje 1992; Glew



Fig. 1. Example of coring setup on the ice. Elevated stands keep equipment out of the snow and ice and can help prevent equipment from freezing.

et al. 2001). The winter researcher will find that steel cables used on coring devices and sounding lines will stiffen and maintain kinks more often under cold conditions. Check cables frequently for kinks or use an alternative, nonstretchable material such as spectra braid line or plastic-coated steel cable. A piston corer cable can be stabilized on the ice surface by wrapping it on a cleat affixed to a piece of nontemperature sensitive material such as lumber that spans the hole. Zorbitrol, a sodium polyacrylate absorbent powder commonly used to stabilize the headwater overlying the sediment (Tomkins et al. 2008), is unaffected by cold weather conditions. To help maintain ambient lake bottom temperature, cores can be stored short-term in a foam-lined box or another insulated wrapping. Elevate equipment on a platform to keep it dry and visible (Fig. 1).

Sounding devices that easily penetrate the lake bottom, such as small condensed weights, are less accurate than those with larger surface areas, such as a Secchi disk, that rest on the sediment surface. A depth measurement with a sounding device will disrupt the sediment surface and should never be used in the same hole where a core for analysis is collected. Hydroacoustics can be used to estimate depth without disturbing the bottom sediment; however, hydroacoustics do not work well when ice thickness exceeds about 1 m due to signature rebound from the sides of the hole.

Sediment grabs such as Ponar, Ekman, and tube (Kajaktype) samplers can work well in winter but may freeze. In addition, larger grabs are difficult to fit through ice holes. Thus, for shallow waters, a petite Ponar is recommended.

Safety considerations

Winter limnology presents three principle safety questions: first is the ice sufficiently thick to support people and equipment; next, do researchers possess the capacity to self-rescue and rescue team members; and finally, can hypothermia and

frostbite be prevented. Temporal and spatial differences in ice thickness can influence the level of risk associated with a given waterbody. For example, midlatitude lakes that experience seasonal melting or those with significant underwater currents present a greater risk than lakes at higher latitudes with sufficient and prolonged ice cover. An ice chisel can be used to test ice thickness; if the chisel breaks through the ice with a single hard thrust, then the ice is not safe. Furthermore, limnologists working in regions where winter air temperatures routinely drop below freezing experience an increased risk of hypothermia and frostbite. Consultation with local experts and resources will provide greater insight into winter-specific and lake-specific safety challenges within the sampling region.

All investigators must be adequately trained and equipped to conduct winter limnology studies (Fig. 2). If one is not prepared to go through the ice, then one should stay off the ice (Giesbrecht 2001). Safety topics to consider include proper workplace communication, personal protective equipment, lake-specific hazards and constraints, and ice load limits. However, some level of risk is always present when working on ice, independent of ice thickness, and no protocol can predict all possibilities. Prior to fieldwork, a briefing should be held to discuss responses to potential emergencies such as falling through the ice or hypothermia. Ultimately, each person is responsible for their safety and that of their team.

Research groups should establish protocols associated with winter limnology work and any lake-specific constraints. If local safety resources are unavailable, consult ice safety protocols (e.g., Canadian Council Ministries of the Environment 2011; Rescue Canada 2013; Ontario Ministry of the Environment and Climate Change 2017) and internet resources (US EPA 2009). Protocols can include information on mandatory safety equipment and training, lake-specific considerations and dangers, and limitations on when field work is and is not permissible based on recent weather and ice conditions.



Fig. 2. Examples of winter field equipment: pulka, motorized ice auger, sieve, shovel, snowshoes, and safety rope.

Also, distribute a field itinerary among those involved in field-work, including a safety contact. The field itinerary should include contact information, site locations, departure and anticipated return times, a timeline and means of communication, and an emergency response procedure. Local emergency services can be notified of field work schedules and expected return times for added safety.

Winter field work should never be conducted alone. All individuals should be properly equipped for winter weather, be prepared for a fall through the ice or losing their way, and ready to cope with transportation failure. At the minimum, safety equipment should include a personal floatation device, ice "claws" or "picks," a charged communication device in a waterproof container, a rescue throw-rope, spare clothing, and a waterproof first-aid kit (Fig. 2). Full, wet-immersion floatation suits and survival kits are ideal. Survival kits (e.g., Canadian Council Ministries of the Environment 2011; Government of Alberta 2013), personal protective equipment, and appropriate field clothing (Rescue Canada 2013) are a necessity in winter. A portable shelter and portable space heater can significantly improve working conditions and reduce the risk of frostbite and hypothermia. Finally, all team members should be aware of symptoms of frostbite and hypothermia in themselves and others, and be prepared to treat the symptoms (Giesbrecht 2001; American Red Cross 2007).

Unfortunately, lake-specific hazards make the development of universal safety protocols difficult. Ice is rarely uniform across an entire lake surface and its thickness can vary considerably over short distances. Underwater currents, inlets, springs, breakwalls, and docks can produce thin, unsafe ice. Acquire additional information on lake-specific dangers from local resources such as winter sporting shops, government agencies, other researchers, or local recreational users. For example, a popular North American forum, *iceshanty.com*, is used by anglers to report lake-specific ice conditions. Similar resources can provide insights into real-time, lake-specific hazards and improve decision making accordingly.

Lake-specific constraints may not be exclusively physical in nature. Use of ice-covered lakes by the public may create challenges. For example, many events such as snowmobile, automobile, ice skating, and ski races take place on ice-covered lakes. Such activities can interfere with research projects, especially if a research structure or a specific location is part of the research plans. Semi-permanent structures, in place overnight or longer, may require a local permit or license. Research projects should not interfere with or present dangers to other lake users. Investigators should mark any sampling hole conspicuously with flags, tree branches, or reflective markers. For example, a 20-cm diameter hole may expand to a 1-m hole within a few weeks by water discharging on the top of the ice, and be hidden by snow and/or a thin layer of ice that does not support a person. To ensure safety, some regions have regulations that limit hole size. Check with the appropriate authorities to

be within legal limits; be informed and observe other lakespecific constraints prior to any winter field work.

The load (weight of equipment and personnel) at which ice is compromised is based on thickness, morphology, and temperature. Publications such as Army Corps of Engineers (1996), WorkSafe Alberta (2008), and Government of Alberta (2013) provide means to calculate safe loads on ice (*see* Eqs. 1, 2).

$$H = (0.5 \times T \text{ white ice}) + T \text{ clear ice.}$$
 (1)

where H is effective ice thickness and T is ice thickness based on morphology (WorkSafe Alberta 2008).

$$P = A \times H^2 \tag{2}$$

where P is the allowable load in kilograms and A is a parameter based on the strength of the ice (safety factor). Gold's formula (Gold 1971), including the values for A, is conservative. Values for A vary according to relative risk: 3, 4, 5, and 6 for low, tolerable, moderate, and substantial risk, respectively (Government of Alberta 2013). An *effective* ice thickness of H = 20 cm is sufficient to support the weight of humans and moderate equipment loads (< 500 kg; Fig. 3).

Stationary loads, i.e., those remaining in place for more than 2 h, require greater ice thickness than a moving load (Government of Alberta 2013, sec 4.1.5). Further, recent snowfall can add weight to the ice and must be included in load calculations. Load limits vary based on ice morphology (Table 2). Air temperatures should be consistently below freezing for approximately 1 week prior to sampling; otherwise, load calculations must be adjusted accordingly.

Measurements of physical conditions

Photosynthetically active radiation

In addition to the typical factors associated with assessing the light environment in the water column in the open-water season (e.g., irradiance, attenuation by particles in the water

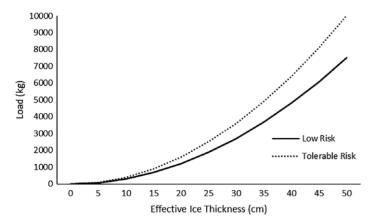


Fig. 3. The relationship between relative risk of point load limits and ice thickness. Redesigned from Government of Alberta (2013).

column, euphotic depth), a number of other factors should be measured during the winter. Factors which affect light attenuation in winter include albedo effects on incident irradiance, snow cover (thickness, quality, and distribution), and ice thickness and characteristics. Snow thickness has the most influence on under-ice photosynthetically active radiation (PAR) (Bertilsson et al. 2013); snow depth and quality can strongly influence light limitation of under-ice primary producers (Pernica et al. 2017) and subsequent reduction in growth rates (Jewson et al. 2009).

Under-ice PAR varies temporally and spatially due to ice thickness and the patchiness of wind-blown snow. An augered hole can influence under-ice PAR measurements made by a light meter; however, as ice thickness increases the hole-effect becomes negligible (Schneider et al. 2016). To reduce the effect of light from the hole, one can extend the PAR sensor beyond the influence of the hole by using an extended folding arm adjustment or by deploying the PAR sensor through a narrow slit in the ice made by an ice saw. Alternatively, the hole could be covered to resemble in situ ice surface conditions. Local snow disturbance should also be minimized. To accurately measure under-ice PAR, take an incident irradiance reading (above ice and snow) and account for albedo effects. Then place the light meter directly under the ice and take incremental measurements to the desired depth.

To observe variable conditions on and under the ice, frequent monitoring is essential. Continuous monitoring is ideal. For example, satellite imagery can measure albedo while in-lake, automated light sensors measure high-frequency temporal variation in light conditions. For more information, see the "Sensor deployments under ice" section below.

Convective mixing and under-ice fluxes

The timing and depth of convective mixing in ice-covered lakes will depend on ice characteristics, regional climate, and density gradients in the near-surface layer. Under-ice convective mixing is influenced by changes in solute concentrations (e.g., Belzile et al. 2001), incoming solar radiation through the ice, incoming meltwaters (Cortés et al. 2017), and heat transfer from lake sediments (Welch and Bergmann 1985). Given the dynamic processes which govern vertical flows in ice-covered lakes, inverse stratification can occur. How variable the stratification may be throughout the winter is unclear. The consequences of such physical dynamics have been illustrated for under-ice PAR and corresponding phytoplankton biomass (Pernica et al. 2017).

Turbulent fluxes are most often estimated using high-frequency measurements of temperature throughout the water column, although high-resolution thermistors (accuracy of $\pm~0.001^{\circ}\text{C}$) are necessary when temperature gradients are small and the role of dissolved solutes is important. Direct measurements of convective turbulence can be made with specialized in-lake instruments such as microstructure profilers, acoustic Doppler current profilers, and acoustic Doppler velocity meters

(Kirillin et al. 2012 and papers therein). Moreover, remotely operated and autonomous underwater vehicles are increasingly used as platforms to characterize physical dynamics in ice-covered lakes (Katlein et al. 2017). Aquatic eddy covariance systems, which make concurrent high-frequency measurements of current velocities, temperature, conductivity, and dissolved oxygen, have been successfully used in lakes and under sea ice to measure turbulent exchanges and heat or solute fluxes at the ice—water interface and the sediment—water interface (McPhee 1992; McGinnis et al. 2008; Else et al. 2015). The specific approach used to quantify turbulent exchanges of heat or solutes should be dictated by the research questions.

Measurement of chemical conditions

Collection of water beneath the ice for most determinations of carbon and nutrients does not require any special winter sampling techniques (see winter-specific sampling conditions). However, CO_2 , CH_4 , and oxygen $[O_2]$ gases, especially immediately below the ice, may be compromised during winter if an ice auger disturbs the water surface. Disturbance of the surface water can be minimized by using a hand drill or ice saw.

GHG sampling during open water, such as the headspace technique for $\rm CO_2$ and $\rm CH_4$ (e.g., Cole et al. 1994), can be applied with modifications during the ice-covered period (Table 1). However, the use of GHG sampling techniques in cold conditions is often difficult; glass storage vials and syringe and needle connections that contain liquids can easily freeze and break. For the headspace technique, record water temperature when the "headspace" is introduced (if different from ambient temperature) so GHG concentrations can be back-calculated to in situ conditions. Also, handheld automated sensors can be used to measure gases below ice (Table 1).

The ice-covered period offers a unique opportunity to target and quantify CH₄ ebullition (i.e., bubble-mediated transport of CH₄ from anoxic sediment to the surface waters). In icecovered lakes, CH₄ ebullition results in CH₄ bubbles being trapped in the ice and at the water/ice interface (Walter et al. 2006; Ricão Canelhas et al. 2016). Methane bubbles at the water/ice interface can be captured and quantified (Huttunen et al. 2003) with the use of bubble gas collectors submerged below the water surface (Huttunen et al. 2001). In addition, the amount of gas trapped in lake ice can be quantified on melted water samples, using the headspace technique noted above, where ice cores are sealed in airtight vessels fitted with serum stoppers (Phelps et al. 1998). Where clear ice conditions persist, photographic inventories of lake ice bubbles have been used to scale CH4 ebullition across the lake (e.g., Walter Anthony et al. 2010). In cases where hotspot seep sites persist, bubble traps can also be deployed to quantify winter CH₄ flux (e.g., Greene et al. 2014).

Few studies have published direct measurements of ${\rm CO_2}$ and ${\rm CH_4}$ emissions during ice melt (reviewed in Wik et al. 2016 and Denfeld et al. 2018), which reflects the logistical difficulties

in sampling during the dynamic ice-melt period. One way to estimate temporally resolved ice-melt emissions, especially when ice conditions are unsafe, is to use in situ carbon gas sensors combined with modeled gas exchange (Huotari et al. 2009; Denfeld et al. 2015). An eddy covariance tower on the lake shore, which enables direct measurements of GHG emission at ice melt within the tower footprint, is another option (Anderson et al. 1999; Huotari et al. 2011; Jammet et al. 2015) but requires expensive instrumentation and extensive data post-processing.

Measurement of biological conditions

Organisms associated with the ice

Techniques for sampling the underside of ice are unfamiliar to most limnologists because such studies in freshwater are rare. Fortunately, research in polar sea ice systems has tested and described appropriate methods to investigate the under-ice microhabitat. To sample organisms associated with the ice, the ice should remain undisturbed as much as possible. An ice saw or SIPRE coring system can be used to cut an intact ice core of known volume of ice. The sampled ice can be melted and organisms preserved for analysis or, before thawing, the core can be sectioned horizontally to examine the spatial distribution of organisms in discrete layers throughout the ice (Horner et al. 1992; Foreman et al. 2011; Bondarenko et al. 2012). A limitation of this method is that algae associated with the bottom of the ice, but not firmly attached, may be dislodged and lost from the sample. A variety of techniques can be used by divers, including standard periphyton sampling methods to scrape and collect organisms from a known area (e.g., Loeb 1981), or gentle suction to sample known volumes of the nearice planktonic community (reviewed in Welch et al. 1988; Melnik et al. 2008). Diving under ice requires special training and certification, a dive team both on the surface and submerged, site preparation, and facilities for post-dive care to avoid hypothermia. Finally, cameras have been used successfully to observe the presence or abundance of ice-associated organisms, the manner in which the organisms are associated with features of the ice, and how they are disturbed by water movement (Mundy et al. 2007).

Primary productivity

With sufficient light penetration through ice and snow, water columns can be surprisingly productive during the ice-covered season (Salmi and Salonen 2016). Even more than with open water measurements of primary productivity, experimental results may be severely affected by exposure of samples to light sources above the water surface. Therefore, when samples are to be brought above the ice, erect a shelter over the auger hole to maintain a dark working area and prevent exposure of samples to ambient daylight.

Careful consideration should be given to the selection of a sampling site. High-traffic areas should be avoided both for

safety and because footprints and vehicle tracks can influence light penetration and consequently alter light levels within in situ experimental arrays measuring primary productivity. To sample sites with undisturbed snow cover, approach from the north (south in the southern hemisphere) to limit disturbance to overlying snow on the sunny side of the auger hole. At stations where snow is to be cleared from the ice surface, the area should be cleared to the south of the hole (north in the southern hemisphere). The size of the cleared area is dictated by the depth to which the experimental array will be deployed.

While many of the popular methods to determine photosynthetic rates (Δ dissolved oxygen (Δ DO), ¹⁴C; Wetzel 1965; Hall and Moll 1975) can be used beneath the ice, winter conditions favor techniques that use a marker that is both easily deployed and measured in the laboratory. In situ incubation of ¹⁴C-spiked bottles (Steemann Nielsen 1952) is a preferred method by many winter limnologists. In low-light conditions, bottles are often spiked with radiocarbon tracers of greater activity, but dosing with approximately 3.7×10^5 Bq ¹⁴C-bicarbonate mL⁻¹ has been found adequate (Vollenweider et al. 1974). Spiked bottles should be deployed at depths suitable for characterizing the entire light profile, with one bottle below the euphotic depth.

Plankton

Plankton net sampling can be conducted in winter using techniques similar to ice-free conditions, although some challenges associated with winter operations remain. The diameter of the ice hole must be sufficient to fit the mouth of the net. Large ice augers (20–25 cm diameter) create holes large enough for small nets such as small Wisconsin plankton nets. However, when larger nets are used, multiple holes must be augered side by side, with an ice chisel or ice saw used to remove the remaining ice between holes. An alternative is a pull-up cord attached to a flexible ring and main tow line that can vertically orient the ring opening, which allows the net to be retrieved through an oval hole. An additional alternative would be collapsible plankton nets with flexible openings constructed of cable (Fig. 4).

Wet nets will freeze if exposed to subzero temperatures. The nets must be rinsed, the cod end removed quickly, and sample rinsed into sample bottled. Alternatively, in subzero temperatures, tows could be conducted in a shelter to prevent freezing when the net is brought out of the water. The hole in the ice should be thoroughly cleared of any ice particles as they can interfere with the sieving of plankton through the mesh and be a nuisance when removing the cod end.

An ice hole allows light to penetrate a normally light-limited environment. Anecdotal evidence suggests an increase in localized light may attract or repel plankton into the area. Thus, estimates of biomass, density, and community composition may be biased. To reduce such bias as a result of phototaxis, sample as soon as the hole is created, cover the hole until sampling begins, or work in a shelter.

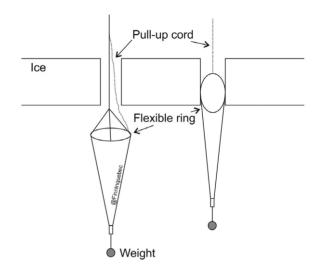


Fig. 4. Flexible ring on tow net which enables deployment and retrieval of a net with a mouth diameter larger than the hole diameter in the ice.

Fish

Ice cover and winter conditions present inherent equipment limitations for fish collection. If fish have reduced their movement, passive equipment will catch less fish than active equipment. Data on fish activity, aggregation, and behavior can be obtained qualitatively using remotely operated vehicles or a simple "inverted periscope" (Magnuson and Karlen 1970), or quantitatively using an acoustic telemetry array (e.g., Hanson et al. 2008) or echosounder (e.g., Jurvelius and Marjomäki 2008; Ahrenstorff and Hrabik 2016). Minnows traps can be placed on the lake bottom or suspended in the water column to investigate fish distributions and collect specimens (Magnuson et al. 1985). For larger fish, fyke nets, gill nets, and seines can be set under the ice. Deployment of a seine, however, requires that large holes be cut throughout the sampling area (Turunen et al. 1997). Large or grouped holes can be a safety hazard and may be illegal on certain lakes. Under-ice diving to assess fish or service experiments is also feasible (Horns and Magnuson 1981). Lønne and Gulliksen (1989) ambitiously used a dipnet mounted on a telescoping pole to collect fish while SCUBA diving between and beneath ice floes. However, the majority of published underice fish studies have used gill nets. An ice jigger is submerged and "crawls" beneath the ice to string a gill net from one hole to another. Detailed tutorial videos on how to operate an ice jigger are available online. The jigger may be obscured by snow cover but electronic locators are available to find the jigger through the ice. An ice jigger can be purchased online or custom built simply from wood and styrofoam.

Catch per unit effort (CPUE), when derived from gill net catches, will change based on the time of year and target species. Fish have lower metabolic activity in winter and are likely to be less mobile than during other seasons (Fry 1971). CPUE will also change based on the target fish species because thermal tolerances vary. Thus, a longer deployment time may be

needed in winter relative to other seasons to obtain sufficient numbers of fish according to sampling goals. In addition, fish may inhabit different areas in winter compared to summer based on temperature, light, dissolved oxygen (Magnuson and Karlen 1970), or prey densities (Klemetsen et al. 2003).

Stomach content analyses for prey identification may be complicated by how fish are sampled in winter. Longer deployment of gill nets increases the potential for loss of diet data because of digestion, although cold temperatures will slow digestion rates. If stomach content analysis is required, gill nets should be retrieved frequently. Preliminary experimentation can be used to determine how long a gill net should be deployed for particular species, depending on species-specific digestion rates across typical winter water temperatures.

Acquisition of fish caught by anglers is a cost-effective and convenient method to sample fish in winter. However, quantification of CPUE from angling in any season is challenging. The techniques, lures or bait used, time of day, and other variables are likely to vary among anglers (Moraga et al. 2015). In addition, angling targets specific size and age classes, which may skew demographic results. Angling, however, can be a useful method to assess fish health and contaminant levels and can provide tissue samples and data on size, age, and growth. In addition, winter creel surveys assess angling pressure during ice-covered periods.

Sensor deployments under-ice

The deployment of continuous data loggers (e.g., temperature, light, O₂, and CO₂) and automated sampling equipment (e.g., sediment traps) in lakes enables analysis of under-ice processes during ice cover, including formation and break up. Until recently, the technological capabilities of aquatic sensors were limited to the open-water season, but recent advances in technology have permitted the deployment of in situ aquatic sensors that can continuously measure physical and chemical properties of water under the ice. However, compared to the open-water period, continuous measurements under ice and at ice-melt are currently limited in the literature (e.g., Baehr and DeGrandpre 2002, 2004; Denfeld et al. 2015; Zdorovennova et al. 2016; Cortés et al. 2017; Obertegger et al. 2017; Maier et al. 2018). However, several papers provide novel insights on under-ice dynamics and demonstrate that automated loggers, including thermistor chains and buoys, and other sampling equipment, can be successfully deployed during the ice-covered period. However, deployment of sensors and equipment is limited by cold temperatures and battery life, and potential damage from the ice. By taking precautionary steps, as discussed below, such risks can be minimized.

Power supply

Battery power is required by handheld sensors, in situ loggers, and automated sampling equipment. Battery life and function are drastically reduced in cold temperatures. Batteries designed for specific equipment (e.g., laptops or sondes) are often expensive and should be protected from the cold. For other equipment which uses off-the-shelf batteries, carry spare batteries, keep batteries warm, or increase battery size to reduce the effects of cold temperatures.

For batteries that are charged using solar panels, shorter day lengths and regions with generally overcast conditions during winter months can be a challenge. At midlatitude locations, the daily average shortwave radiation in summer can approach $400~\rm W~m^{-2}$, but in winter may drop below $50~\rm W~m^{-2}$ (J. A. Rusak unpubl. data). Charge potential of batteries can be substantially reduced when even a small area of solar panels is covered by ice or snow. Solutions to low power situations include a reduction in the frequency of data collection and transmission. Sensors can also be programmed to turn off when battery voltages drop below a threshold. Batteries can be permanently damaged or become increasingly difficult to recharge when voltages drop below recommended ranges.

Automated sensors and samplers

Below we offer a few examples of aquatic sensor deployment and setups but acknowledge that other solutions exist. Furthermore, the chosen setup will likely depend on several factors including lake characteristics (e.g., small vs. large and shallow vs. deep) and location (remote vs. local), scientific question and available funding. Researchers interested in automated sensor deployment in ice-covered lakes should modify their setup to meet their needs.

If aquatic sensors are deployed prior to ice-on, they should be suspended at depths below the expected maximum icecover depth to avoid damage (Fig. 5A-C). A sensor with internal power can be deployed at the desired depth using an anchor and float system (as is done during the open water season, e.g., Salonen et al. 2014). However, ice break-up may pose the risk of damaged lines and floats, thus the float should also be deployed at a depth below the maximum ice depth with sinking lines. In large lakes, wind and waves can push ice into piles that are several meters thick (Assel 1999). Lines should be rated for freezing conditions and be strong enough to withstand abrasion from moving ice. Lines vary by material and durability and can break under freezing conditions; light steel cables are ideal. In addition, wet lines may freeze when removed from the water. Sampling should be done at a sufficient distance from sensor platforms to avoid rope entanglements and equipment disturbance. Suggestions to ease sensor recovery at ice-melt include placing pop-up markers, such as floating lines, a colorful float frozen into the ice (Fig. 5B), or submerged floats with automatic pop-up timers. In addition, a flexible vertical plastic rod, which absorbs heat more than the adjacent ice and creates a mini-hole above the sensor unit in late winter, slides into the water if the ice moves preventing drift and loss of the sensors (Fig. 5C).

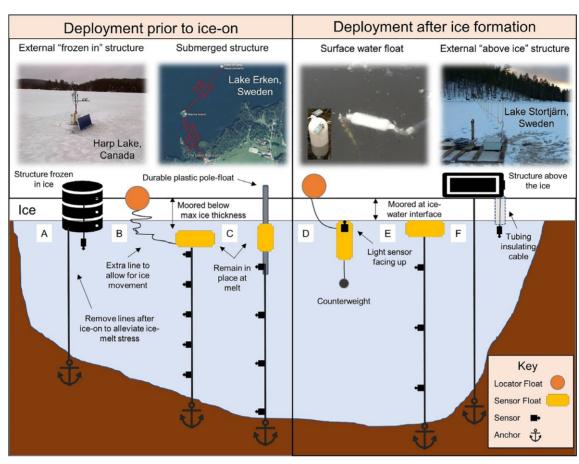


Fig. 5. Example setups of automated sensor deployment prior to ice-on (A–C) and after ice formation (D–F). Note, float and anchor shape and size can vary, and the float-anchor set up should be tested for stability prior to deployment. Picture insets show examples of setups currently used by Global Lakes Ecological Observatory Network (GLEON) sites.

Sensors which require external power must be equipped with an ice-proof power supply and structural support that can withstand winter conditions. If monitoring support structures are left to freeze in the ice (e.g., Harp Lake, Fig. 5A), the greatest risk of damage to equipment occurs during ice breakup, especially on large lakes. Wind events can transport large ice floes that are capable of submerging anchored buoys or dragging sensors if ice movement overcomes the mooring system. One solution is to remove anchor lines after ice-on to reduce the potential for ice to submerge the buoy. However, the float and sensor set-up will move at ice-melt, and thus retrieval efforts in spring should be prompt. An inexpensive GPS unit added to the monitoring hardware above the water is very useful to detect when a monitoring buoy begins to drift from its original position. We speculate that movement could be an additional mode of detecting ice-out but do not know of any such uses to date. Another solution to deploy externally powered aquatic sensors prior to ice formation is to have a cable connection from the land that is completely submerged (D. C. Pierson pers. comm.), as is currently done at Lake Erken (Fig. 5).

Aquatic loggers can also be deployed after ice formation (e.g., Denfeld et al. 2015, Fig. 5D–F). If the in situ sensor

requires external battery power, deployment after ice-on may be advantageous, as a relatively simple and inexpensive floating structure, housing the power supply, can be situated on top of the ice (e.g., Lake Stortjärn, Fig. 5F). The external structure should be sufficiently robust to withstand winter and icemelt conditions. In addition, deployment after ice-on enables sensors to be placed directly below the ice-water interface, which is particularly important for measurements such as light penetration. If an investigator is interested only in surface-water conditions or anchoring is not possible, sensors can be deployed below ice without a sediment anchor, but a colorful float should be placed on the ice (Fig. 5D) so the equipment can be located in the spring or removed prior to ice out. Although deployment of loggers after ice formation offers cheaper structural support solutions and the ability to take measurements at the ice-water interface, early winter conditions are missed, and a winter's worth of data may be lost if the ice never fully forms.

In addition to automated sensors placed beneath the ice, passive sampling equipment, such as (sequential) sediment traps deployed before ice-on, permits processes to be monitored under ice and during ice break-up. In general, such equipment has

rarely been used in ice-covered lakes despite their great potential. Automated equipment not only enables samples to be collected during the ice-covered period but is particularly valuable during the "shoulder seasons" when the formation and thinning of ice make the logistics of sampling more challenging. Sequential sediment traps have adjustable sample resolution and can capture processes during ice breakup that are otherwise impossible to sample manually. For example, sequential sediment traps are advantageous for sampling particle and plankton flux, especially when ice breakup makes manual sampling dangerous (Kienel et al. 2017, Maier et al. 2018, Maier et al. unpubl.).

Conclusion

Winter limnology provides many opportunities to expand our knowledge of the physics, chemistry, and biology of ice-covered lakes. A majority of limnologists, however, are unfamiliar with the challenges that winter introduces to limnological methods. Therefore, the methods we suggest offer instructions on how to effectively and safely explore a wide range of questions. We used the diverse experiences of a globally distributed group of limnologists and relevant published literature to compile this primer to assist those who are new to winter limnology field work. With growing technological improvements and a greater interest in winter limnology, we expect rapid development of more creative methods to study lakes under the ice. Ultimately, increased winter sampling will provide a more comprehensive understanding of how aquatic ecosystems function, particularly in light of changing winter conditions (Magnuson et al. 2000; Jensen et al. 2007; Hewitt et al. 2018). In addition, continued active dialog will help develop creative new methods, lower barriers for researchers to initiate winter work, and facilitate integrative and comparative winter studies across globally distributed lakes.

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Acknowledgments

We would like to thank two anonymous reviewers for their valuable comments which have substantially improved this manuscript. We would also like to thank participants of the Global Lakes Ecological Observatory Network (GLEON) 19 All Hands' Meeting for their initial input and recommendations; partial support for the GLEON 19 meeting was provided by NSF grant EF-1137327. GLEON provided the platform to further develop our ideas and continue the work of the Ecology under Lake Ice working group (NSF DEB 1431428). This project was partially supported by the Great Lakes Fishery Commission with the assistance of Senator Patrick Leahy. J. Ellen Marsden and Ted Ozersky provided helpful comments and reviewed the completed manuscript prior to submission.

Conflict of Interest

None declared.

Submitted 14 September 2018 Revised 05 November 2018 Accepted 16 November 2018

Associate editor: John Smol