The influence of winter lake surface cover conditions on under-ice light regimes and primary productivity in small, hydrologically disconnected lentic systems

by

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We acknowledge with respect the Lekwungen peoples on whose traditional territory the university stands and the Songhees, Esquimalt and W̱SÁNEĆ peoples whose historical relationships with the land continue to this day.

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Abstract

As the Earth undergoes continued climatic change, shifts in the cryosphere are occurring at increasingly rapid rates. As a significant proportion of global freshwater is located in the mid- to high-latitudes of the northern hemisphere and is often seasonally ice covered, it is vital to understand how surface cover quality influences biological activity in these systems. Ice and surface cover conditions have been noted to be very effective at limiting light availability, and therefore influencing the photosynthetically active radiation (PAR) available for primary production. To quantify the relationship between winter surface cover and under-ice hydroecological variables, two complimentary controlled experiments were conducted over two winter periods at the University of Calgary Aquatic Experimental Facility. Above-ground polyethylene mesocosms were utilized to remove the influence of littoral and benthic activity influences on measured biological endpoints. Two paired experimental ponds, that are hydrologically disconnected and located immediately adjacent to the mesocosm enclosures were utilized to further develop the relationships, while including a slightly more complex food web structure. Surface cover manipulations were done by either adding snow (snow-on-ice), or slushing snow (white ice), and resulted in distinct differences in under-ice light regimes, dissolved oxygen (DO) levels, but not primary production measured as chlorophyll-α values. However, in the pond systems, surface cover had minimal impact on the DO levels, with both the control and treatment systems trending towards hypoxic conditions quickly after ice-on conditions, as measured using high temporal resolution probes embedded in the ponds. Chlorophyll-α levels in the pond systems, however, was significantly different with the snow-on-ice (control) pond

having lower values than the pond where snow was mechanically removed, over two adjacent winter observation periods. In the second observation period, dissolved organic carbon (DOC) values were also manipulated in the mesocosm systems, with the elevated DOC systems exhibiting a decrease in DO values when compared to control systems. The utilization of controlled experimental systems and high-resolution data loggers allowed this study to offer unique insights into the relationship between ice and surface cover composition, under-ice light regimes, and corresponding biological activity under-ice. As shifts in annual winter climatic and associated meteorological conditions are predicted to continue to occur, generally towards increased precipitation and warmer mean air temperatures, the relationships derived in the study will be valuable in understanding and potentially predicting the implications of climate variability and change on seasonally ice-covered systems

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Dedication

This thesis is dedicated to all those who have helped my get to this stage in my life, but particularly to Maddie Bemrose who has been a rock throughout this graduate study experience, helping to ground me and remind me of the important things in life – dogs, friends, fresh air, and lively discussion.

"*I Like the cold weather. It means you get work done.*"

- Noam Chomsky

1. Introduction

Many freshwater systems located in northern temperate experience seasonal ice cover of varying duration and quantity. Changes in hydroclimatic regimes are already resulting in decreased ice cover duration (Magnuson et al. 2000; Derksen et al. 2012; Benson et al. 2012; IPCC 2019) which is expected to continue under projected climate change (Brown and Duguay 2011; Shuter et al. 2013; Sharma et al. 2019). With later freeze-up and earlier break-up, the overall duration of ice cover is decreasing. On average, the freeze-up date has occurred 5.8 days per 100 years later and break-up has occurred 6.5 days per 100 years earlier, leading to a net decrease in total ice-covered period (Magnuson et al. 2000; Bonsal et al. 2006; Magee ad Wu 2017; Sharma et al. 2022). These shifts in ice cover duration are expected to have effects on lake stratification, overall temperatures, and biological activity (Prowse et al. 2006a; Wrona et al. 2016).

In addition to shifts in ice cover duration, it is expected that ice quality (phenology) and quantity will change based on the increased variability of hydroclimatic conditions (Dibike et al. 2011). With significant rates of warming in the Arctic (Serreze and Barry 2011) and the sensitivity of northern freshwater systems to even small changes in climate (Prowse et al. 2006b), understanding the hydroecology of the region is crucial. If winter precipitation increases, as has occurred over the last century (Smol and Douglas 2007; Krasting et al. 2013; Cooper 2014; Lopez et al. 2019), it can be expected that there will be a resulting change in ice cover characteristics. This would include a potential increase in thickness and the increase in highly reflective white-ice (Dibike et al. 2012). Consequently, this will impact the radiation budgets within ice covered lakes,

with the greatest impact being the loss of photosynthetically active radiation (PAR) availability (Prowse and Stephenson 1986). Decreases in PAR availability and the consequent loss of productivity in ice covered months may lead to significant losses of biological activity.

With the majority of limnological studies taking place during open-water seasons, the importance of the winter season has been historically under-acknowledged (Salonen et al. 2009; Powers and Hampton 2016). Recent studies of cold-regions lakes have highlighted the need for an improved mechanistic understanding of the relationship between winter surface cover and under-ice biological activity (Özkundakci et al. 2016; Hampton et al. 2017; Pernica et al. 2017). Though the linkage between the physical properties of surface cover and under-ice biological activity was made by Prowse and Stephenson (1986), little work has been done to connect them since. Additionally, the connection between dissolved organic carbon (DOC) and under-ice light conditions were not considered by previous researchers and has yet to be quantified. This project aims to further develop and quantify the relationship between surface cover conditions and under-ice biological activity through a combination of in-situ and controlled experiments. To obtain a more wholistic understanding of annual trends in productivity in seasonally ice-covered freshwater ponds, with changing hydroclimatic conditions, a concerted effort must be made to quantify the relationships between physical ice cover properties and under-ice biological activity.

Across the northern mid-latitudes, the Southern Arctic, and the Canadian prairies, shallow freshwater systems are prevalent and in many instances are the dominant freshwater body (Vincent and Laybourn-Parry 2008; Rautio et al. 2011; Wrona et al.

2016). Specifically, the prairie pothole region of Canada is dominated by shallow, hydrologically disconnected pond systems (Haque et al. 2018) that are serve as crucial ecosystems for a variety of waterfowl, vegetation, and terrestrial organisms (Johnson et al. 2005). These shallow systems, which are predominantly filled by overland flow and characterized by their 'fill-and-spill' hydrology, are expected to be disproportionately impacted by climate change due to warming summer air temperatures and shifting annual precipitation regimes (Liu and Schwartz 2012; Niemuth et al. 2014). In winter, these systems freeze rapidly, and have limited to no inflow, generally resulting in a substantial ice cover and are extremely susceptible to surface cover changes as a result of snow accumulation and white ice accumulation as a result of freeze-thaw cycles (Duguay et al. 2003). Given their dominance in the landscape, unique hydrological conditions, and importance for ecosystem health, having a mechanistic understanding of winter processes in these systems is important to assessing the impact of shifting hydroclimatic regimes.

1.1 Literature review

1.1.1 Changing cryospheric conditions

The world is currently experiencing a shift in climate that is unprecedented in recent history (Bates et al. 2008; AMAP 2017; IPCC 2019). As a result, the importance of freshwater resources is becoming increasingly apparent, especially in the northern mid-latitudes and the Arctic, where rates of change are highest (Rouse et al. 1997; Arnell 1999; Schindler and Smol 2006; Kundzewicz et al. 2008; Previdi et al. 2021). These shifts will have very regional and specific implications on the extent, phenology and

volume of freshwater ice (Duguay et al. 2006; Brown and Duguay 2011; Dibike et al. 2012; Surdu et al. 2015).

Northern freshwater systems are considered to be at the highest risk under changing hydroclimatic conditions, due to their sensitivity to small changes in environmental variables including altered snow and ice cover regimes (Wrona et al. 2005, 2006; Smol et al. 2005; Dokulil 2016). Though a mechanistic understanding of the connection between alterations in ice cover quality, quantity and duration and the hydro-ecological processes of cold-regions aquatic systems has been identified as a missing link in the climate change discussion (Salonen et al. 2009; Özkundakci et al. 2016; Pernica et al. 2017; Powers et al. 2017a; Jansen et al. 2021), few attempts have been made to address it. This project aims to fill said research gap through the use of controlled and *in-situ* experiments, addressing the themes discussed below.

1.1.2 Hydrology of shallow pond systems in the Canadian prairies

The southern areas of the Canadian prairies are dominated by shallow, hydrologically disconnected, and seasonally ice-covered ponds, or ‘potholes’. These potholes were formed during the recession of glaciers during the Late Pleistocene era and are dominated by ‘fill-and-spill’ hydrology where overland flow is the main mechanism for hydrologic input (Shaw et al. 2013). These systems are used extensively by waterfowl for nesting and reproduction (Niemuth et al. 2014) and by terrestrial organisms, including livestock (Forcey et al. 2007). Due to their ecological significance and the historical drainage for agricultural purposes, there are extensive efforts by governments, farmers, and environmental organizations to return these systems to the region through restoration efforts (Puchniak Begley et al. 2012). These pothole ponds,

also sometimes referred to as wetlands, are frequently rich in macrophytes, including cattails (genus: *Typha*), which are important sources of nutrients during decomposition (Galatowitsch and van der Valk 1996). They also are shallow enough to be considered entirely littoral, with sunlight and associated PAR reaching the bottom of the depressions unless filtered/removed by surface cover or water conditions.

A defining feature of these pothole lakes is their lack of substantial inflow or outflow, aside from overland flow. Though shallow groundwater storage can provide inflows during summer months, the predominance of clay-rich tills beneath many prairie pothole systems means that deep groundwater inputs are severely limited (Hayashi et al. 2016). Additionally, the limited surface inflow can result in substantial variability in water levels, particularly during summer/warm months where evaporation is high, resulting in the concentration of ions/nutrients in decreasing water volumes. Correspondingly, during the winter period, when sub-zero temperatures and ice/snow cover dominate the landscape, these systems become effectively closed systems from a hydrological perspective. The addition of ice cover effectively acts as a cap on the aquatic system, limiting nutrient, light, and oxygen inputs to the system (Baird et al. 1987). As a result, these systems tend to be rich in organic matter and reach hypoxic conditions very rapidly after the onset of ice cover (Barica and Mathias 1979; Baird et al. 1987). The rapid response of these systems especially important when considering their responses to hydroclimatic variability and the projected change in ice and snow conditions during the winter months (Prowse and Brown 2010).

1.1.3 Influence of winter surface cover and ice properties on under-ice light regimes.

Ice cover as a physical control of under-ice light regimes has been identified as a key component to winter productivity in seasonally ice-covered lentic systems (Pernica et al. 2017). However, to date, attempts to quantify this connection have been data-sparse or done exclusively on large lakes with high spatial variability (Pernica et al. 2017). Seminal work by Prowse and Stephenson (1986) highlighted three primary winter lake covers: 1) black ice, which is highly transparent, 2) white ice, which is highly opaque and 3) snow-on-ice which is highly reflective. Each of the aforementioned lake covers form under different environmental conditions controlled by factors such as air temperature, precipitation and the rate of air temperature fluctuations (i.e., Freeze-thaw) (Prowse and Stephenson 1986).

Each surface cover mentioned has unique albedos and radiation permeability (also referred to as transmittance). The factors controlling the optical transmissiveness of lake cover, are the density, free water content and grain size characteristics (Prowse and Stephenson 1986). Of particular interest for biological activity is the transmission of PAR – wavelengths between 400-700 nm (Williamson et al. 1996). This is due to the fact that many primary producers require light in this spectrum to survive and thrive, particularly in cold region systems, where light availability is highly seasonal, and a good portion of the year is spent ice-covered (Jungblut et al. 2010).

Although snow and ice characteristics will influence the wavelengths of light that enter the water column, the largest effect comes from the limiting of light quality by covers that have high reflectance (Michel and Ramseier 1971; Grenfell and Maykut 1977; Warren 1982). The highest albedo occurs in freshly fallen snow, which can have

an upper albedo value of 0.95 (Warren 1982; Prowse and Stephenson 1986). Albedo values of white ice and black ice are less agreed upon but can range from 0.77-0.3 respectively (Prowse and Stephenson 1986; Saloranta 2000; Dibike et al. 2011). Similarly, the ice thickness plays a significant role in the attenuation of radiation, with greater thickness correlating to greater attenuation (Barica and Mathias 1979; Mathias and Barica 1980; Saloranta 2000). Seasonal variations in ice type and resulting PAR values have been reported for Lake Bonney, a permanently ice-covered lake in Antarctica (Fritsen and Priscu 1999), however similar studies have yet to be completed in the seasonally ice-free lakes of the Canadian Arctic or within the northern midlatitudes.

1.1.4 The impact of winter ice regimes on under-ice productivity and biogeochemistry

As alluded to in previous sections, the extent, timing, characteristics, and quantity of lake-ice is undergoing change in the Canadian Arctic. As an ecosystem driver, primary productivity is key to the health of any system (Rapport et al. 1998). Changes to primary productivity levels can have cascading effects throughout an ecosystem, even resulting in a significant decrease in system health (Flanagan et al., 2003). With projected increases to ice free periods, there is an expectation that cold-region freshwater systems will become more productive in the summer months (Rouse et al. 1997). Although being tough to project, it is expected that increases in lake water temperature leads to increases in photosynthesis rates of planktonic organisms (Wrona et al. 2006). If projected increases in nutrient loading are, in fact, correct, this combined with increased temperatures may lead to a significant net productivity in these regions (Wrona et al. 2005).

The rates of primary productivity are tightly linked with oxygen levels in lakes with frozen ice or snow cover (Barica and Mathias 1979; Babin and Prepas 1985; Prowse and Stephenson 1986; Terzhevik et al. 2009; Fang and Stefan 2009). With ice acting as a semi-permeable barrier between the atmosphere and the underlying water column, ice-covered lakes can be viewed, very simplistically, as closed systems. There is a relatively small amount of oxygen movement from within the ice cover and from the overlying atmosphere; however, this amount is negligible (Prowse and Stephenson 1986). The available oxygen within the water column can be represented by the following equation, modified from Prowse and Stephenson (1986; equation 2):

$$DO = (C + P + Q + F) - (B + R + L)$$

Where: DO = Total Dissolved Oxygen content
C = Initial DO content
P = Production of O_2 from photosynthesis
F = Freeze-out of O_2 during ice formation
Q = Contributing DO from inflowing water
B = Benthic organism respiration
R = Water column respiration
L = Loss of oxygen in outflowing water

On the losses side, the benthic organism respiration term dominates with water column respiration occurring at relatively low rates (Schindler et al. 1974). Of particular interest, however, is the relatively understudied component of water column oxygen production through photosynthesis (*P*). It is expected that this term may fluctuate significantly based on the composition of lake cover (Prowse and Stephenson 1986). Some studies have shown lakes with ice that is snow covered, rates of primary production in the water column are minimal (Schindler 1971; Fang and Stefan 2009)

whereas other studies have shown a significant amount of total annual photosynthetic activity occurs during ice-on periods (Wright 1964; Lougheed et al. 2011). Currently there is no consensus from within the scientific community regarding the importance of the role of snow and ice cover on the photosynthetic productivity in ice-covered lakes.

In addition to DO trends, the controlled experiments will allow for a more robust understanding of how under-ice biogeochemistry is affected by surface cover conditions. This includes trends in nitrogen and phosphorus, which have recently been shown to be elevated in winter when compared to summer (Hampton et al. 2017). Furthermore, the influence of surface cover on parameters such as salinity and conductivity have yet to be examined.

As northern regions continue to experience changes in hydroclimatic conditions, the delivery of terrestrial sources of carbon to freshwater systems are increasing (Zhang et al. 2010; Stanley et al. 2012; Lennon et al. 2013). This is particularly true for shallow pond systems in the Arctic and northern mid-latitudes where permafrost slumping and changes in hydroclimatic conditions are resulting in an increased delivery of allochthonous DOC (Thompson et al. 2012; Diodato et al. 2016). Increased DOC delivery will directly affect water column light transmission through ‘browning’ of water, due to the coloured fractions such as humic acids (Snucins and Gunn 2000; Strock et al. 2017). Additionally, the darkening of the water column has been found to reduce the epilimnion depth due to increased surface water temperatures (Williamson et al. 2015; Strock et al. 2017). The addition of terrestrially-derived dissolved organic matter (DOM) is currently outpacing the increase in autochthonous DOM and is of lower nutritional quality for primary producers (Creed et al. 2018). In northern cold-region systems,

Godwin et al. (2014) determined that DOC was the controlling driver of benthic productivity, explaining 86% of variability in periphyton production, rather than phosphorus. Similarly, Ask et al. (2009) found DOC to be a driver of both benthic and pelagic productivity in northern Sweden.

Experimental approaches are key to better understanding the causal links between DOC, light, and productivity (Lennon et al. 2013). Mesocosm approaches allow for highly controlled manipulations and the removal of complicating factors such as benthic organisms. Additionally, they have been found to be valuable in undertaking small-scale DOC experiments (Klug 2005; Lennon and Cottingham 2008) due to the difficulty in whole-system manipulations (Lennon et al. 2013). To address the challenge of upscaling manipulative DOC experiments, Lennon et al. (2013), posit the use of agricultural humic solutions (i.e.., SuperHume) as an efficient method of enrichment; though with the caveat the SuperHume does have slightly different optical properties than DOC introduced from terrestrial sources.

To simplify and standardize the method of measuring colour, and by extension coloured DOC, Cuthbert and del Giorgio (1992) propose the use of a spectrophotometer measuring light absorbance at 440 nm; a method adopted by numerous studies since (Belzile et al. 2001; Klug 2005; Karlsson et al. 2009; Thompson et al. 2012; Lennon et al. 2013; Torremorell et al. 2015; Zwart et al. 2016). The method has been shown to be robust, and localized relationships to DOC have proven to be strong (Lennon et al. 2013) though they may not necessarily comparable inter-annually due to variability in DOC sources.

1.1.5 Current work and modeling

While under-ice limnology has only recently become increasingly acknowledged as an important and understudied are of freshwater science (Powers and Hampton 2016), some efforts have been made to model both physical lake ice quality evolution (i.e., MyLake, Dibike et al. 2012) and biological composition (Phytoplankton Ecology Group, Sommer et al. 2012). There are multiple reasons as to why researchers have historically omitted seasonal ice cover from their lake modeling efforts. They include: 1) a paucity of data from the ice-on period (Hampton et al. 2017) resulting from, 2) the difficulty of obtaining data in the winter due to sampling challenges (Block et al. 2018), and 3) the perception that under-ice biological activity is limited and viewed as a 'dormant' during ice-on periods (Hampton et al. 2015).

The objective of this research was to quantify the mechanistic relationships between the physical control of ice cover, and simple water quality and biological endpoints, specifically dissolved oxygen (DO) and pelagic primary production measured as chlorophyll-α. These endpoints were utilized due to their ease of sample collection and the ability to continuously monitor in some instances. Additionally, though DO is a chemical and water quality endpoint, it is substantially influenced by biological activity and is an important environmental variable, when considering food web function. By utilizing controlled experiments, both in mesocosm environments and hydrologically disconnected ponds, the intention is to create a quantitative relationship between ice cover quality and under-ice biological activity. These relationships could then be built into ecological models, including the aforementioned MyLake (Saloranta and Andersen 2007). This novel approach allows for greater control of influencing factors, such as

hydrologic inputs, surface cover composition, and ice cover depth. This type of controlled experimental approach is common in ecological studies (Beisner et al. 1997) and biogeochemical studies (Flanagan et al. 2006) and have been completed at the University of Calgary Aquatic Experimental Facility for over 25 years.

1.2 Objectives and hypotheses

The broad objective of this research is to quantify how changes in ice cover quality and composition affects under-ice biological and geochemical responses. Using a series of integrated, manipulative studies, the research is designed to improve the ability to predict how changes in future climate, and by extension surface ice cover conditions, influences water quality and related basal food web structure and function in cold regions lacustrine ecosystems. Furthermore, the outcomes of the research will contribute to advancing cold regions lake modeling efforts aimed at improving the ability to predict how changes in physical and chemical controlling factors in seasonally ice-covered lakes will affect autotrophic productivity both under-ice and during the following ice-free period.

Three primary hypotheses were tested, and are the underpinnings of the chapters in the thesis:

1. The presence of snow on ice is negatively correlated with under-ice PAR availability when compared to black, translucent ice.
2. A shift towards white ice or snow-on-ice conditions will result in decreased primary production as a result of PAR limitation, resulting in decreased dissolved oxygen and pelagic chlorophyll-α levels.

3. The addition of DOC will result in decreased PAR availability and will provide increase nutrients for heterotrophic activity resulting in a net increase in respiration rates and a corresponding decrease in dissolved oxygen levels.

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2 Methods

Controlled experiments utilizing two experimental ponds alongside multiple 700L polyethylene above-ground mesocosms were undertaken at the Aquatic Experimental Facility, located on the University of Calgary campus, in Alberta, Canada (51°N, 114°W). The facilities had no public access and were used only for research and teaching activities, limiting anthropogenic interference in the experiments. Wildlife, in the form of deer, coyotes, and hares were common in the facility. The experiments occurred over two consecutive field campaigns spanning October-April over the 2015-2016 and 2016-2017 winter seasons.

The paired experimental ponds had surface areas of approximately 185 m^2 and were hydrologically disconnected. The maximum depth was approximately 2m, with the bathymetry being a simple bowl shape (Figure 2.1). The ponds had abundant macrophytes within the system and the semi-aquatic plant *Typha* (commonly known as cattail or bulrush) were dominant in the littoral zone (Figure 2.2). As the systems were hydrologically disconnected, water was added through the University of Calgary irrigation system in the first field season (2015/16). This water was added in the springtime, to allow time to equilibrate prior to the initiation of the experiment. The source water for the irrigation system is supplied from the Bow River and is not manipulated aside from temperature controlling; no chemical treatment was done.

The pond systems are reflective of the dominant prairie pothole type system in the Southern regions of the Canadian Prairies. They are shallow systems that freeze rapidly once air temperatures drop below 0°C. Ice onset results in freezing to the bottom of the shallower littoral zones, and maximum ice thickness can be more than 1/3 of the

maximum depth (Figure 2.1). Due to the presence of *Typha* there is a substantial amount of decaying vegetation that enters the water column as well as gets frozen into ice during formation. The small size overall size of the ponds also results in a relatively uniform ice quality, and limited snow redistribution due to wind. The ponds are not expected to be representative of larger ice-covered lacustrine systems due to their unique hydrology and physical characteristics, and thus upscaling of the results found in the following studies should only be done with careful consideration regarding the unique hydrological conditions of these systems.

Previous studies conducted at the facility suggest that the food webs within the experimental ponds are simple but well-established due, in part, to the age of the ponds which were established in the late 1980's. DOC values were 8.9 and 9.2 mg/L (n=1) in the paired ponds at the beginning of the experiments when taken via a single grab sample. The Bow River source water, which was added to the ponds to account for losses from summer evaporation, is low in DOC, with a mean value of <1 mg/L between 1989-2018 (Alberta Environment and Parks, Bow River at Cochrane station AB05BH0010). As such, it is not expected to influence the overall pond DOC levels significantly over the study periods.

The above ground mesocosms (700 L polyethylene tanks) were located within large greenhouses immediately adjacent to the experimental ponds. Prior to use, the tanks were mechanically cleaned using a large scrubbing brush along with irrigation supply water for rinsing and then left to dry prior to filling. Filling was done by utilizing a pump drawing water from the experimental ponds. The water was passed through a 60 µm

mesh filter to remove invertebrate predators and large zooplankton, as per (Beisner et al. 1996).

To prevent the mesocosms from freezing entirely to the bottom, and; a) damaging monitoring equipment, and b) affecting the biological community composition, low-voltage heating bands were wrapped around each tank, approximately 10 cm from the bottom. Additionally, during periods of prolonged cold temperatures (i.e.., cooler than -15 °C), an electric heater was used to help prevent complete freeze-up. A PAR pyranometer was also installed in the upper part of the greenhouse to allow measure attenuation of light through the plastic sheeting of the greenhouse.

Details of the manipulations and analyses are separated out by project component below. Additional details can be found in the respective chapters/papers.

2.1 Ice cover as a driver of under-ice light regimes and biogeochemistry

This experiment was conducted in both the 2015/16 and 2017 field seasons and utilized both the controlled mesocosms and experimental ponds. Manipulation of surface covers in both environments were undertaken alongside frequent under-ice PAR measurements.

2.1.1 Mesocosm experiments

The mesocosm component of the experiment included triplicate replication of surface cover, though sampling was not conducted on all replicates at each sample collection period. Not all replicates were sampled at each period to minimize disturbance on light measurements and YSI multisonde measurements as the sampling of under-ice water slightly disturbed the surface cover conditions. Two surface cover

condition treatments were applied in an attempt to replicate the natural formation of white ice, and snow-on-ice, with three replicates for each treatment. Additionally, a control (no manipulation) triplicate was utilized.

In the first field campaign, prior to initial freeze-up, underwater PAR pyranometers were attached to a plastic arm which was attached to a 2 x 4 crossbeam placed across the mesocosm. The PAR sensors were placed in a single replicate of each treatment and were logged using an LI-1400 datalogging unit. Measurements were taken hourly throughout the experimental period. In addition to light sensors, three multi-sondes (YSI 6600 V2) were deployed with temperature, conductivity, pH, blue-green algae, and turbidity sensors onboard. These were also set to log hourly in tandem with light measurements.

Following Besiner et al (1996), surface cover manipulation of the mesocosms occurred after a two-week period allowing for stabilization of the abiotic and biotic conditions. To simulate white ice, approximately 0.05 m^3 of fresh snow from within the ecoreserve area was added to the surface ice, and then mechanically mixed with approximately 1 L of de-ionized water until a slush was created. The snow-on-ice treatment was accomplished by adding approximately 0.1 m^3 (or approx. 5 cm depth) fresh snow and distributing it evenly across the surface. The control mesocosms received no manipulation. These manipulations occurred every two-weeks for the duration of the experiment during both field seasons.

Water samples for biogeochemical analyses were collected through a borehole created using a 5-foot auger drill bit. A triple DI-rinsed small gauge tube was then inserted into the borehole through the ice to the underlying liquid. The required volumes

of sample were then siphoned using the tubing, and all collection bottles, where possible, were triple rinsed with sample prior to collection. Biogeochemical analyses were conducted through an analytical laboratory (Maxxam Analytical) for a comprehensive set of parameters. Sample collection followed the required procedures required by the analytical laboratory, including delivery of the sample within a 12-hour window after collection.

2.1.2 Experimental ponds

The paired experimental ponds were also used to assess the influence of surface cover variability on under-ice light regimes and biogeochemistry. One pond was kept as a control, with snow allowed to accumulate throughout the field season, while the other was mechanically cleared of snow. Snow removal occurred within a 48-hour period from when the snow began to accumulate once the ice reached a thickness that was safe for foot travel. Mechanical removal was accomplished using a combination of an electric leaf blower and a traditional snow shovel. The intention of the mechanical removal of snow was to create black ice conditions; however, that approach had mixed results given the ambient weather conditions in the first field season.

Access to open water under-ice was gained using a SIPRE-style (Hughes and Terasmae 1963) ice-corer with a battery-operated drive unit. The titanium shaft extracted 5-inch diameter cores and created an opening of approximately 6 inches in diameter. Once the first borehole was completed, grab samples were obtained at approximately 60 cm from the top of surface cover, prior to any other sampling being conducted, to limit turbulent mixing. Grab samples were obtained using standard methods (CCME 2011) and stored in coolers to limit temperature fluctuations and the

influence of light. Where applicable, samples were delivered, alongside those collected from mesocosms, to an analytical lab for biogeochemical assessment. Once the grab samples were obtained, a calibrated multisonde (YSI 6600 V2) was attached to a constructed winch system and a depth profile was obtained.

Above-ice incoming PAR measurements were taken using a planar underwater-capable PAR meter (Li-Cor LI-190R), handheld at shoulder height. Both incoming and reflected PAR were measured and recorded. Upon completion of the multisonde profile, a second borehole was augered, immediately adjacent to the initial one. This allowed for the light meter to be attached to an underwater lowering frame and placed in the water. Two measurements were made using the handheld meter readout, 1) at the surface of the water (immersed), and 2) immediately under-ice. Measurements of ice depth and snow depth (control pond) were taken and recorded at this time as well. Samples were collected at a 2-week interval from the ponds.

2.1.3 Statistical analysis

The experimental design included triplicate replication of surface cover conditions in the mesocosms. This was done, in part, to provide comparable conditions for the installation of continuously logging light meters in one mesocosm, a continuously monitoring multisonde in a second mesocosm, and sub-surface water sampling occurring in a third mesocosm. This prevented the disturbance of ice cover conditions that occurs during the drilling process associated with obtaining under-ice water samples. This provided a relatively undisturbed surface cover for the light and multisondes (water quality) measurements, while concentrating disturbance related to drilling to a single mesocosm. The temporal analysis component was altered to reflect

early winter (before January 21) and late winter (after January 21) to reflect the sampling intervals and observed winter conditions over the sample periods. This was also reflective of the other components of the project that included mesocosm analyses.

Analysis of under-ice light variability was assessed utilizing an ANOVA, using the R statistical package (R Core Team 2019). A Tukey's multiple means test was also conducted for the light values. A Students' t-test was also used to compare difference between ponds, after testing for normality using the Shapiro-Wilks test. All graphical outputs were made using the 'ggplot' R package.

2.2 The impact of winter light regimes on under-ice primary productivity

To examine the connection between surface cover conditions, and their corresponding effects on light regimes and primary productivity, the previously mentioned experimental ponds were used in conjunction with the above-ground mesocosms. A description of the experimental ponds, located within the University of Calgary Aquatic Experimental Facility is included in the section previous to this one. The experimental set-up was congruent with that previously described, as the experiment was run alongside that outlined in the previous section.

To quantify primary production, a combination of dissolved oxygen and pelagic chlorophyll-α concentrations were used as response endpoints. To quantify DO, a HOBO brand dissolved oxygen sensor (U26) was immersed in each mesocosm environment via cable to an overhead support beam. The sensors were positioned 10 cm from the bottom, to prevent them from freezing into the ice. In the ponds, additional sensors were placed at depths of 10 cm, and 50 cm from the bottom of the pond. In the 2015/16 field campaign, an additional sensor was deployed at a depth of 100 cm from

the bottom of the experimental ponds. The sensors logged values every 30 minutes throughout the experimental period. A YSI multi-sonde (6600-V4) was also deployed in 3 mesocosms (one of each surface cover), using a support cable and overhead beam. These multisondes recorded numerous parameters of interest, including dissolved oxygen and blue-green algae (phycocyanin) every 60-minutes. Probes on the sonde that utilized optical sensors were self-cleaning via a mechanical wiping action and equilibrated for approximately 1-minute prior to recording a value.

Three Li-Cor branded spherical light sensors (LI-193) were deployed in the mesocosms; one in each surface cover condition. Surface cover manipulation treatments were initiated after 10 mm of ice cover formed on the surface of the tubs.

In the 2016/17 field season, dissolved organic carbon total concentration was also manipulated in the mesocosm environments to determine its effect on a) light transmission, and b) primary production. The manipulations were done via the addition of a humic acid fertilizer, Black Earth Organo Hume ULTRA (http://www.hortimax.biz/BlackEarth/BlackEarth-Organo_Hume_ULTRA.pdf). DOC was added to half of the treatment mesocosms so as to have both elevated and control. Elevated DOC treatments had approximately 100 ml of humic acid fertilizer added to them prior to freeze-up. This amount was based on a target DOC value of 20 mg/L following Curtis and Adamas (1995) and Lennon et al. (2013). This resulted in a total of 6 treatment conditions, with 3 replicates each (Table 2.1).

2.3 Dissolved organic carbon and surface cover composition as drivers of under-ice productivity regimes

This component of the project utilized the experimental mesocosms exclusively. The previous experimental set-up from the 2015/16 experiment was used, with supply water being sourced from the adjacent experimental ponds in the University of Calgary Aquatic Experimental Facility. The mean DOC level in the experimental ponds during the 2015/16 field season was 12.2 ± 2.3 mg/L, though the irrigation-supplied water, was sourced from the Bow River, which has low DOC levels (<1 mg/L).

To create an environment with elevated DOC levels, a commercially available humic acid fertilizer was used (Organo Hume ULTRA; http://www.hortimax.biz/BlackEarth/BlackEarth-Organo_Hume_ULTRA.pdf). An elevated DOC condition was applied to 9 of the 18 experimental mesocosms, so there were elevated and control DOC conditions for each of the surface cover manipulation conditions. The humic acid addition is comprised of 24% humic and fulvic acids and also contains 3.7 g/L of suspended solids (manufacturer specified). The DOC enrichment was undertaken prior to freeze-up, but within 2 weeks of ice onset. Mechanical mixing was undertaken after the addition occurred, to ensure equal distribution of the substance, though some rapid settling of the suspended solids was observed rapidly after addition.

Samples were obtained using the previously described auger drill bit method, followed by syphoning. Samples to be analyzed for chlorophyll-α were collected in opaque 1 L Nalgene brand bottles and were transported back to the lab for filtration within 8 hours of sample collection. A subsample from the 1 L Nalgene bottles was

processed through a spectrophotometer to determine light absorbance at the 440 nm wavelength (Cuthbert and del Giorgio 1992; Lennon et al. 2013). These absorbance values were then compared to samples processed for DOC at an analytical laboratory (Maxxam Analytical, Calgary), to create a relationship between absorbance at 440 nm (a440) and DOC concentrations. Once established, a DOC value was calculated for each sample throughout the experimental period.

Processing of chlorophyll-α samples occurred at the aquatic ecology lab at the University of Calgary. A standard acetone-extraction method (Arar and Collins 1997) was utilized. All samples were vacuum filtered through GF/C membrane filter with 24-hours of collection, after which the filter papers were wrapped in foil and stored in a -20 °C freezer until processing could occur. Mechanical homogenization of the filters along with acetone occurred, prior to centrifugation. The samples were then processed through a calibrated fluorometer (Barnstead Turner Quantech). Additionally, samples were also processed for TSS and AFDM using standard procedures (Franson et al. 1998).

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2.5 Figures and tables

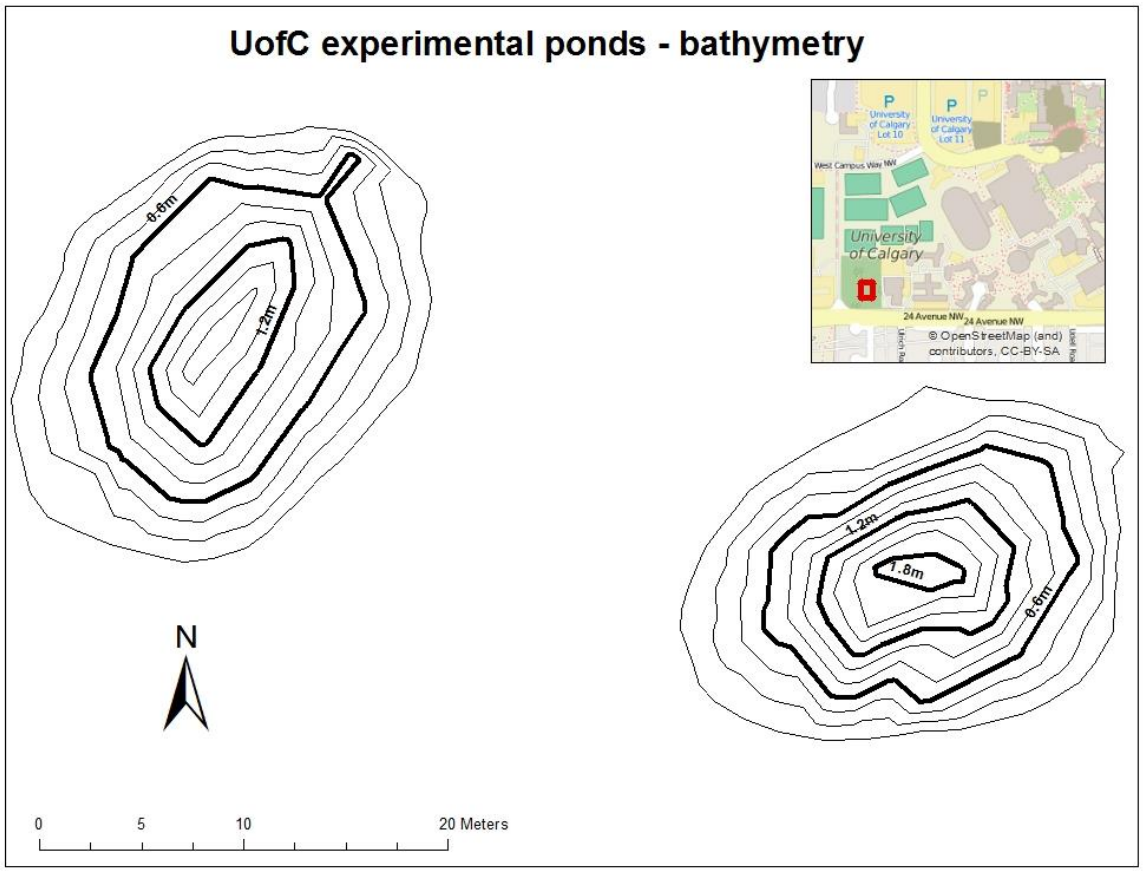

Figure 2.1 Experimental ponds bathymetry, Aquatic Experimental Facility University of Calgary, Alberta, Canada. Completed in fall 2015.

Figure 2.2 Fall view of the second experimental pond, showing the presence of extensive Typha growth around the littoral zone

Table 2.1 Experimental mesocosm treatment key for 2016-17 experimental season where DOC was manipulated (Control vs High) alongside surface cover conditions (Control/no manipulation, white/slushed ice, and the addition of snow on ice).

	Control surface cover (No manipulation; CS)	White-ice (W)	Snow-on-ice (S)
Control-DOC (No DOC added; CD)	CD-CS	CD-W	CD-S
High DOC (HD)	HD-CS	HD-W	HD-S

3 The influence of surface cover and ice properties on under-ice radiation regimes and biogeochemistry in mid-latitude, seasonally ice-covered ponds and controlled mesocosm environments

3.1 Abstract

Seasonally ice-covered lake systems dominate much of mid-latitude North America, with ice duration comprising at least 4 months of the year. In spite of this, the winter period is often viewed as ecologically insignificant, due to light limitation and cold temperatures. To evaluate the impact of surface cover condition, 9 experimental mesocosms and two small experimental ponds were utilized over two consecutive winter seasons (2015/16 and 2016/17). Within the mesocosm environments, controlled experiments were done in which surface cover was manipulated to include white (opaque), black (translucent), and snow-on-ice conditions. Correspondingly, the ponds were either left untouched, or had snow removed from the surface. Under-ice light measurements found that the extinction coefficients of the surface covers were 1.66 ± 0.22, 9.99 ± 4.06, and 20.98 ± 16.87 for black ice, white ice, and snow-on-ice respectively. Under-ice light sensors recorded higher light transmission through white ice on mesocosms, by 18.4% when compared to snow-on-ice conditions, though this varied over the sample periods, with the greatest difference occurring in late-winter. No significant differences were found in measured biogeochemical parameters in the mesocosm experiments. In contrast, significant differences in dissolved chloride, iron, sodium and sulphur were found between the treatment and control pond systems, though mean values were very low. Vertical profiles of conductivity and salinity also differed significantly between snow-removed and control ponds. This study further advances our understanding of how alterations in surface cover quality affects under-ice light regimes and related biogeochemistry in response to changing hydroclimatic conditions on mid-latitude, seasonally ice-covered lacustrine systems.

3.2 Introduction

The world is currently experiencing a shift in climate that is unprecedented in recent history (Bates et al. 2008; AMAP 2017; IPCC 2019). As a result, the importance of freshwater resources is becoming increasingly apparent, especially in the northern mid-latitudes and the Arctic, where rates of change are highest (Rouse et al. 1997; Arnell 1999; Schindler and Smol 2006; Kundzewicz et al. 2008; Prowse et al. 2015 Wrona et al. 2016). These shifts will have very regional and specific implications on the extent, phenology and volume of freshwater ice (Duguay et al. 2006; Brown and Duguay 2011; Dibike et al. 2012; Surdu et al. 2015).

Northern freshwater systems are considered to be at the highest risk under changing hydroclimatic conditions, due to their sensitivity to small changes in environmental variables including altered snow and ice cover regimes (Wrona et al. 2005, 2006; Smol et al. 2005; Dokulil 2016). Though a mechanistic understanding of the connection between alterations in ice cover quality, quantity and duration and the hydro-ecological processes of cold-regions aquatic systems has been identified as a missing link in the climate change discussion (Salonen et al. 2009; Özkundakci et al. 2016; Pernica et al. 2017; Powers et al. 2017a), few attempts have been made to address it. Some work has been done to identify the role of ice cover on hydrodynamics and corresponding biogeochemistry, however the majority of it has been undertaken in large lake systems (Jansen et al 2021).

Ice cover as a physical control of under-ice light regimes has been identified as a key component to winter productivity in seasonally ice-covered lentic systems (Pernica et al. 2017). However, to date, attempts to quantify this connection have been data-

sparse or done exclusively on large lakes with high spatial variability (Pernica et al. 2017). Seminal work by Prowse and Stephenson (1986) highlighted three primary winter lake covers: 1) black ice, which is highly transparent, 2) white ice, which is highly opaque and 3) snow-on-ice which is highly reflective. Each of the aforementioned lake covers form under different environmental conditions controlled by factors such as air temperature, precipitation and the rate of air temperature fluctuations (i.e., Freeze-thaw) (Prowse and Stephenson 1986).

Each surface cover mentioned has unique albedos and radiation permeability (also referred to as transmittance; Jansen et al 2021). The factors controlling the optical transmissiveness of lake cover, are the density, free water content and grain size characteristics (Prowse and Stephenson 1986). Of particular interest for biological activity is the transmission of PAR – wavelengths between 400-700 nm (Williamson et al. 1996). This is due to the fact that many primary producers require light in this spectrum to survive and thrive, particularly in cold region systems, where light availability is highly seasonal, and a large portion of the year is spent ice-covered (Jungblut et al. 2010).

Although snow and ice characteristics will influence the wavelengths of light that enter the water column, the largest effect comes from the limiting of light quality by covers that have high reflectance (Michel and Ramseier 1971; Grenfell and Maykut 1977; Warren 1982). The highest albedo occurs in freshly fallen snow, which can have an albedo of 0.95 (Warren 1982; Prowse and Stephenson 1986). Albedo values of white ice and black ice are less agreed upon but can range from 0.77-0.3 respectively (Prowse and Stephenson 1986; Saloranta 2000; Dibike et al. 2011). Similarly, the ice

thickness plays a significant role in the attenuation of radiation, with greater thickness correlating to greater attenuation (Barica and Mathias 1979; Mathias and Barica 1980; Saloranta 2000). Seasonal variations in ice type and resulting PAR values have been reported for Lake Bonney, a permanently ice-covered lake in Antarctica (Fritsen and Priscu 1999), however similar studies have yet to be completed in the seasonally ice-free lakes of the Canadian Arctic or within the northern midlatitudes.

3.3 Methods

3.3.1 Experimental ponds

Experiments were conducted at the University of Calgary Aquatic Experimental Facility, Alberta, Canada over two winter seasons (2015/16 and 2016/17) and consisted of utilizing two experimental ponds, with a surface area of approximately 185 m^2 and a max depth of 2m, and nine 700L polyethylene mesocosm tanks. Source water for the experimental ponds came from the nearby Bow River, and the University irrigation system. Aside from modulating temperature, no additional processing of the source water is undertaken prior to entering the ponds systems. The supply water from the Bow River is low in DOC, with a mean value of <1 mg/L from 1989-2018 at the Alberta Environment and Parks, Bow River at Cochrane Long Term River Station (AB05BH0010). In both years of the study, additional water was added in early summer months, to replace volume lost to sublimation over the winter, and evaporation from the previous summer. This water was then allowed to stabilize over the summer months, prior to freeze-up in the fall. The pond systems are well established and were in place for approximately 35 years, prior to the experiments being conducted. While no fish are present, there are abundant members of the pelagic and benthic macroinvertebrate

food webs, including zooplankton, oligochaetes, Diptera larvae and Coleoptera. The ponds also frequently have small waterfowl present and occasionally used by coyotes and deer for drinking water.

The treatment condition of the experimental ponds included the mechanical removal of snow on one pond (within 48-hours of snow accumulation beginning), with the control pond accumulating natural snowfall. This contrasts with the mesocosm experiments where control systems had no manipulation, but due to being housed inside a greenhouse, did not accumulate snowcover. Due to frequent temperature changes (recurring above - 0°C air temperatures) and corresponding freeze-thaw cycles in the first sample period (2015/16), the manipulated pond was predominantly composed of white ice. In the second sampling season, air temperatures dropped more rapidly at the onset of fall and were more consistent, resulting in fewer freeze-thaw cycles and the formation of black ice (Figure 3.1).

To gain access to the under-ice environment, a SIPRE-style (Hughes and Terasmae 1963) ice-corer with a battery-operated drive unit was utilized. The corer obtained 5” diameter cores while providing access to the underlying water, thus allowing for grab sampling (not reported) and light measurements to be taken. Cores were removed from the titanium corer barrel and placed in a half-round PVC receptacle and the depth of white vs black ice was observed and recorded. They were then placed into a polyethylene bag and sealed, prior to being transported back to the laboratory and stored in a -20° C freezer. To limit spatial variability, cores were taken from the same area (within approximately 4 m^2 area) at each sample interval. Under-ice mixing and

slushing of ice surface was limited through careful extraction of the ice core and slow introduction of the under-ice light sampling equipment.

For both experimental ponds, PAR measurements were taken approximately every two weeks throughout the sampling period. Measurements were done using a Li-COR 192, underwater PAR sensor and were taken 1) at shoulder height (approximately 150 cm), measuring incoming radiation, 2) at shoulder height, measuring reflected radiation, 3) in water- at surface of ice, and 4) immediately under ice surface. From these measurements, albedo and ice transmittance were calculated.

A YSI multisonde (6600V2) was used to collect vertical profiles of temperature, conductivity, pH, and turbidity of the pond systems using a winch and the aforementioned auger bore-hole. Water column dissolved organic carbon concentration was quantified using a standardized grab sampling technique (CCME 2011) and processed at an analytical laboratory (Maxxam Analytical). Additionally, water chemistry analyses were conducted using the grab samples, by the analytical laboratory; this included conductivity, pH, dissolved inorganic carbon, total nitrogen, and total phosphorus (Figure 3.7).

3.3.2 Mesocosms

Mesocosms were housed inside a fully enclosed greenhouse immediately adjacent to one of the experimental ponds and were filled, in the early fall, with water drawn from said pond. Removal of invertebrate predator organisms was done via filtering the source water through a 60 µm mesh filter during filling of the mesocosms. This was done to limit top-down predation impacting the results, as per (Beisner et al. 1996) To prevent the freeze-up of the entire volume of water included in the mesocosm tank, low-voltage

heating cables were attached around the bottom 10cm of all containers. Once an initial ice cover was present on all nine replicates, surface cover manipulations were carried out to result in snow on ice conditions (3 replicates), white ice, via slushing (3 replicates), and no manipulation control (3 replicates). Though the surface cover manipulations did result in slightly differing ice depths over the season, the overall impact was negligible when compared to the overall ice depth. To maintain snow on ice conditions, approximately 0.1 m^3 of snow was added to each of the three manipulated mesocosms every two-weeks, resulting in a snow cover depth of approximately 5 centimeters. White ice conditions were created by mechanically mixing approximately 0.05 m^3 of fresh snow with approximately 1 L of de-ionized water. The resulting slush is distributed evenly across the frozen surface and allowed to re-freeze, resulting in white ice formation. In season one, spherical underwater PAR light sensors (LI-193) were installed via a custom designed mounting arm into one of each surface cover replicates and recorded at 30-minute intervals via a LI-1400 datalogger. Solar radiation was also measured inside the greenhouse to enable to calculation of light transmission. To access liquid water for sample collection during the experimental period, a 5-foot auger drill bit was used in conjunction with a hammer drill. Once through the ice, a clean length of small gauge tubing was inserted and liquid water was siphoned from immediately under the ice surface, as required, into sample collection bottles.

Under-ice turbidity values were assessed using a YSI 6600-v4 multiparameter sonde. Sondes were embedded in three of the mesocosms (one of each surface cover) in the initial sampling season and had a sample interval of one hour. Additionally, a

suite of similar water quality parameters was collected from the ponds and mesocosms (Figure 3.7).

In the second sampling period, following Lennon et al. (2013) and Cuthbert & del Giorgio (1992), a relationship was established between dissolved organic carbon measured via analytical chemistry and absorbance of light at the 440 nm wavelength, using a spectrophotometer. This allowed a relationship between DOC and colour to be established. All samples throughout the collection period were analyzed for colour and subsequently DOC values were calculated using the relationship. This relationship and the specific effects of DOC on primary productivity under different surface cover conditions are explored in greater detail in Chapter 3.

Analysis of under-ice light variability in mesocosms was assessed using an ANOVA, along with a Tukey's multiple means test in R 3.5.0 (R Core Team 2019). Boxplots and other statistical analyses were also completed in R with the 'ggplot' package. Albedo differences in ponds were statistically tested using a students' t-test after testing for normality using the Shapiro-Wilks test.

3.4 Results

3.4.1 Ponds

In the initial sampling period (2015-16), the experimental ponds had average albedos of 0.32 ± 0.12 and 0.61 ± 0.20 (n=12) for the treatment (snow-removed) and control (snow-on-ice) pond, respectively (Figure 3.2). Multiple freeze thaw cycles resulting in frequent crack formation and upwelling, resulting in the formation of white

ice on the pond where snow was removed. Light transmission through the manipulated and control ponds during the same period was 0.03 ± 0.02 and 0.002 ± 0.001 respectively (Figure 3.2). The second sampling period (2016-17) had more consistent sub-zero temperatures, resulting in the formation of black ice. During this period, the control pond (snow-on-ice) had an albedo of 0.74 ± 5.59, compared to 0.33 ± 0.05 for the treatment (snow-removed) pond. Transmission through pond surface covers during the same period was 0.04 ± 0.02 and 0.25 ± 0.06 for control and treatment ponds respectively Figure 3.3. Albedos between the mesocosms were assessed and found to be significantly different between all treatments ($p < 0.05$) except the control and white ice relationship ($p = 0.99$), using a one-way ANOVA, Transmittance between the two ponds was also significantly different (Students' t-test; $p = 0.037$).

Vertical profiles of the pond systems illustrated expected patterns, based on salt extrusion principles, in salinity values between the treatment and control ponds. When snow was removed, and thicker ice formation occurred, under-ice salinity levels increased relative to when snow was allowed to accumulate on the surface cover, and ice thickness was correspondingly less. The difference between profiles taken on the same dates were significant for all taken when ice cover was present and returned to being not significantly different after ice breakup (Figure 3.6, Students t-test, $p<0.05$, $\alpha=0.05$).

3.4.2 Mesocosm experiments

Temporal variability was observed in under-ice light regimes within mesocosm environments, based on surface cover type (Figure 3.4), with some periods, particularly at the beginning of the ice-on period, having comparable values. Using an analysis of

variance approach, with a Tukey post-hoc test, light penetration under white ice did not differ significantly ($p=0.99$; $\alpha=0.05$) from the control but were significantly different than snow-on-ice conditions ($p=0.008$; $\alpha=0.05$).

As expected, diurnal cycling is visible in all mesocosm applications, along with respective shortening or lengthening of daylight hours (seasonality), and an increase in incident solar radiation (Figure 3.4) based on season. These trends are visible due to collection of high-frequency (30 minute-interval) data from the fully immersed spherical PAR sensors. Though ice depth measurements for the mesocosms were not collected in an effort to limit surface disturbance, ice never encapsulated the spherical sensors and remained approximately 20 cm or greater from the bottom of the containers at a minimum. On average, over the entire ice-on period, light transmission through the control mesocosm surface cover was 18.4% higher than that in the snow-on-ice treatment.

Albedos varied substantially between snow-on-ice and other treatment conditions. The addition of fresh snow atop of an already frozen surface cover resulted in a mean albedo of 0.56 ($n=11$) during the winter period, compared with albedos of 0.33 and 0.35 for slushed (white ice) and control surface covers respectively (Figure 3.2). The transportation and deposition of snow into the greenhouse environment, along with slightly milder temperatures, resulted in a lower albedo than what was measured on the undisturbed ponds adjacent to the mesocosm locations.

Extinction coefficients, calculated using equation one from Roulet & Adams (1986), were 1.66 ± 0.22 ($n=5$; 2016/17) for black ice and 9.99 ± 4.06 ($n=4$; 2015/16) for white ice. Snow-on ice resulted in a considerably higher extinction coefficient of 20.98 ± 16.87

(n = 5; 2015/16 & 2016/17). These values were comparable to those reported in the literature, with black and white ice having extinction coefficients of 0.5-1 and 6-6.25 respectively. In addition to affecting light attenuation, the snow-on-ice covering resulted in warmer water temperatures (Figure 3.5) because of the insulating properties of snow.

Dissolved organic carbon (DOC) values in the mesocosms ranged from 10-13 mg/L and varied slightly under surface cover manipulation conditions. DOC in the pond systems averaged 12.2 mg/L for both the treatment and control ponds (n= 5) but ranged from 9-17 mg/L in 2015/16. Turbidity values were very low in the mesocosm environments, ranging from 0.1 – 0.6 NTU. Similarly, turbidity in the pond systems was negligible (mean <1 NTU) and did not show any temporal nor depth-related trends.

3.4.3 Water quality

No significant differences were found in using a Student t-test (α=0.05) in mesocosm biogeochemistry results from 2015/16 grab samples (Table 3.1). In contrast, in the pond systems, significant differences in dissolved chloride (p=0.01), dissolved iron (p=0.0002), dissolved sodium (p=0.008), and dissolved sulphur (p=0.025) were found between pond treatment and control, though mean values were very low (all <1 mg/L). All other parameters in the ponds were found to not be statistically significant (Figure 3.7). When comparing between early (prior to January 21) and late (post January 21) samples using a two-way ANOVA and a Tukey's post-hoc test, significant differences in hardness (white ice mesocosm treatment; p = 0.014), DOC (white ice mesocosm treatment; p = 0.012), and TN (control pond; p=0.016). For all other chemical parameter and treatment combinations, no significant differences were found between early winter and late winter samples.

3.5 Discussion

The differences in hydro-climatic conditions between the two experimental winter periods (2015/16 and 2016/17) provided additional insights into the role of changing ice-cover quality on under-ice physical/chemical and ecological properties in 2015/16, the winter was very mild and had a substantial number of freeze-thaw cycles. This resulted in the increased formation of white ice, due to slushing and freeze-thaw processes (Michel and Ramseier 1971; Prowse and Stephenson 1986). In contrast, the second experimental period in 2016/17 had a rapid drop and consistently cooler air temperatures, resulting in the formation of black translucent black ice (Figure 3.3). This pattern was observed in both the mesocosm and pond systems.

As a result of the natural formation of white ice, the light transmission values in mesocosms were similar in white and control conditions, with only the snow-once-ice treatment exhibiting a significantly lower amount of light transmission. This suggests that under the increased precipitation and air temperature conditions forecast for the majority of mid-latitude North America (Dibike et al. 2012), it can be expected that there will broadly be a decrease in light transmission to the underlying water column with resulting consequences on biogeochemical and ecological processes.

Light availability is extremely important for photosynthetic organisms, and correspondingly aquatic food web structure. With the greatest difference in light transmission occurring in the late winter season, when organisms are already experiencing light limitation (Syväranta and Rautio 2010; Dokulil et al. 2014), could result in increased mortality rates. This light limitation would be exacerbated at higher latitudes where daylight is shorter, thus decreasing an already limited amount of light for

photosynthetic organisms. Where nutrient limitation is often cited as the controlling factor (Karlsson et al. 2009), this may not be the case during winter months in seasonally ice-covered freshwater systems. Additional top-down food web pressures are present in the form of zooplankton predation; however, Hrycik & Stockwell (2021) identified light as the main driver in phytoplankton growth in *in-situ* mesocosm experiments. This indicates that both mechanisms could be operating concurrently to control observed autotrophic production levels.

Though light measured in the mesocosms were controlled by surface cover composition (Figure 3.4), values were well above the thresholds for light limitation reported by Gosselin et al. (1985) and supported by Pernica et al. (2017). This is likely in part due to the above-ground nature of the mesocosm experiments combined with the use of spherical light sensors, allowing light to penetrate the tank walls, thus increasing measured and available light. In contrast, light limitation was found to be occurring in the control pond system (snow) with 8 out of 9 samples falling below the 7.6 $\mu mol/m^2/s$ threshold for photosynthetic activity, and all samples falling below the 20 $\mu mol/m^2/s$ threshold for biomass increase. Increased light penetration through the manipulated pond, did result in the thresholds for both activity and biomass growth being exceeded in black ice conditions (4 out of 6 times). However, under white ice conditions, the biomass growth threshold was never reached, and the photosynthetic activity threshold was only exceeded 1 out of 4 times. Notably, the relationship between snow depth and light availability presented by Pernica et al. (2017) did align with the observed values in this study, though the smaller scale of the pond systems and presence of the semi-aquatic plant *Typha* along the littoral edges of the ponds likely

resulted in decreased wind-blown snow transport (Kang et al. 2014), and in differences in snow metamorphosis due to air temperature (Colbeck 1982).

Biogeochemical differences as a direct result of surface cover manipulation were found to be limited in this study and statistical differences could be considered as biologically insignificant as a result of the low values. Though dissolved organic carbon is a very efficient attenuator of light (Thrane et al. 2014), the results of this study suggest that the limited amounts of light present under-ice did not result in shifts in DOC due to photodegradation (Stanley et al. 2012) as might be expected. However, watershed scale environmental changes are expected to result in the browning of freshwater systems in the mid- and high-latitude regions of North America (Hobbie et al. 1999; Zhang et al. 2010) and will likely further diminish under-ice light availability as a result of DOC addition. Salinity values were different between the treatment and control pond, with higher values being observed in the treatment pond (Figure 3.6). This is likely due to the lack of thermal insulation from snow on the treatment pond, resulting in a thicker ice cover, the corresponding extrusion of salts, and effective increase in concentration within the underlying water-column (Leppäranta 2015). Additionally, the lack of significant differences in biogeochemistry when compared temporally (early- vs late-winter), suggests that the most rapid rate of change in both the mesocosms and small pond systems may be occurring during initial ice cover formation. Additionally, this highlights the limitation of the mesocosm environment where the lack of benthic sediments does not provide any source or sink of DOC.

Elevated levels of TN and TP were observed in the mesocosm treatments with the snow-on-ice surface cover but were found to be not significant (Table 3.1). Additional

replication and sampling would be required to determine if the observed trends combined with elevated levels of dissolved nitrate support the findings of Powers et al. (2017a) that suggest that nitrification is not limited by light availability, but the consumption of nitrate by microbial organisms may be. (Giannelli et al. 2001). Though it is possible that the elevated values of TN and ammonia may be due to the sampling location, near the ice-water interface, where nitrate accumulation occurs most rapidly due to the availability of oxygen (Powers et al. 2017a)

3.6 Conclusion

The results of this study support the previously published research indicating that snow cover, specifically, has a significant effect on under-ice light regimes, due to its high albedo and low transmittance (Prowse and Stephenson 1986; Leppäranta 2015; Pernica et al. 2017). Extinction coefficients of white ice, black ice, and snow-on-ice reported in this study are also in line with those reported in Prowse and Stephenson (1986) and suggest that under shifting hydroclimatological conditions, accelerated changes in under-ice light regimes due to surface cover shifts will be observed. The complete time-series of winter light regimes presented in this study further emphasizes the importance of surface cover on seasonally ice-covered systems, especially with regards to the late-season (February/March) time period, where dissolved oxygen levels may already be low (Barica and Mathias 1979; Mathias and Barica 1980; Baehr and DeGrandpre 2002). In small lentic systems, similar to the experimental ponds in this study, dissolved oxygen levels already frequently reach values low enough to result in fish kill (Barica and Mathias 1979) and it is expected that this may be exacerbated as a

result of decreased light and photosynthetic activity occurs due to surface cover. This also will likely result in a change of phytoplankton community composition when ice-off occurs, and therefore may affect subsequent open water conditions, including the timing and prevalence of algal blooms. Additionally, surface cover composition, and particularly the presence of snow-on-ice, results in variations in under-ice biogeochemistry, which will have cascading effects on food web structure and function.

The highly variable nature of hydroclimatic conditions in the mid-latitudes, particularly in the study area resulted in two contrasting hydro-climatic regimes for comparison. This provided a very useful proxy for white ice and black ice surface cover conditions in our experimental ponds. While additional research is required, mesocosm-based controlled experiments, augmented by *'in situ'* monitoring information collected in natural systems may be extremely useful in informing the development of predictive models that describe the mechanistic relationships between surface cover and under-ice productivity regimes. Additionally, this research further supports the findings of Hampton et al. (2017) which suggest that under-ice ecology needs to be considered to fully understand the role of hydro-climatic drivers in affecting the community composition and functioning of aquatic ecosystems.

3.7 References

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3.8 Figures

Figure 3.1 Manipulated (snow removed) pond ice cover conditions with visible black ice (left; 2 December 2016) and white ice (right; 7 January 2016)

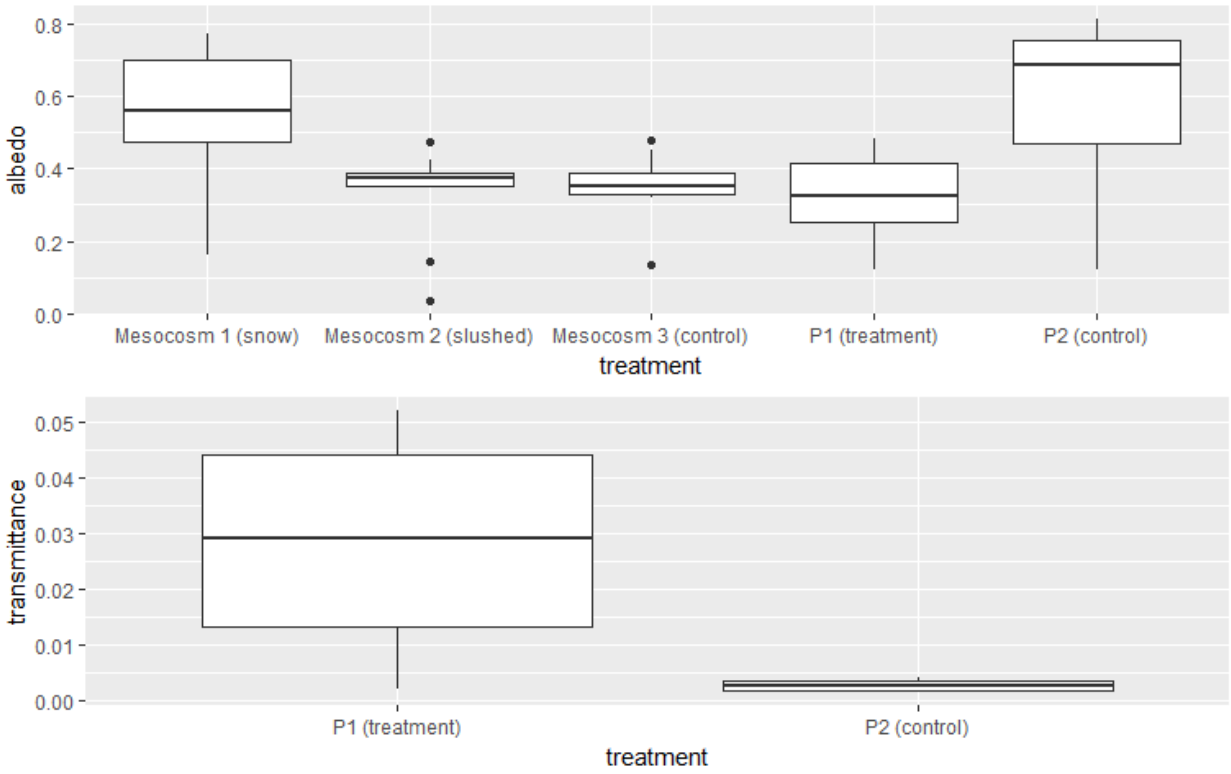

Figure 3.2 Albedo and transmittance values through mesocosm and pond surface cover environments in winter 2015/16, where white ice dominated the control systems. Line is median value, box spans the 25th and 75th percentiles, and whiskers are 1.5 x IQR (box). Control conditions varied between ponds and mesocosms, with P2 being exposed to the elements and correspondingly having snow accumulation, in contrast to the control mesocosm which was located in a greenhouse and had no snow accumulation nor surface cover manipulation. Albedo was significantly different between all treatments (one-way ANOVA Tukey's post-hoc test, $p < 0.05$) except for the white ice mesocosm-control mesocosm ($p = 0.99$); comparisons between mesocosms and ponds not analyzed. Pond transmittance was significantly different (paired Students' t-test; $p = 0.037$).

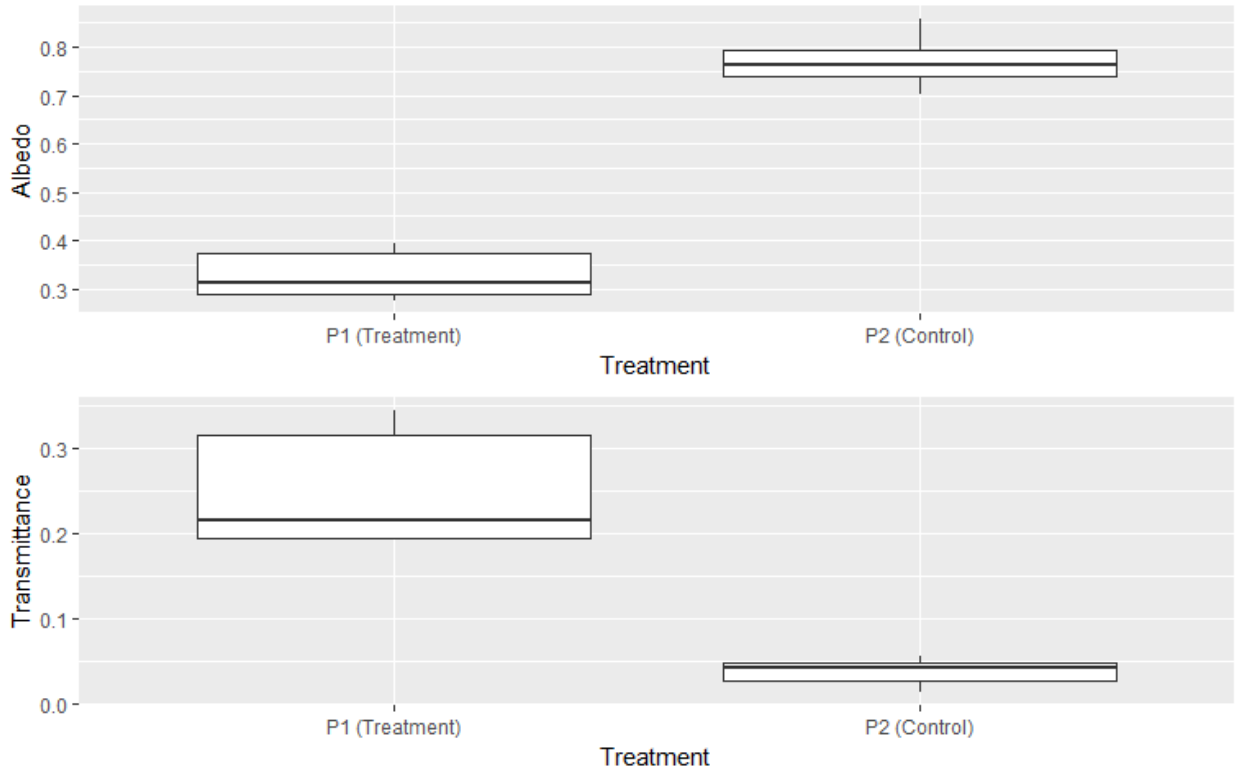

Figure 3.3 Albedo and transmittance values through pond surface cover environments in winter 2016/17, where black ice dominated the control systems. Line is median value, box spans the 25th and 75th percentiles, and whiskers are 1.5 x IQR (box) P1=Pond 1, P2=Pond 2; Differences significant; p < 0.05; Student-t test)

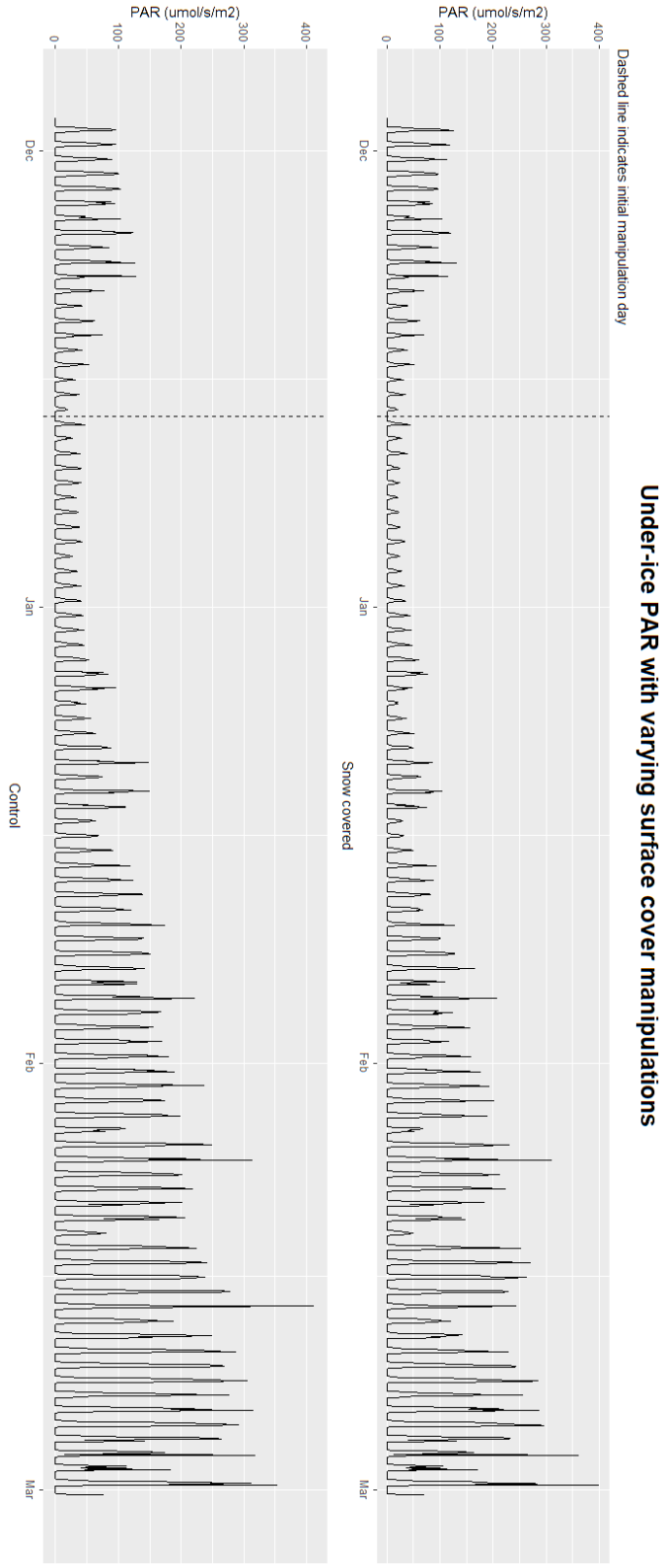

Figure 3.4 Continuous PAR measurement values under two surface cover conditions in mesocosm systems (top/right) and the difference between control and snow-on-ice water column PAR values (bottom/left)

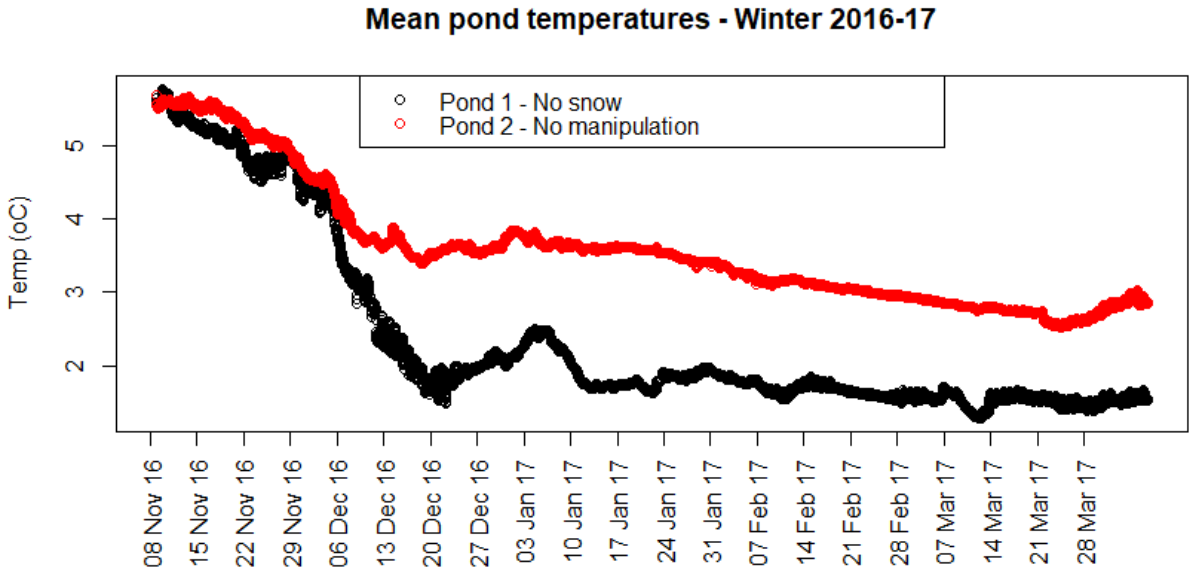

Figure 3.5 Continuous mean under-ice temperature regimes in two experimental ponds from two depths (50cm and 10cm) from bottom collected using a HOBO brand dissolved oxygen logger

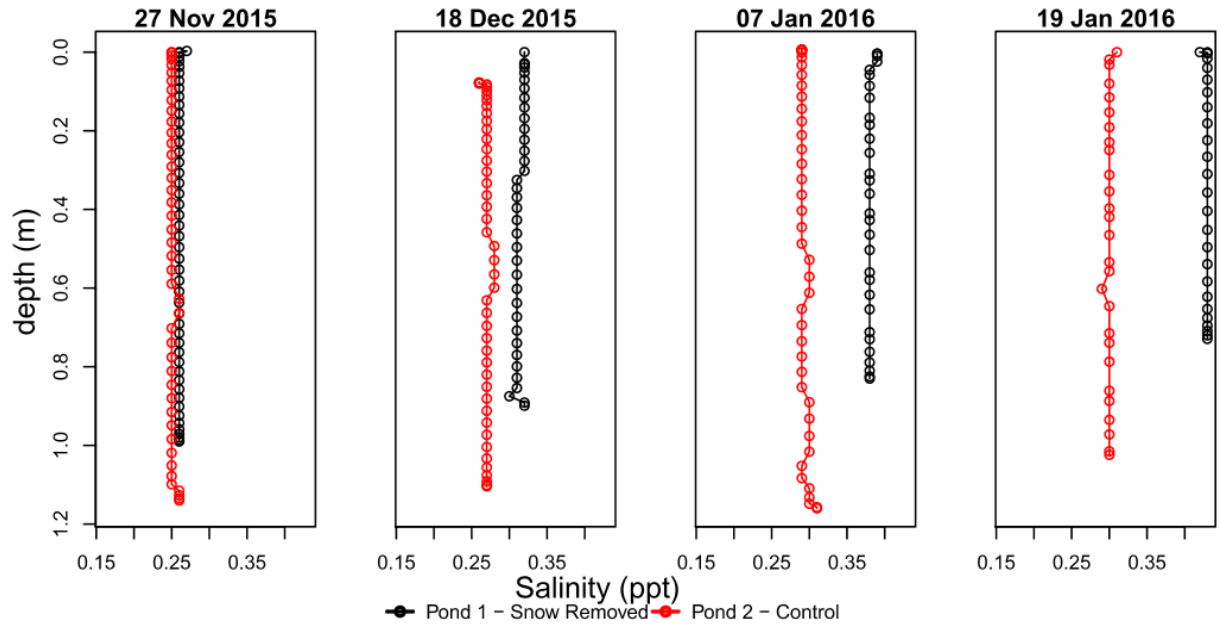

Figure 3.6 Vertical salinity profiles in the two experimental ponds in the 2015/16 sampling period. Collected using a YSI multisonde illustrating the effect of ice thickness differences and salt extrusion.

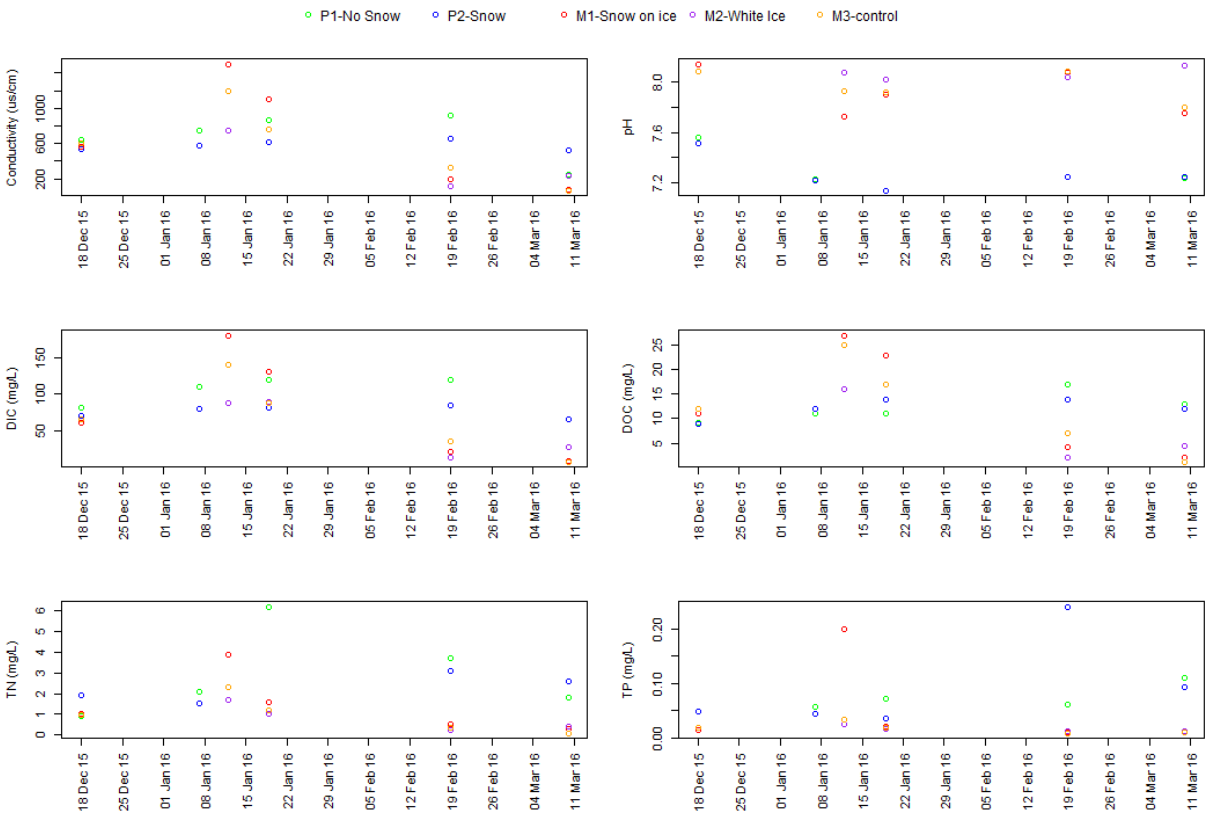

Figure 3.7 Under-ice biogeochemistry including conductivity, pH, DIC, DOC, TN, and TP, from both ponds and mesocosms for 2015/16 sampling period. Data processed by Maxxam Analytical Laboratories in Calgary, Alberta.

Table 3.1 Mean and standard deviation of biogeochemistry values (n=5) from 2015/16 season, under different surface cover conditions in pond and mesocosm environments. Statistical significance (ANOVA and Tukey's post-hoc test) of $p < 0.05$ was not found in any comparisons between respective environments (ponds and mesocosms analyzed separately).

	Hardness (mg/L)	**Conductivity (µs/cm)**	**pH**	**TN (mg/L)**	**TP (mg/L)**	**TDS (mg/L)**	**DIC (mg/L)**	**DOC (mg/L)**
Mesocosm – Control	317.6 ± 273.2	589.8 ± 498.4	7.96 ± 0.11	0.98 ± 0.99	0.018 ± 0.012	335.4 ± 290.4	67.1 ± 58.6	12.4 ± 10.6
Mesocosm – White	277.8 ± 176.3	494.0 ± 296.6	8.07 ± 0.04	0.85 ± 0.58	0.017 ± 0.005	284.2 ± 179.2	56.8 ± 34.8	10.3 ±6.7
Mesocosm – Snow	388.2 ± 353.9	685.8 ± 605.0	7.92 ± 0.19	1.46 ± 1.45	0.051 ± 0.083	407.6 ± 378.1	80.1 ± 73.2	13.4 ± 11.2
Pond – Control (snow)	310.0 ± 26.5	580.0 ± 52.4	7.27 ± 0.14	2.14 ± 0.67	0.092 ± 0.086	310.0 ± 21.2	76.4 ± 8.0	12.2 ± 2.1
Pond – snow removed	354.0 ± 146.4	684.0 ± 265.2	7.28 ± 0.16	2.94 ± 2.08	0.070 ± 0.024	370.0 ± 141.2	91.8 ± 39.4	12.2 ± 3.0

4 The impact of winter surface cover on under-ice dissolved oxygen and autotrophic production in shallow mid-latitude seasonally ice-covered systems

4.1 Abstract

Under predicted climate change, it is expected that winter hydroclimatic conditions will shift towards increased air temperatures, greater snow deposition, and will consequently result in shifts to watershed-scale dissolved organic carbon (DOC) transport in the northern mid-latitude regions of North America (Thackeray et al. 2019; Woolway et al. 2020). In the Canadian prairies, small, shallow, hydrologically disconnected lakes and ponds are common and are seasonally ice-covered for at least 4-5 months of the year. To evaluate the relationships between surface cover composition (white ice, black ice, and snow-on-ice) and under-ice autotrophic production (as measured by chlorophyll-a), a series of controlled experiments were conducted over a 2-year period. Mechanical surface cover manipulation – snow addition, removal, or slushing - was undertaken in a paired pond experiment, along with the use of 700 L above-ground polyethylene mesocosms. DOC concentrations were manipulated in the mesocosm environments through the addition of a humic acid liquid fertilizer. The presence of snow-on-ice resulted in significantly lower autotrophic production as measured by chlorophyll-α values in pond systems, compared to either white or black ice (snow removed) treatments. Though both ponds became anoxic very quickly after ice-on, the removal of snow did result in some diurnal dissolved oxygen (DO) cycling that was not observed under the snow-on-ice treatment. In mesocosms, the addition of DOC did not result in significant changes to under-ice autotrophic production but did result in decreased DO concentrations. These results suggest that predicted future hydroclimatic conditions in temperate, mid-latitude regions may result in changes in under-ice primary productivity and dissolved oxygen regimes that could

affect subsequent ice-off water quality conditions and aquatic community composition and succession patterns.

4.2 Introduction

Current research around climate change and it's impacts on hydroecological processes in the northern midlatitude region has predominantly been focused on ice-free (summer) months (Powers and Hampton 2016; Hampton et al. 2017). The Canadian Prairies are dominated by shallow, hydrologically disconnected pothole lentic systems which are seasonally ice-covered for at least 4 months of the year (Magnuson et al. 2000). While ice cover is often viewed as uniform spatially and consistent over the winter season and is correspondingly treated as a physical 'cap' limiting the interaction of the water column with overlying atmospheric conditions, the composition and quality of ice cover can vary extensively. Ice cover quality is influenced by air temperature, wind conditions, and precipitation amounts and types (Leppäranta 2015). Three different surface cover types are possible when examining seasonally frozen lentic systems: 1) black (clear) ice which forms under a rapid cooling and limited surface disturbance, 2) white (opaque) ice, which forms as a result of freeze-thaw cycles, hydrostatic upwelling and consequent re-freezing, or slushing of precipitation on the ice cover, or 3) snow-on ice (Prowse and Stephenson 1986). The factors driving the creation of each of these types of lake ice cover are unique (Liston and Hall 1995) and rely on a specific suite of conditions being present to promote their formation. The optical properties of each respective cover type vary widely and is of particular interest when examining food web structure as primary productivity via photosynthetic activity is

a critical portion of ecological energy pathways. much of the biological activity in ice-covered lakes relies on primary productivity through photosynthesis.

When modeled using the one-dimensional process-based model MyLake (Saloranta and Andersen 2007) coupled with a range of global climate models, Dibike et al. (2012) found that, due to increases in winter precipitation values in much of the prairie regions, both snow and white ice thicknesses are expected to increase in occurrence by 2050. Additionally, warmer winter air temperatures are likely to result in an increased number of freeze-thaw cycles, particularly in areas such as southern Alberta, Canada, which often receive above 0°C temperatures as a result of mountain air masses. This increase in freeze-thaw cycles will result in the formation of additional occurrences of white ice conditions. The modeling completed to this point has focused on the physical factors that are expected to shift as a result of climate change, however the under-ice biological conditions are less well understood (Bižić-Ionescu et al. 2014; Garcia et al. 2019).

While there is a growing body of information on how the timing of lake ice cover formation and duration influences under-ice phytoplankton community structure and succession patterns (eg. Sommer et al. 2012; Özkundakci et al. 2016; Hampton et al. 2017), limited information exists on the role of lake ice optical properties in affecting these relationships (Pernica et al. 2017). It is also increasingly recognized that antecedent under-ice conditions (e.g., photosynthetically active radiation (PAR), nutrient levels; phosphorus, nitrogen, DOC) may collectively play an important role in the development and succession patterns of planktonic communities in the under-ice and subsequent open-water periods (Domis et al. 2013). Contributions of either autochthonous or catchment-derived humic substances (i.e.., DOC) can affect both

under-ice light transmittance and carbon availability to heterotrophic and autotrophic pathways (Karlsson et al. 2009; Thrane et al. 2014; Diodato et al. 2016; Strock et al. 2017).

While the connection between primary production, the DO conditions present under-ice, and the influence of environmental conditions (ice cover type/quality and DOC addition) have been acknowledged (Couture et al. 2015), the direct correlation between said parameters have not been quantified. This is of particular concern for lentic systems that frequently experience anoxia (Baird et al. 1987) and are susceptible to eutrophication (Mathias and Barica 1980). To better understand the mechanistic relationships between DO and surface cover conditions, manipulative experiments in controlled (mesocosm) and semi-controlled (hydrologically disconnected) experimental ponds were used.

4.3 Methods

Two experimental ponds, with surface areas of approximately 185 m^2 and max depth of 2 m, and eighteen, 700 L polyethylene above-ground mesocosm tubs were used in this study. Both the ponds and mesocosms were located at the University of Calgary Aquatic Experimental Facility in Alberta, Canada (51°N, 114°W). Data collection occurred over two consecutive winter seasons in 2015/16 and 2016/17. Both mesocosms and ponds were filled using the same source water, supplied from the Bow River, and passed through a temperature-controlled irrigation network, with no additional treatment. Once ponds were filled in spring (April/May), they were allowed to equilibrate over summer months, prior to mesocosms being filled directly from the

ponds via pump. A 60 µm mesh filter was used to filter out invertebrate fauna as the mesocosms were being filled, as per (Beisner et al. 1996). Notably, the Bow River is low in dissolved organic carbon (DOC) with a mean value of <1 mg/L between 1989-2018 (Alberta Environment and Parks, Bow River at Cochrane station AB05BH0010) and therefore it was expected that the majority of DOC contributions would be from decaying autochthonous or allochthonous material in the ponds.

The above-ground mesocosms were located within a fully enclosed greenhouse that allowed for limited temperature control, along with a controlled ice surface cover manipulation. Low-voltage heating bands were wrapped around the mesocosms approximately 10 cm from the bottom of each tank to ensure complete freezing of the water column was avoided. To access under-ice water, a small diameter, 135 cm long drill bit was utilized as a micro-auger. Samples were then acquired via syphoning with triple rinsing using deionized water occurring in between samples.

Prior to freeze-up, one HOBO brand DO logger (U26) was immersed in each mesocosm attached via cable to an overhead support beam, so that the sensor was located 10 cm from the bottom of the tank. In the ponds, sensors were placed at 10 cm and 50cm from bottom (to prevent freezing), with an additional one deployed at 100 cm from bottom in the 2015/16 sampling period to provide data on stratification and a vertical profile. All sensors logged at 30-minute intervals through the entire ice-on period. In addition, YSI 6600-v4's were deployed in 3 mesocosms during the first sample period, with a 60-minute sampling interval. Probes on the YSI instruments included temperature, conductivity (µs), pH, turbidity (NTU), and dissolved oxygen (mg/L). Other calculated values were also output by the sensor.

Additionally, in 2015/16, 3 spherical Li-Cor brand photosynthetically active radiation (PAR) light sensors (LI-193) were placed into one replicated of each of the surface cover conditions. These sensors reported under-ice radiation at 1-hour intervals. Incoming solar radiation within the greenhouse was also measured using a Li-Cor brand light meter at matching 1-hour intervals.

After ice-cover was established on the mesocosms to a minimum depth of 1 cm, surface cover manipulations were started. Two ice surface manipulations were conducted on an ongoing basis with no more than 14 days between treatments throughout the experimental period: 1) the addition of approximately 5cm of snow (snow-on-ice) and 2) the addition of approximately 3 cm of snow, mixed with deionized water (white ice). Each surface cover type was applied to three replicate mesocosms, with a set of three control mesocosms with no surface cover manipulations also established. In 2016/17 the same surface cover manipulations were utilized, however DOC manipulation, through the addition of a humic acid fertilizer (Black Earth Organo Hume ULTRA; http://www.hortimax.biz/BlackEarth/BlackEarth-Organo_Hume_ULTRA.pdf) was also performed, with a total of 18 mesocosms being utilized.

Using the two experimental ponds available for this project, surface cover manipulation (the mechanical removal of snow) was applied to one, while the other pond was used as a control. Snow was cleared from the manipulated system within 48-hours after snowfall, once ice safety was validated. Autumn/Winter environmental conditions in 2015/16 resulted in the treatment being mostly white ice (frequent freeze-

thaw cycles), while 2016/17 had more consistently cold temperatures resulting in predominantly black ice.

Once surface-cover manipulations were initiated, a bi-weekly sampling regime was conducted that included above-ice light measurements (albedo and incident radiation) and under-ice measurements, including a multi-sonde depth profile, the collection of an ice core, and under-ice radiation measurements. Water column grab samples were also obtained and transported the same-day to an analytical laboratory (Maxxam Analytical/Bureau Veritas) and analyzed for carbon (dissolved and total), phosphorus, nitrogen (see previous chapter; Table 3.1; Figure 3.7). One litre samples were also collected in opaque polyethylene bottles (Nalgene brand) and processed for chlorophyll-α, using a fluorometer and acetone extraction techniques (Arar and Collins 1997). All statistical analyses were conducted using the R analysis program (R Core Team 2019). Additionally, Estimation statistics (Ho et al. 2018) were used due, in part, to a large sample size and also due the ability to identify/visualize overall trends in the data in a simple manner.

4.4 Results

The two consecutive winter sampling periods (2015-16 and 2016-17) had very different hydrometeorological conditions, which correspondingly influenced ice cover formation, duration, and condition. Between October and March (inclusive), the mean daily minimum air temperatures were -5°C and -9°C for the first and second experimental periods respectively (Alberta Climate Information Service interpolated weather data). During the same periods the mean daily maximum air temperatures were

6 and 2°C and total precipitation amounts (snow-water equivalence) were 44.5 and 87.0 mm for 2015-16 and 2016-17 respectively. As a result of the warmer weather, and more frequent freeze thaw cycles in the initial sample period, the manipulated (snow removed) pond and control mesocosms were dominated by white ice as opposed to the overall cooler temperatures in the second experimental period resulting in a dominance of black, translucent ice. The elevated precipitation in the second year also corresponded with an increased thickness of snow accumulation on the control pond system, with a maximum depth of 27.6cm measured in 2015/16 as opposed to 43.8cm in 2016/17.

Mean chlorophyll-α values were higher, though not significantly ($p = 0.07$ in 2015/16, and 0.06 in 2016/17), in the snow-removed pond systems, regardless of whether the dominant ice cover type was white (opaque) or black (translucent). Under black ice conditions, mean chlorophyll-α in the ponds was 58.9 µg/L ± 50.9, compared to 25.4 ± 22.3 under snow (Figure 4.3). Under white-ice conditions, chlorophyll values were comparable, with a mean of 58.4 ± 24.1 µg/L and a corresponding mean of 23.7 µg/L ± 20.6 in the control system (Figure 4.2). However, the differences in means were not significant in either sampling period with p values of 0.058 and 0.072 ($df1 = 1$, $df2 = 38$) for the first and second experimental seasons respectively using a Students' t-test with a significance level of 0.05. Notably, chlorophyll-a values over the 3-months after ice-off (initial sampling season) reacted differently corresponding to ice-on surface cover manipulation, with a mean value of 44.7 ± 24.5 µg/L for the previously snow-on-ice pond, and 22.9 ± 16.6 µg/L for the snow-removed pond. When grouped into early to mid-winter (before January 21) and mid to late winter (after January 21) regardless of

sample year, mean chlorophyll-α values were not significantly different between the two periods (early vs late; p = 0.0553), or sample time and treatment level (p = 0.279), but a significant difference was found between treatments (snow removed or control; p = 0.003) when analyzed using a two-way ANOVA.

Dissolved oxygen levels in the ponds were low, if above 0 mg / L, at deepest points (10 cm above benthic sediment) in both sampling seasons (Figure 4.5 and Figure 4.6). Some small increases in DO were observed at sensors placed 50cm above the benthos, particularly in the second season, when black ice was present on the manipulated pond system (Figure 4.6). When values were above 0 mg L, strong diurnal cycles were present with peaks occurring around 14:00. Under white ice conditions, some limited diurnal cycles were present under mid-winter ice cover (Figure 4.5), though the peaks were below 1 mg / L.

Maximum DO values under black ice conditions were an order of magnitude higher than those under white ice conditions (Figure 4.6 and Figure 4.5, respectively). However, both ice types saw diurnal DO peaks that were not present in the control ponds, which had snow accumulation, where the DO concentration remained at or near 0 mg/L throughout the entire ice-on period.

Water temperature in the control pond was significantly higher (students t-test; p values <0.05, df = 198) at all depths in both experimental periods when compared to manipulated surface cover (snow-removed) conditions with mean values of 3.88 ± 0.78 °C and 2.97 ± 1.13 °C respectively (2016-17), at a depth of 10cm from benthic sediments (Figure 4.8). At shallower depths (50cm from the bottom), the difference in mean temperature was greater, with the manipulated pond have a mean temperature of

1.82 °C and the control pond averaging 3.31 °C. This overall pattern was comparable to that observed in the 2015-16 experimental period.

The addition of humic acid, via liquid fertilizer, to mesocosms resulted in no significant differences in chlorophyll-α values, under any surface cover treatment, though the variability was high. In both seasons, the control and white ice treatments had similar mean chlorophyll concentrations and also had comparable temporal trends, as a result of frequent freeze-thaw events at the experimental location.

Through both sample periods, the mesocosm environments had lower chlorophyll values than the neighbouring ponds. Additionally, the ice was thicker on the mesocosm systems, as a result of being located above-ground. Ice was not allowed to reach the bottom of the mesocosms, theoretically allowing for ongoing biological activity in the remaining water beneath the ice.

DO was significantly higher in mesocosm environments, when compared to pond environments (two-way ANOVA and Tukey's post-hoc test). This includes the lowest values which were found early after the onset of ice and were <11 mg/L across all treatment conditions (Figure 4.7). Peaks in DO were observed throughout the ice-on period with the maxima occurring in early January across all treatments. However, the peaks were highly variable with DOC and surface cover manipulations resulting in different DO concentrations; The addition of DOC resulted in lower DO values when compared with the same surface cover treatment controls (i.e., white ice, DOC added vs. white ice, control). The variability was considerably less, particularly in later winter, in DOC enriched environments, with diurnal trends being more noticeable in control systems. Estimation statistics show the similar means and population distribution

between white ice and control systems, while highlighting the differences between surface cover and DOC levels (Figure 4.10). Estimation statistics (Ho et al. 2018) are particularly helpful in this analysis due, in part, to a large sample size and also due the ability to discern overall trends of multiple treatments simplistically.

4.5 Discussion

In the mesocosm and experimental pond systems, snow cover was found to significantly influence under ice productivity as measured by DO and chl-α endpoints. The presence of snow on top of a pre-existing ice cover is known to be a very effective attenuator of solar radiation (Warren 1982). In the controlled pond system, the presence of snow on ice cover resulted in anoxic conditions at depths of 50 and 100cms from the bottom, while the removal of snow resulted in some diurnal cycling of DO (Figure 4.6, Figure 4.7). This is likely as a result of the limitation of photosynthetically active radiation through the snow layer (Bolsenga et al. 1996) alongside the warmer temperatures in the snow-covered system (Figure 4.8) resulting in increased heterotrophic respiration of available DO (McGuire et al. 2000). In the mesocosm systems, the control conditions were comparable to white ice conditions, as a result of the frequent above-freezing temperatures traditionally experienced in Calgary, and many other mid-latitude seasonally ice-covered systems. White ice is an effective limiter of light transmission (Prowse and Stephenson 1986) and is expected to increase in prevalence in the coming decades due to shifts in precipitation and air temperature in cold-regions systems (Dibike et al. 2012).

The lack of significant differences between early- and late-winter chlorophyll-a values likely are due to the rapid shift in environment that occurs once ice is present on the systems. The hydrologic disconnection of the ponds systems from other freshwater sources results in a closed system that, combined with limited available water under ice, results in an environment where autotrophic activity is severely limited (Hampton et al. 2017). Correspondingly, these findings suggest that ice/surface cover quality is more important than time when evaluating under-ice productivity as measured by chlorophyll-α.

Though the manipulation of surface cover did not result in statistical differences in chlorophyll-α values within the mesocosm systems, this may be related to the above-ground experimental design which allowed light penetration through multiple sides, as opposed to exclusively through the surface cover. Additionally, the lack of an established benthic substratum as a potential source of nutrients and primary production however limited (Horner and Schrader 1982), may suggest that pelagic biological activity under ice cover is reliant on mixing layers and the vertical transport of nutrients and other relevant biogeochemical parameters (Pernica et al. 2017).

When scaled up to the experimental ponds, differences in chlorophyll values become increasingly apparent (Figure 4.2, Figure 4.3) with the presence of snow on ice, resulting in a decrease in mean chlorophyll-a values. This is likely due to the availability of DOC and the contributions of benthic organisms (Godwin et al. 2014) resulting in altered rates of productivity. Additionally, the greenhouse enclosure in which the mesocosms were housed, did reduce overall incoming solar radiation, which may have further reduced overall available PAR within the mesocosm systems. The in-ground

nature and maximum depth of the ponds at approximately 2 meters, allowed for a smaller percentage (though still substantial amounts) of the aquatic system to be frozen, when compared to the mesocosm environments. While there is evidence of photosynthetic activity within ice cover, it is extremely limited and expected to occur at much higher rates in aqueous environments (Fritsen and Priscu 1998). Notably, chlorophyll values obtained in the winter were comparable to, or slightly higher than, those obtained from the same systems in ice-free months (*unpublished data*). This may be a limitation of the sampling method employed in the winter, which obtained pelagic grab samples from only the uppermost meter of the ponds, compared to summer samples which could be taken at a slightly greater depth and could be affected by lake turnover or other mixing (George and Edwards 1976). Additionally, predation from grazing zooplankton may be decreased during the winter months, as a result of limited vertical movement within the water column, and decreased water temperatures (Thorisson 2006). This seasonal difference is in contrast to that found by Hampton et al. (2017) which found an average of 50% decrease in winter chlorophyll values compared to open water; though this may be partially explained by the difference in size of water which were evaluated.

When DO was examined as the measured endpoint, the differences between system scale, and treatment became more apparent. This is particularly true at shallower depths in the pond systems and is highly controlled by surface cover quality and composition. The trend of anoxia occurring first near the benthos found by Prowse and Stephenson (1986) and Babin and Prepas (1985), was found to occur in our experiments, however the rates of oxygen depletion occurred much more rapidly in our

considerably smaller experimental ponds. The diurnal fluctuations also suggest that there is ongoing photosynthetic activity, however additional work measuring respiration and production rates explicitly would aid in our understanding of these trends. Additionally, as temperature and biogeochemical factors influence DO levels, further work utilizing equipment such as respiration chambers would be valuable in isolating the biological influence (respiration/production) on DO trends.

While the control surface cover composition of both systems (mesocosm and ponds) varied between sample periods, this is reflective of the variable air temperatures traditionally experienced regionally at the sample site, which frequently are above zero for periods of time while ice is still present. The result is the formation of opaque white ice with low transmissivity, which is extremely effective at filtering out PAR wavelengths (Roulet and Adams 1986). Though white ice was shown to be effective at reducing the transmission of PAR (Pernica et al. 2017) (see: chapter 1 – The influence of surface cover and ice properties on under-ice radiation regimes and biogeochemistry in mid-latitude, seasonally ice-covered ponds and controlled mesocosm environments), it still allowed a great deal more light transmission than snow-on-ice treatments (mesocosm) and the control pond. This lack of PAR, combined with little to no overlying atmospheric interaction, resulted in the rapid decrease of DO levels observed in all environments and sample periods. Though surface cover is known to be the major controlling factor of under-ice radiation regimes (Prowse and Stephenson 1986), additional factors, including the ‘browning’ of lakes through increases in delivery of allochthonous DOC, will also alter the transmission of PAR, particularly within the water column itself (Karlsson et al. 2009).

The addition of humic acid substances has been shown to be an effective experimental surrogate for allochthonous DOC in controlled systems (Lennon et al. 2013). However, based on the literature review conducted as part of this thesis, this is the first instance of DOC manipulations being completed in this way with a focus on under ice processes. The addition of DOC in mesocosms resulted in alterations to seasonal DO trends under all surface cover treatments, with the greatest differences occurring towards the end of the ice-on period. This is of importance for organisms reliant upon DO, particularly when anoxic conditions are experienced, as was observed in both pond systems. While ice cover duration has been shown to increase fish kill due to increased chances of anoxic conditions (Mathias and Barica 1980; Robarts et al., 2005), there are a variety of factors that influence the DO depletion (Mathias and Barica 1980). However, the effect of dissolved organics on the rates of oxygen consumption and overall DO levels is not agreed upon in the literature. For example, Mathias and Barica (1980) suggest that DOC have limited effect on oxygen consumption under-ice. In contrast, when examined with a focus on light penetration in nutrient-poor systems, Karlsson et al. (2009) suggest that light penetration is the key driver of biomass growth and is directly influenced by the 'browning' effect of coloured DOC materials. In the above ground mesocosm systems, it is suspected that the inhibition of photosynthetic activity due to greater light attenuation may have a larger effect than in natural systems.

It is expected that under future climatic conditions, an increase in watershed-derived allochthonous material will be delivered to lacustrine systems in the mid-latitude cold-regions systems, resulting in the browning of surface waters (Roulet and Moore 2006) and the corresponding scattering of important PAR. In addition to the browning of

waters, it is predicted that an increase of opaque white ice in North America, as a result of increased precipitation and warmer temperatures (Dibike et al. 2012), will further diminish available PAR under ice-on conditions, potentially increasing the prevalence of anoxic conditions, particularly in shallow, nutrient-rich systems. This relationship is further complicated by the trend of shorter ice cover duration (Sharma et al. 2019) and reduction in overall lake ice thickness (Surdu et al. 2014).

Additional factors influencing DO values in seasonally ice-covered systems include biogeochemistry and atmospheric/meteorological conditions. While the relationship between phosphorus and lake productivity is well studied (i.e., Schindler et al. 2016), the relationship under-ice is comparatively less studied (Hampton et al. 2017). Similarly, a limited understanding of Nitrogen dynamics under winter conditions is only recently starting to be addressed (Powers et al. 2017b, a). It is suggested that additional controlled experiments examining the linkages between winter surface cover, biogeochemical, and biological community composition be undertaken. This is especially important as it is becoming increasingly apparent that the under-ice conditions of seasonally ice-covered lakes may affect primary producer community composition, and the subsequent ice-off conditions (Özkundakci et al. 2016).

Under future climate conditions, it is expected that the relationships between climate, seasonal ice cover, and under-ice productivity will continue to change. To the knowledge of the authors, the only study predicting shifts in ice cover composition/quality as a result of climate change were done by Dibike et al. (2012) using the MyLake one-dimensional model (Saloranta and Andersen 2007). Integrating this work along with predictive work on harmful algal blooms (*ie.* Wells et al. 2015) is

required to better understand the dynamic relationships between seasonally ice-covered lacustrine systems and seasonal phytoplankton community dynamics. To further understand these complex relationships, it is suggested that additional controlled experiments be undertaken with a focus on the inter-seasonal connections between surface cover, DOC, and plankton community structure and function. A long-term monitoring program with the capacity to detect inter-annual shifts in community composition is required to build robust predictive capabilities. Ideally, this would also be integrated with a comprehensive watershed scale analysis, linking stressors, landuse/disturbance, and hydroclimatic regimes to primary production trends year-round.

Given the unique nature and hydroecology of the small pond systems, the extension of these findings to larger water bodies should not be undertaken without additional sampling. Scaling up to a larger lacustrine system would likely result in substantially different hydrology and limnological processes being present and dominant. The findings of this study are particularly relevant for similar small systems that dominate the southern Canadian Prairies, in the prairie pothole region.

4.6 Conclusion

This study clearly indicates the importance of surface cover quality on the under-ice productivity as measured by DO and Chlorophyll-α. The presence of snow on ice, is an effective inhibitor of PAR transmission, thereby generally decreasing chlorophyll concentrations, in manipulated pond systems, though the connection is less clear in above-ground mesocosms. The presence of white-ice, as suggested by Prowse and

Stephenson (1986), also limits the transmission of PAR, but the results on underlying productivity were hard to decipher in this study, due to frequent freeze-thaw conditions over the duration of the experimental period. The relationships drawn from this study are more directly applicable to shallow ponds with limited winter in-flow or outflow, such as those common across the southern Canadian prairie pothole landscape.

An increase in dissolved organic carbon delivery to lentic systems, as a result of predicted changes in hydrometeorological conditions will further alter under-ice productivity in shallow systems. It is expected that the decreased light penetration through the pelagic zone will depress oxygen production, therefore reducing DO and altering planktonic community composition. This potential shift in food-web structure may result in alterations to ice-off community dynamics, and may result in changes to the presence, duration, and intensity of potentially harmful algal blooms. Additional research is required to improve quantifying the linkages between biological community composition responses as a result of alterations in DOC and surface cover conditions under climate change. This would aid in building an improved capability of predicting seasonal plankton dynamics under varying climate scenarios.

4.7 References

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4.8 ***Figures***

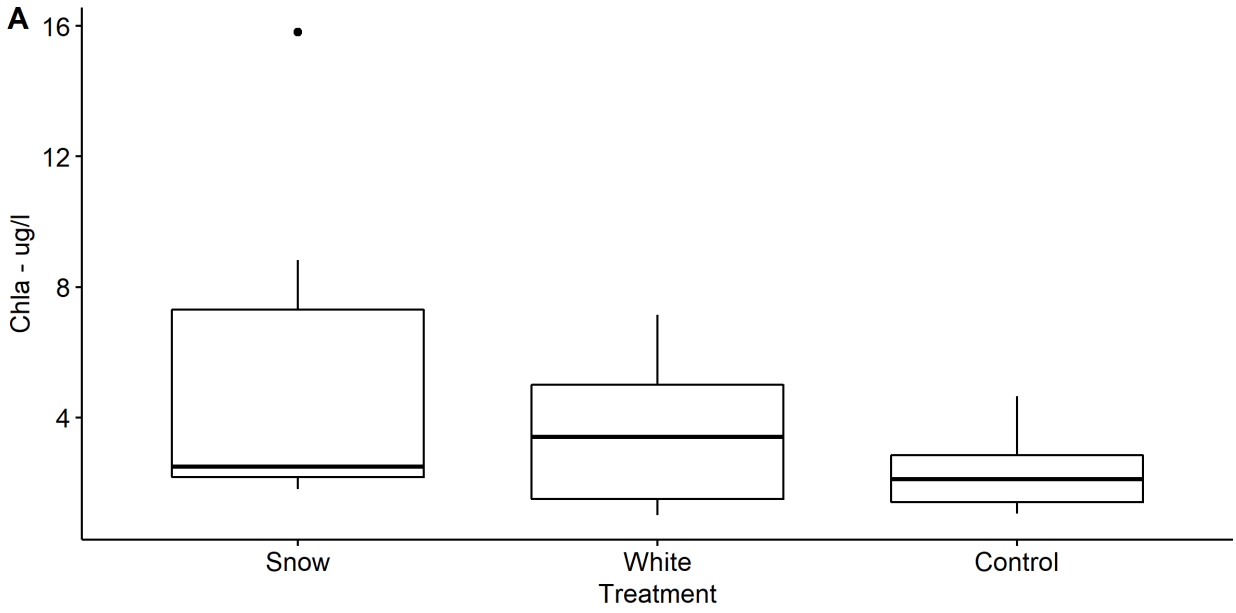

Figure 4.1 Chlorophyll-α values from mesocosm systems with three different surface cover conditions in the 2015/16 sampling period where white ice dominated the control systems Box plots line is median value, box spans the 25th and 75th percentiles, and whiskers are 1.5 x IQR (box). No significant differences present (ANOVA and Tukey's post-hoc test; all p-values > 0.05.

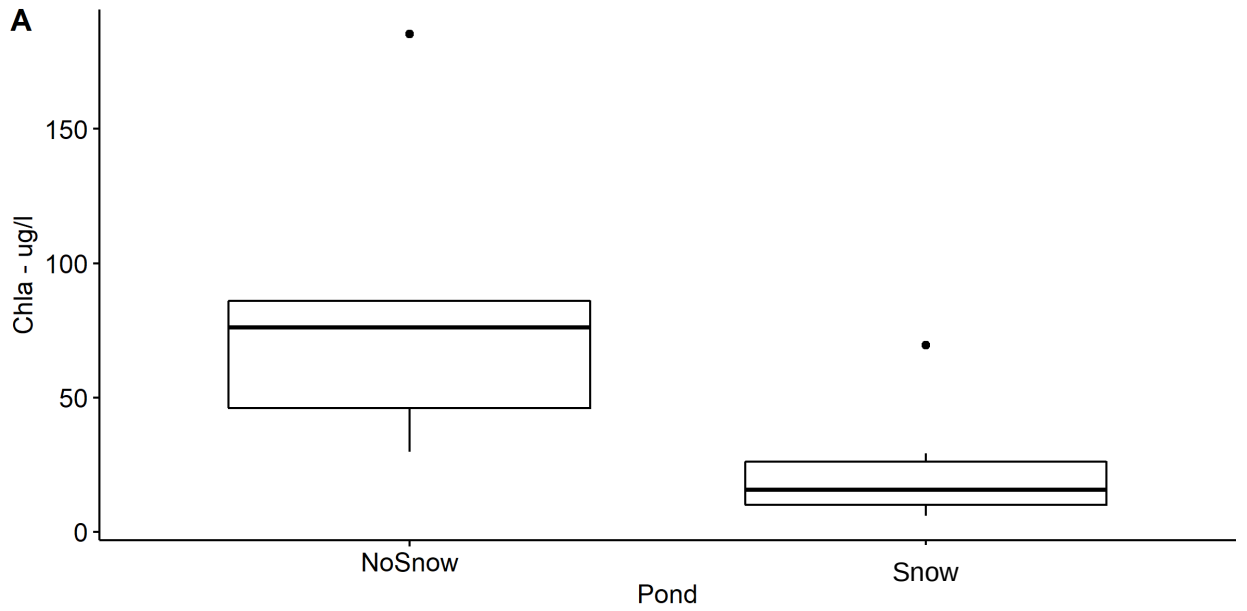

Figure 4.2 Chlorophyll-α values from experimental pond systems with a treatment (no snow) and control (snow) surface cover condition in the 2015/16 sampling period where white ice dominated the control systems. Box plots (top) line is median value, box spans the 25th and 75th percentiles, and whiskers are 1.5 x IQR (box). No significant differences observed using a Students' t-test (p = 0.07).

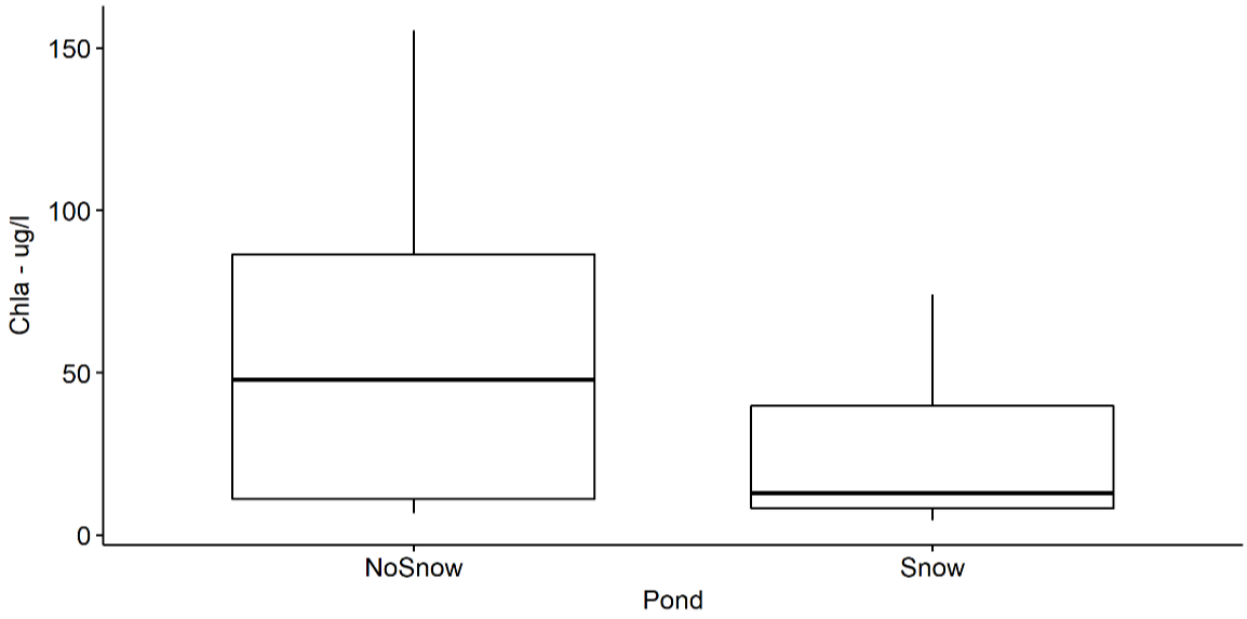

Figure 4.3 Chlorophyll-α values from experimental pond systems in the 2016/17 sampling period where black ice dominated. Box plots line is median value, box spans the 25th and 75th percentiles, and whiskers are 1.5 x IQR (box).

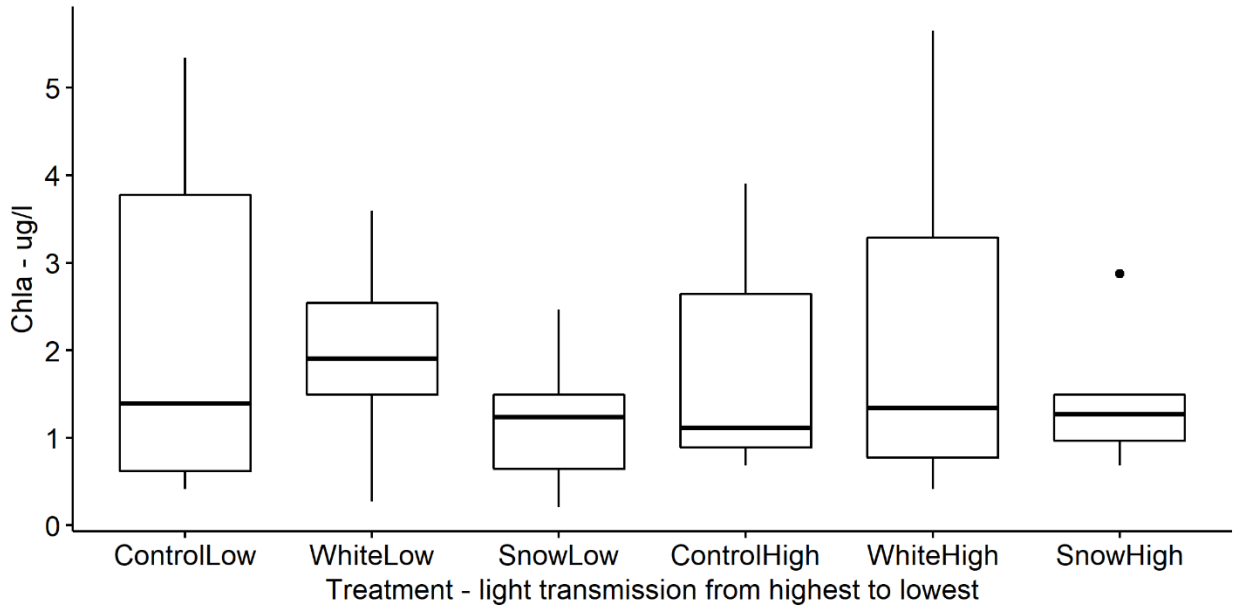

Figure 4.4 2016/17 Chlorophyll-α values from mesocosms. Mesocosms had both surface cover manipulation (control, snow added, white ice) and DOC manipulations (High or Low [control]) 2016/17 sampling period where black ice dominated the control systems Box plots line is median value, box spans the 25th and 75th percentiles, and whiskers are 1.5 x IQR (box). No significant differences present (ANOVA and Tukey's post-hoc test; all p-values 0.83 or higher)

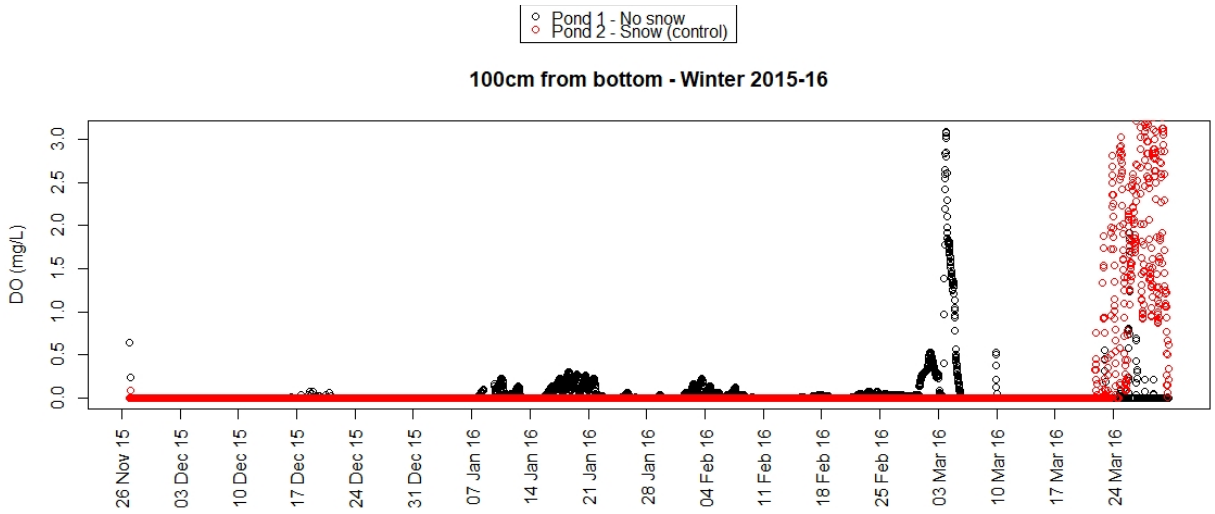

Figure 4.5 Dissolved oxygen levels in the experimental ponds at 100cm from pond bottom in the 2015/16 period where black dots represent the snow-removed pond, and red dots represent the control (snow present) system.

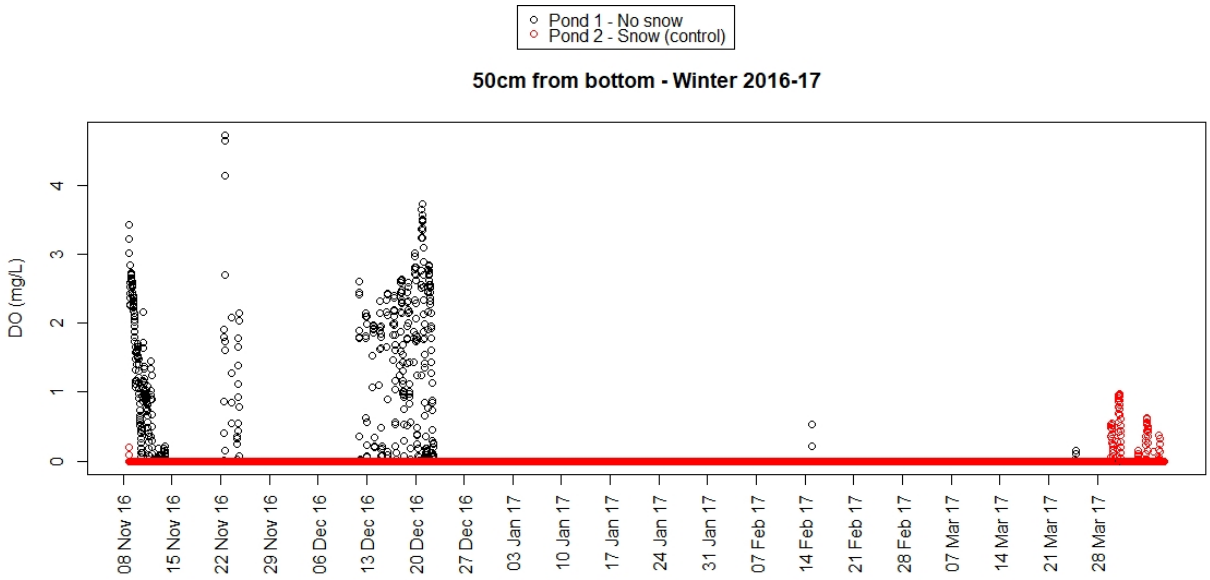

Figure 4.6 Dissolved oxygen (DO) levels in the experimental ponds at 50cm from pond bottom in the 2016/17 period where black dots represent the snow-removed pond, and red dots represent the control (snow present) system.

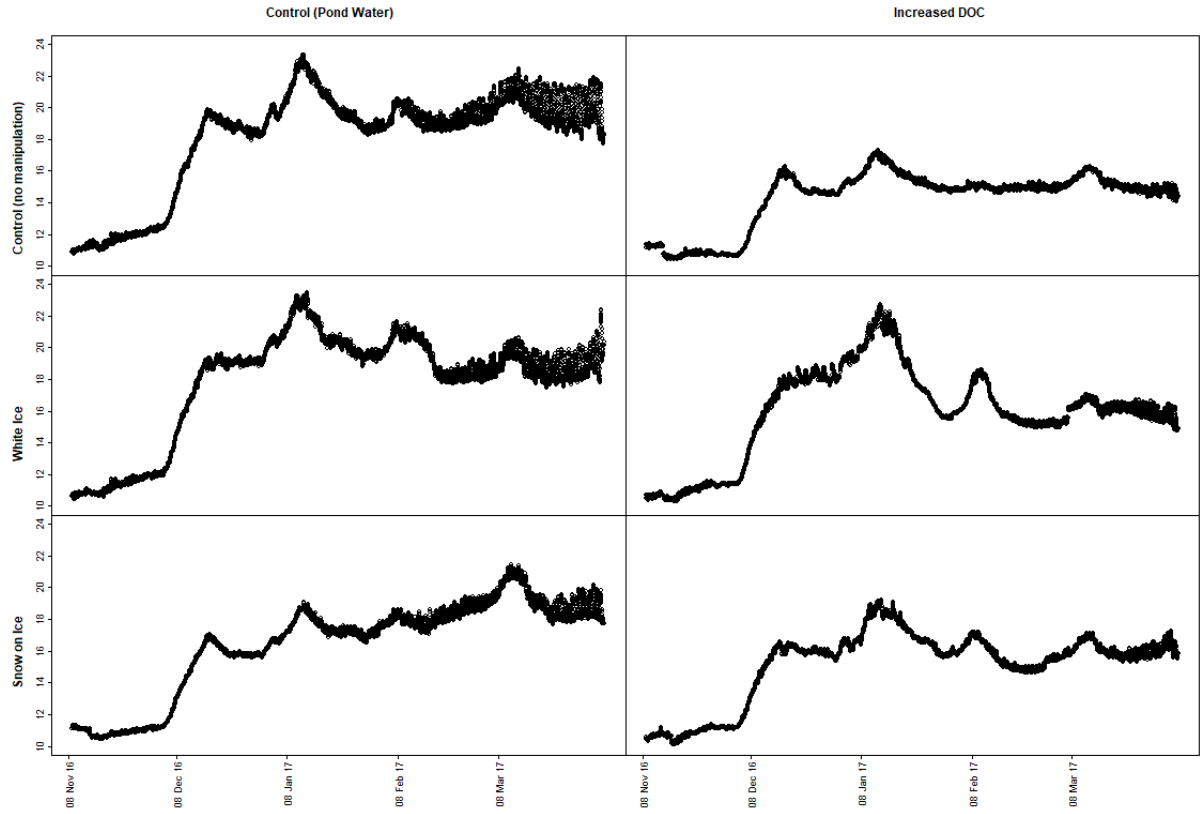

Figure 4.7 Dissolved oxygen levels in mesocosms under differing treatment surface cover conditions (rows) and the addition of Dissolved Organic Carbon (DOC) (columns). All treatments were significantly different when weekly aggregated means were used, using one-way ANOVA on ranks and Tukey's post-hoc test ($p<0.001$)

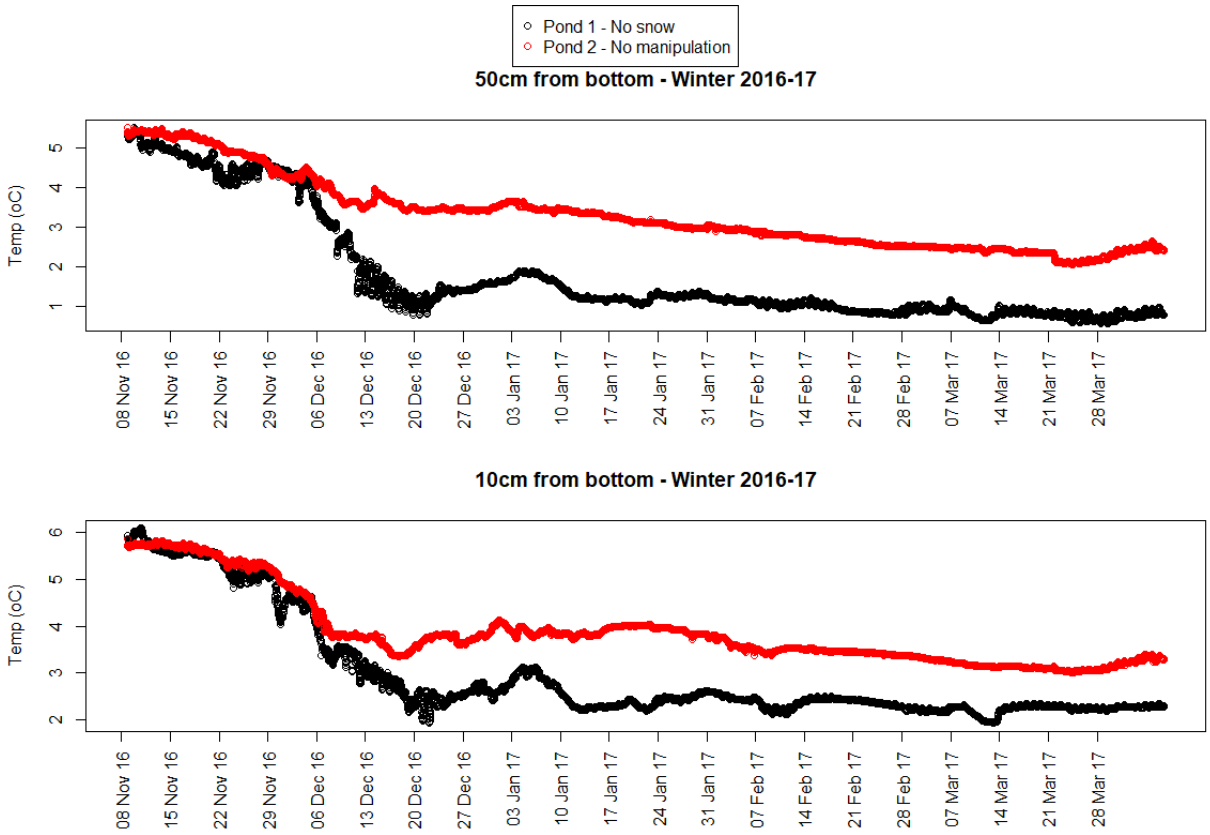

Figure 4.8 Pond temperature under two surface cover scenarios: 1) control (snow-on) and 2) snow mechanically removed. Differences between treatments were significant ($p<0.05$) using Students' t-test for analysis for both depths.

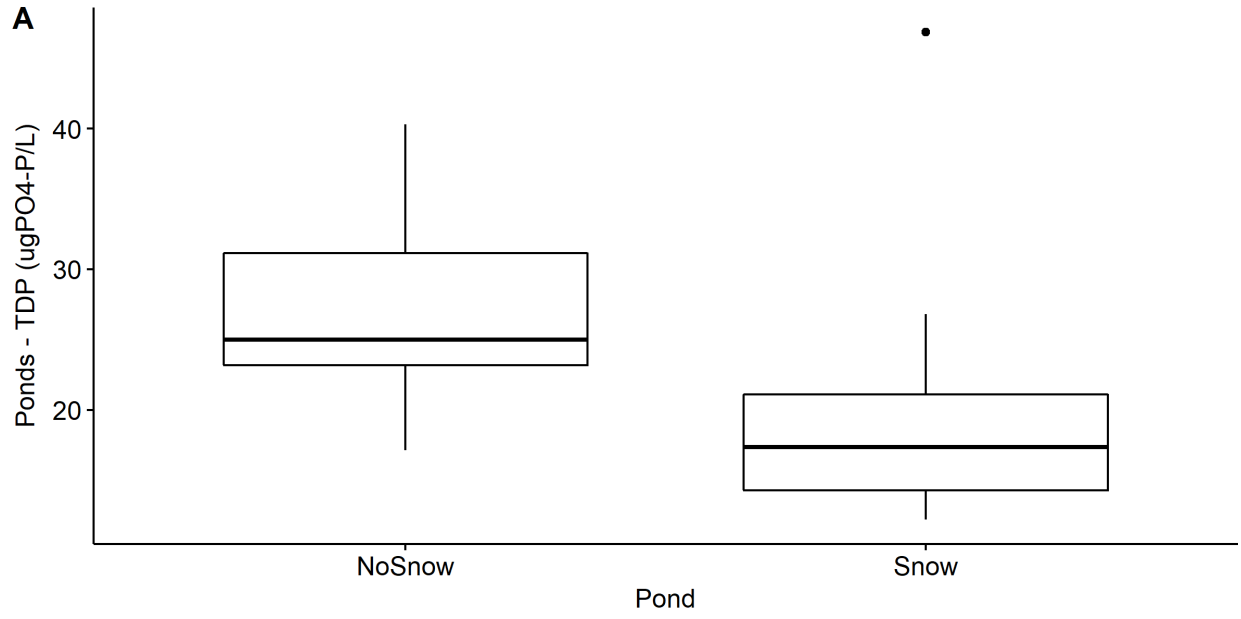

Figure 4.9 Box plots (top) line is median value, box spans the 25th and 75th percentiles, and whiskers are 1.5 x IQR (box). Mean values along with variance (bottom). Significant difference between ponds identified using a Students' t-test ($p = 0.04$).

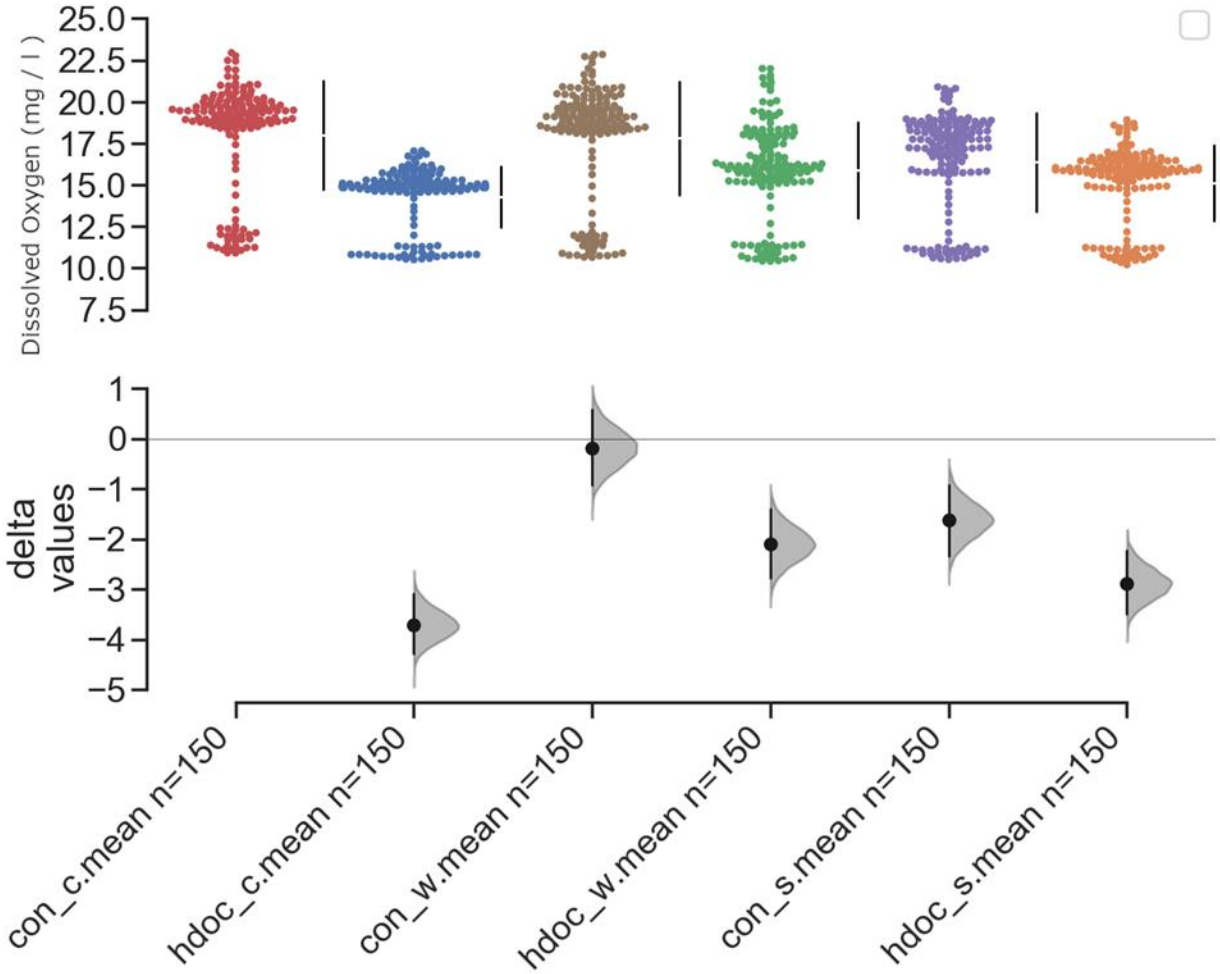

Figure 4.10 Daily averaged mesocosm DO differences using estimation statistics where: con=control, hdoc = high DOC, w = white ice, s = snow on ice, c = control (surface cover). Mean and standard deviation are shown by gapped lines to the right of each sample set, and effect size and 95% confidence intervals included in the lower component of the figure. Estimated distributions provide a visual on how different treatments are when compared to the control-control (DOC-surface cover) treatment.

5 Dissolved organic carbon and surface cover composition as drivers of under-ice productivity

5.1 Abstract

Seasonally ice-covered lentic systems are undergoing rapid change because of changes in both hydroclimatic conditions, as well as watershed processes that are altering the delivery of dissolved organic carbon (DOC) and nutrients. The influence of both temperature and watershed processes have been noted during summer months; however, only limited work has been conducted in winter conditions when ice and snow are present. Both surface cover conditions (quality and quantity), and the presence of DOC in the water column have direct effects on the photosynthetically active radiation available for pelagic autotrophic production. This study evaluates the impacts of changes in ice surface cover quality and DOC on under-ice biological activity, using the controlled experiment approach in both pond and above-ground mesocosm systems. Surface cover manipulations were conducted on 18 above-ground, polyethylene mesocosms as well in a pair of experimental ponds, in an attempt to replicate three surface cover conditions (black ice, white ice, and snow-on-ice). Additionally, DOC manipulations were conducted in the mesocosm systems, using a humic acid substance to create an elevated DOC treatment. Using dissolved oxygen (DO) and chlorophyll-α as water quality and biological endpoints, it was found that both surface cover and DOC concentrations significantly influenced under-ice biological activity. In pond systems, chlorophyll-α values were significantly lower when snow was present as a surface cover. The same pattern was not statistically significant in the mesocosm environments. Though the ponds became anoxic quite rapidly after the onset of ice cover, the removal of snow resulted in increased DO production, causing some mid-winter diurnal fluctuations to be observed. The addition of DOC in mesocosms resulted in the

decrease of DO, likely because of increased heterotrophic respiration processes. This study highlights the importance of understanding mechanistic relationships between physical drivers and biological responses during ice-on periods in lacustrine systems.

5.2 Introduction

Cold-region freshwater systems are set to experience a high rate of hydroclimatic change in the coming 50-100 years (Rouse et al. 1997; Duguay et al. 2003; Prowse et al. 2006a; Mueller et al. 2009; O'Reilly et al. 2015). These systems are sensitive to hydroclimatic changes, due to how small shifts in conditions can drive numerous ecological processes, particularly with regards to snow and ice cover (Wrona et al. 2005; Smol et al. 2005; Hampton et al. 2015, 2017). Recent studies focused on seasonally ice-covered lentic systems have highlighted the need for a more mechanistic understanding of how shifts in ice cover will impact under-ice ecological processes (Salonen et al. 2009; Özkundakci et al. 2016; Hampton et al. 2017; Pernica et al. 2017) especially alongside measured shifts in dissolved organic carbon (DOC) and nutrient regimes (Hudson et al. 2003; Couture et al. 2015). Crucially, total DOC involves both the mass component of dissolved carbon and a coloured component (cDOC) with cDOC being measured by absorbance Cuthbert and del Giorgio (1992). The cDOC fraction of total DOC is primarily influenced by humic substances which appear brown and can alter water column colour and optical properties (Molot and Dillon 2011). Contemporary lake-ice studies have been with a focus on the observed trend of decreased ice-cover duration (Magnuson et al. 2000; Benson et al. 2012) with little consideration given to changes in ice quality and quantity (i.e., thickness and extent).

With regards to seasonally ice-covered systems, surface cover conditions have the potential to influence a suite of biological responses (Salonen et al. 2009; Benson et al. 2012; Hampton et al. 2017).

In addition to light extinction being modified through lake surface cover, water column transmission can vary widely, influenced largely by the concentration of coloured-DOC (cDOC) (Thrane et al. 2014; Seekell et al. 2015). Increased cDOC concentration generally results in water-column browning as a result of the addition of humic acids, thus decreasing light transmission. Correspondingly, increases in cDOC have been found to suppress total chlorophyll a concentrations and phytoplankton abundance (Nicolle et al. 2012). Extending this relationship further, it could be expected in natural systems that increases in cDOC may result in a decrease in oxygen production due to decrease PAR availability and primary producer activity. This trend is occurring more frequently in freshwater systems around the world, as a result of changing hydroclimatic conditions (Roulet and Moore 2006; de Wit et al. 2016). Nutrient availability is also related to total DOC enrichment, with additional nutrients often being available as a result of increased cDOC (Williamson et al. 2015).

Coloured DOC is often discussed more simply as water colour in reference to the browning effect it has on lacustrine systems (Karlsson et al. 2009). Measurements of colour are done using a spectrophotometer, to measure the absorbance of light at a specific wavelength. With regards to cDOC, measuring the absorbance of light at 440 nm wavelengths has been used to measure colour (Reche and Pace 2002; Lennon et al. 2013; Burford et al. 2022).

Historically, ice-covered periods have been viewed as times with negligible biological activity, especially for primary producers (Wilhelm et al. 2014; Leppäranta 2015). This assumption is reflected in some of the early plankton models, such as the original Plankton Ecology Group model (Sommer et al. 1986). Though only 2% of peer-reviewed freshwater literature has considered under-ice processes (Hampton et al. 2015), recent work has highlighted the importance and complexity of under-ice ecological processes (Salonen et al. 2009; Twiss et al. 2012; Bertilsson et al. 2013; Powers and Hampton 2016; Powers et al. 2017b, a) and new understandings have been incorporated into updated models (Sommer et al. 2012).

Mid-latitude seasonally ice-covered lentic systems have highly variable ice conditions as a result of differences in the regional hydroclimatic conditions present when the ice is forming. A rapid freeze, along with limited precipitation, and horizontal mixing, allows for a translucent, or ‘black’ ice to form (Prowse and Stephenson 1986; Bolsenga et al. 1996). In contrast, fluctuating air temperatures, horizontal mixing, or precipitation result in the formation of highly opaque ice, or ‘white’ ice (Prowse and Stephenson 1986; Dibike et al. 2012). The addition of snow on ice cover and consequent slushing resulting from ice surface cracks and resulting upwelling also results in white ice formation (Prowse and Stephenson 1986).

The importance of photosynthetically active radiation (PAR) as a driver of primary production in lentic systems is widely documented (Bolsenga et al. 1996; Karlsson et al. 2009; Seekell et al. 2015). Less widely acknowledged is the role of role of winter surface (ice-snow) cover on the attenuation of PAR. Initial work by Prowse and Stephenson (1986) found a significant controlling effect of surface cover type and

quality on light transmission, and corresponding dissolved oxygen (DO) levels. The role of ice as a restrictive layer for atmosphere-lake interactions, is of particular importance for DO levels, in addition to light attenuation (Prowse and Brown 2010). The addition of fresh snow on top of an established ice cover was found to reduce the under-ice radiation to approximately 0, with a corresponding drop in DO (Prowse and Stephenson 1986). Spectral absorbance across the PAR wavelengths was also found to be influenced by surface cover quality and composition (Roulet and Adams 1986).

Coloured dissolved organic carbon (cDOC) is also widely acknowledged to have a substantial effect on PAR attenuation, with an inverse relationship between cDOC and PAR being present (Ask et al. 2009; Karlsson et al. 2009; Thompson et al. 2012; Godwin et al. 2014; Thrane et al. 2014; Seekell et al. 2015). Increases in cDOC delivery in watersheds across the globe are being measured and are expected to continue with predicted climatic change (Roulet and Moore 2006; Kissman et al. 2013; Williamson et al. 2015; de Wit et al. 2016). In Scandinavian lakes, Thrane et al. (2014) determined that the coloured fractions of cDOC and, more widely, coloured dissolved organic materials, attenuated the largest fractions of PAR, when compared with phytoplankton pigments and total phosphorus. Compounding the effect of cDOC on light attenuation in the associated delivery of nutrients from terrestrially-derived DOC contributions (Lennon et al. 2013; Kissman et al. 2017). In the Arctic environment, while terrestrial ‘greening’ is occurring, the browning of lakes due to permafrost thumping and watershed-related DOC (and corresponding cDOC) delivery is resulting in decreases in overall lake productivity (Moquin and Wrona 2015) and further highlights the complex relationships between changing hydroclimatic conditions as a driver of lake productivity.

To better understand the relationships between hydroclimatic and the resulting cryospheric conditions on lakes, models, such as the MyLake model (Saloranta and Andersen 2007) have been developed. When future climate conditions are applied to the freshwater systems North America and modelled using MyLake, increases in white ice thickness and slight decreases in snow depth are predicted; with a significant spatially variability visible (Dibike et al. 2011, 2012). At more northern latitudes, increased precipitation (snow) will result in increases in white-ice thickness. However, the inter-decadal trend of decreasing ice cover duration in freshwater systems (Duguay et al. 2006; Prowse et al. 2006a) further complicates the relationship between climate change and lentic productivity relationships. Additionally, the predicted shifts in allochthonous DOC/cDOC need to be considered and modelled alongside climate and lake physical conditions to determine its impact on biological activity under ice cover conditions. A better mechanistic understanding of these relationships will allow for increased predictive capabilities and adaptive measures.

The objective of this controlled manipulative study is to develop a better understanding of the interplay between winter surface ice-snow cover condition with cDOC and total DOC concentrations in affecting under-ice PAR and primary productivity relationships as measured by changes in autotrophic production (measured as Chlorophyll-a) and DO regimes. Building a better understanding of these relationships in experimental mesocosm environments will guide additional sampling approaches and experimental designs applicable to natural systems in future work.

5.3 Methods

This study utilized eighteen, 700 L polyethylene mesocosms, located at the Aquatic Experimental Facility (University of Calgary) in Alberta, Canada. The facility has two small experimental ponds which are supplied with water derived from the Bow River through the University of Calgary irrigation system. The mesocosms were all housed in a greenhouse to help limit environmental influences on treatments. All mesocosms were filled with water pumped from the pond systems at least 2 months post addition of irrigation water and as close to ice-on duration as reasonably possible. The pond systems supplying the mesocosms are known to be high in DOC with the mean winter concentration in 2015/16 being 12.2 ± 2.30 mg/L though the pond supply water (Bow River) has mean DOC concentration below 1.0 (1987-2018; Alberta Environment and Parks; Station AB05BH0010). Mesocosms were wrapped with a low-voltage heating cable to ensure some freeboard remained in the systems to a) prevent complete die-off of any organisms; and, b) prevent damage to the installed instrumentation from occurring.

Manipulation of DOC levels was achieved using approximately 300ml of an agricultural humic acid solution (Organo Hume ULTRA; http://www.hortimax.biz/BlackEarth/BlackEarth-Organo_Hume_ULTRA.pdf) in 9 mesocosms. The humic acid solution has 24% humic and fulvic acid content and contains 3.7 g/L of suspended solids. Mesocosms which received the humic acid addition are referred to as “high DOC” treatments. All manipulations were performed prior to freeze-up period and were mechanically mixed to ensure equal distribution of the solution.

During the ice-on period, a 5-foot auger drill bit was used to create a borehole for obtaining water grab samples. Samples were taken using flexible tubing and the syphon technique and all instruments and materials were triple rinsed in distilled water prior to the collection of another sample. Additionally, all sample containers were triple rinsed with sample, prior to collection. Chlorophyll-*a* samples were collected in opaque 1 L Nalgene bottles and were transported in a temperature-controlled environment back to the laboratory on the same day for filtering and processing.

After the addition of the humic acid substance, grab samples were collected, following all prescribed handling procedures, and processed through an analytical laboratory (Maxxam Analytical, Calgary) to obtain DOC concentrations. Each grab sample was also analyzed using a spectrophotometer to determine light absorbance at the 440 nm wavelength (Cuthbert and del Giorgio 1992; Lennon et al. 2013). This allowed a relationship between total DOC and colour (absorbance at 440 nm) to be established. All samples throughout the collection period were analyzed for colour and subsequently DOC values were calculated.

To simulate different surface cover conditions, snow was added to 6 mesocosms and deionized water and snow (slush) was added to an additional 6 mesocosms. The slush was created by mechanically mixing snow and de-ionized water on top of current ice and resulted in the creation of white ice conditions. The combination of manipulations resulted in 4 treatment and 2 control replicates (Table 5.1. Incoming and PAR measurements were taken to calculate the albedo of treatment and control surface covers.

To measure under-ice productivity, one HOBO brand DO sensor was placed at a depth of 10 cm from the bottom of the container bottom with a logging interval of 30 minutes. Additionally, water column chlorophyll-*a* samples were taken from each treatment application approximately every two weeks during the ice-on period. Additional samples were obtained prior to freeze up to provide background values.

Estimates of chlorophyll-*a* concentrations were obtained using the acetone-extraction method following Arar and Collins (1997). Filtration of samples through GF/C filters occurred within 24-hours of collection and filter papers were then wrapped in foil and stored in a -20°C freezer for subsequent analysis. To complete the analysis, filters were mechanically split into smaller pieces and ground into a slurry with acetone. The samples were then centrifuged in additional acetone and then read using a calibrated fluorometer. All grab samples were also processed for total suspended solids and ash-free dry mass, using standard procedures (Franson et al. 1998).

All statistical analyses, including ANOVA's, Students' t-tests, and summary statistics were conducted using the R analysis program (R Core Team 2019) or Sigmaplot 14. Due to the ability of estimation statistics (Ho et al. 2018) to better illustrate effect size, particularly with large sample sizes, the method was employed when examining the observed DO values. The use of estimation statistics also allowed for high resolution temporal data to be viewed in a way that emphasized differences based on treatment type.

5.4 Results

Similar to Cuthbert and del Giorgio (1992) and Lennon et al. (2013), the DOC-colour (as measured by absorbance at 440 nm) relationship was calculated using n=10 samples, with 6 from mesocosms and 4 from source ponds (Figure 5.1). Paired samples were analysed for both colour in the laboratory and DOC utilizing an analytical chemistry lab (Maxxam Analytical/Bureau Veritas). This allowed a significant linear relationship to be derived (R^2=0.52; p = 0.019). As this relationship was found to be significant, it was consequently used to estimate subsequent DOC values throughout the sample period.

The addition of a humic acid solution was intended to alter total DOC concentrations and decrease photosynthetically active radiation (via an increase in the cDOC fraction) within each treatment mesocosm equally. Utilizing grab samples taken at the onset of the experiment (i.e., within the first month of ice-cover formation) and the modelled relationship in Figure 5.1, the initial DOC concentrations between control (no DOC added) and elevated DOC treatments were found to be significantly different using a Student's t-test (P<0.001; Table 5.1). Similarly, the differences between calculated DOC values (pooled across surface cover conditions) were significantly different (P = 0.0481) between control DOC mesocosms and elevated DOC mesocosms (Table 5.1).

The calculated differences in DOC, as measured from a440, were also apparent visually, with the elevated DOC systems being notably darker in colour than the control systems (Figure 5.2). When analysed across the entirety of the sampling period, differences in DOC values were only statistically significant between the white ice control and white ice-high DOC treatment (Students t-test; P = 0.0434) once log-

calculated DOC were utilized. The control and snow added manipulations had no significant differences in log-DOC observed values across the experimental period with P-values (Students t-test, df = 5) of 0.09611 and 0.7075 respectively and a high variability in the data is evident (Figure 5.3).

A two-way ANOVA on log transformed values revealed no significant differences ($P>0.05$) in average chlorophyll-a concentrations either by DOC treatment or surface cover conditions over the experimental period, and no significant interaction was observed between the two treatments (Table 5.2). The boxplots in Figure 5.4 illustrates the variability in measured chlorophyll-a values across the experimental treatments.

In contrast to the observed lack of significant differences in average chl-a values across experimental treatments (Table 5.2), patterns in the rates of change and cumulative levels in under-ice dissolved oxygen were found to be significantly different depending on ice-cover type and water column DOC levels (Figures 5.5, 5.6, 5.7). By categorizing the continuous DO recording data according to the pre- and post- winter solstice periods, significantly different patterns were observed between the ice-cover treatment combinations and the addition of DOC.

For the experimental period preceding the winter solstice, using linear regression model fits for inter-comparative purposes, both the control ice-cover and white-ice treatments showed similar rates of DO accumulation (Figure 5.6: A, B), and a decrease in the rate of DO accumulation associated with the addition of DOC (Figure 5.6: D, E). In contrast, the snow-on-ice treatment displayed a lower rate of DO accumulation than the other two ice-cover treatments, and the addition of DOC did not change the observed rate of accumulation (Figure 5.6: C, F). Moreover, observed peaks in the DO

levels in control mesocosms with no surface cover manipulation were approximately 1.5x higher when compared to DOC added treatments (Figure 5.5).

During the post-solstice experimental period, the DO concentration in the control mesocosms were consistently above those in DOC-supplemented treatments, regardless of ice surface-cover (Figure 5.5; Figure 5.7). Mean post-solstice DO values were 19.73 ± 1.11 mg/l, 19.58 ± 1.24 mg/l, and 17.97 ± 1.25 mg/l for the control, white ice, and snow on ice treatments, respectively, with no DOC added (Figure 5.7). Correspondingly, the values over the same period for DOC added mesocosms were 15.19 ± 0.59 mg/l, 17.15 ± 1.85 mg/l, and 16.23 ± 0.94 mg/l for control, white ice, and snow-once ice, respectively, for mesocosms with additional DOC added (Figure 5.7). Both the control (no manipulation) and snow-on-ice cover treatments showed a significant ($P<0.05$) low and positive rate of increase in the DO accumulation over the post-solstice period (Fig 5.7 A, C), while all other treatment combinations displayed a decreasing trend (Figure 5.7 B, D, E, F).

These observed patterns are further reinforced when daily averaged DO values were examined using estimation statistics (Ho et al. 2018; Soukup 2019). differences were further evident, especially between DOC treatment levels (Figure 5.8), with DO levels in DOC added treatments being lower than those with no DOC manipulation. The differences between white ice and control surface cover were minimal, while the addition of snow on ice resulted in decreased DO levels, when compared to other surface cover treatments. Though the estimation statistics do not utilize the p-value statistic, confidence intervals are used instead and provide useful insight into the precision of the measured difference (Ho et al. 2018). As mentioned by Ho et al. (2018),

estimation statistics provide a visualization by which both magnitude and precision of changes can be observed and differences in distribution of values in different populations is easily identified. This allows for large datasets to be easily visualized and differences in population distributions and helps to emphasize the gradient of a distribution curve (Ho et al. 2019).

5.5 Discussion

The addition of humic acid fertilizer solution was undertaken to evaluate the responses of under-ice productivity to varied DOC concentrations alongside a range of ice surface cover conditions. Due to the nature of controlled mesocosms, some assumptions were required. Namely, the mesocosms represent pelagic water-column environments only, as the systems lack a sediment/benthic component of lacustrine systems. While this limits the applicability of the results to natural systems, it does provide an improved mechanistic understanding of the relationships between manipulated conditions and resulting biological endpoints. The observed statistically significant effect of adding a DOC supplement (Table 5.1) corresponds with the observed browning and discolouration observed the treated mesocosms (Figure 5.2). This further supports the findings of Lennon et al. (2013) who found that the use of humic acid fertilizers to produce elevated DOC levels in experimental systems works well.

The lack of benthic organisms may have limited the resuspension of settled cDOC (Hawley and Lesht 1995) therefore suppressing values slightly, especially in the post-winter-solstice period. Additionally, the above-ground nature and the type of mesocosms utilized in this experiment allowed for deeper ice formation than occurred in adjacent ponds. This resulted in a more rapid freezing, and as a result, may have increased the extrusion of DOC (Giannelli et al. 2001) and expedited settling due to flocculation (Von Wachenfeldt and Tranvik 2008).

The method of calculating DOC values from optical properties (absorbance at a specific wavelength, in this case 440 nm), worked well in this study during the

experimental period (Figure 5.1). This contrasts with other studies (Lennon et al. 2013) that have found this relationship to be only partially useful and highly variable. However, the relationship between water colour and DOC concentrations was found to not be consistent inter-annually (unpublished data), so caution is recommended when using this method, and it is suggested that the relationship be quantified annually to account for differences in DOC composition. When tested in years beyond the scope of this project, the initial linear regression equation did not maintain significance, and therefore was not used. As such, caution is urged for using the same equation between seasons or between locations (unpublished data).

To further strengthen the relationship between measured a440 values and cDOC/DOC in this environment, it is suggested that additional replication be utilized in future studies. Additionally, when the data is analyzed using estimation statistics, differences in mean and distributions of DOC values between control and treatment mesocosms becomes more evident (Figure 5.3). A more comprehensive sampling regime with additional replication would be of value to help strengthen relationships and further support identified trends. Additionally, the role of cDOC in comparison to total DOC needs to be evaluated further. Dissolved oxygen concentrations varied both temporally and when evaluated at over the entire ice-on period (Figure 5.5; Figure 5.8) with the greatest differences occurring in treatments where DOC was added. The addition of DOC appears to have resulted in a suppression of photosynthetic activity, likely as a result of the system browning and the corresponding degradation of light quality and overall quantity. However, observed differences in pre-solstice accumulated DO values under different surface covers also suggests that snow-on-ice to be a critical

factor seemingly suppressing under-ice photosynthetic activity as measured by DO rate of change over the pre-solstice period (Figure 5.5; Figure 5.6). The observed pattern in DO is likely a result of two different processes being affected by the addition of coloured DOC: 1) the humic substance solution used as an additive promoting increased heterotrophic activity due to increased nutrient availability (Steinberg et al. 2006), and/or 2) the coloured DOC solution further inhibiting photosynthetic activity via the diffusion of light (Ask et al. 2009; Thrane et al. 2014; Seekell et al. 2015). The effects of DOC addition on DO were very apparent, with all treatment mesocosms seeing a decrease in DO, under all surface cover conditions when the humic acid fertilizer was added (Figure 5.8). This illustrates the effect of DOC addition on under-ice primary productivity, though it requires additional controlled experiments to strengthen the relationships found here.

Notably, the lack of differences in chlorophyll-a concentrations across treatments suggests that comparable amounts of photosynthetic organisms are present in the different treatments, and that basal food web is perhaps more affected by light quality/quantity carbon availability through the addition of DOC. Further to this, the shift in dissolved oxygen values and rates of increase/decrease prior to and following the winter solstice (shortest day of the year), and the extremely high DO values suggest that organisms may reach a saturation point and, due to the inability for gas to escape through the surface water, reduce production (Long et al. 2020). The result of this capping process in the mesocosms is evident when evaluating water quality parameters (see previous chapters) where ice extrusion of nutrients and salts is apparent.

Current large-scale discussions of climate change suggest that increased productivity will occur in cold-regions due to increased mean air temperatures (Macias-

Fauria et al. 2012). Correspondingly, it is suggested that the increased availability and delivery of allochthonous carbon to freshwater systems will result in greater productivity as due to increased nutrient availability (Finstad et al. 2016). However, experiments conducted in the boreal region of central Canada have found dissolved organic carbon delivery to lakes to have decreased (Schindler et al. 1997). This suggests that the simplified arctic amplification/greening notion does not universally apply across all cold-region systems. This is further influenced by the complex relationships between Arctic warming and the addition of cDOC and sediments from thawing permafrost, which can result in “Arctic browning” as identified by Wrona et al. (2016). The identified process of browning due to the addition of sediments, results in more DOC and sediment can drive lake colour change (and correspondingly, cDOC) along with a clearing of lakes, due to flocculation of sediments. However, application of these results to Arctic systems would require further study due to the different environmental conditions influencing the region.

While humic substances do provide an energy source directly to food chains (Jones 1992), there are a variety of other physical factors, such as light availability, pH, and UV penetration, that are influenced by organic material in the water column (Creed et al. 2018). Mesocosm experiments in this study showed decreased DO levels in environments enriched with DOC (Figure 5.5) suggesting either, or a combination of, a) increased heterotrophic activity consuming available DO, or b) decreased photosynthetic activity due to limitation of PAR. As summarised by Creed et al. 2018, the relationship between dissolved organic matter, and by extension DOC, is complex (see Creed et al 2018; figure 3).

Water column primary production is driven by phototrophic organisms that utilize the 400-700 nm PAR wavelengths (Kirk 2010). Though it is noted that the aquatic medium results in some light attenuation (Kirk 2010), especially in relation to DOC content, the extinction coefficients of black ice (~0.5 – 1) and white ice (~6 – 6.25) found by (Roulet and Adams 1986) are of much greater influence. Samples taken from ponds adjacent to the mesocosm studies, during other sampling periods were calculated at 1.66 ± 0.22 (n=5; 2016/17) for black ice and 9.99 ± 4.06 (n=4; 2015/16) for white ice. Snow-on ice resulted in a considerably higher extinction coefficient of 20.98 ± 16.87 (n = 5; 2015/16 & 2016/17). Black ice in the mesocosm environments was not completely transparent, due to the temperature fluctuations during the sample periods, which may account for the comparatively high light extinction rates found in this study. Reduced photosynthetic activity is evidenced by the suppressed DO levels in mesocosms with manipulated surface covers (Figure 5.5).

Due to the size of the mesocosm environments, an ice core was unable to be retrieved and, therefore, no analysis of ice composition aside from albedo and transmittance, were able to be collected. Additional information on the quality of the ice cover and the control it exerts relative to water column extinction rates, would aid in understanding how productivity relationships may change under future hydroclimatic conditions.

5.6 Conclusion

Seasonally ice-covered lentic systems are experiencing increasing rates of hydroclimatic variability, including decreases in ice cover duration (Duguay et al. 2006; Lopez et al. 2019), quality (Prowse et al. 2006a), and quantity (Brooks et al. 2013; IPCC 2019). These shifts have resulted in changes to winter surface cover characteristics and carbon cycles. Experiments conducted in this study using controlled mesocosm environments found surface cover quality (white ice vs black is vs snow-on-ice) to have limited effects on the overall biomass of chlorophyll-α with no significant differences in chlorophyll-α biomass occurring as a result of surface cover manipulation or DOC/cDOC manipulation being observed. In contrast, the addition of cDOC also was found to affect under-ice dissolved oxygen levels with cDOC-enriched mesocosms seeing decreased DO, likely a result of a reduction in PAR values with a secondary driver perhaps being increased heterotrophic activity, driven by the addition of labile carbon nutrients. High DO values alongside the relatively static chlorophyll-α biomass values measured among treatments, suggests that the supersaturation of the mesocosm environments, as a result of the presence of an ice ‘cap’ on the tops of the systems, may also have complicated the interpretation of results in this study.

The work conducted in this study is preliminary in nature and needs additional experimental work to further support and validate the results conveyed. Controlled experiments conducted in larger systems, such as larger experimental ponds that have a more complex food web structure and have some greater level of permeability with above-ice processes would be beneficial in further developing the productivity relationships observed in this study. In-ground or in-pond mesocosms would also help

to address the issue of light ingress and would be closure to natural conditions. Additional work quantifying the role of heterotrophic/microbial respiration specifically is also required to better understand the productivity-respiration balance under varying surface cover conditions and improving water management decisions related to adapting to future climate change scenarios.

In future, it can be expected that cold regions lake systems will experience decreases in DO, as a result of decreased light availability (increased white ice and snow-on-ice), and increased delivery of cDOC. These shifts in under-ice processes will likely have a corresponding effect on ice-free periods, though more research is needed to address the role antecedent conditions play on lake system trophic relationships.

5.7 References

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5.8 Figures and Tables

Table 5.1 Student t-test results comparing calculated DOC levels for control and DOC-added mesocosm treatments using the derived DOC-a440 relationship (Figure 5.1) at both onset (November 2016: first month of ice-on) and at the end of the ice-on experimental period (March 2017) across surface condition types.

DOC Treatment	N	Mean (mg/l)	Std Dev	Time Period
Control (No DOC added)	6	8.720	0.631	Initial
High (DOC added)	6	13.080	2.116	
Two-tailed P-value	<0.001			
Student-t (10 df)	4.837			
Control (No DOC added)	3	6.397	2.618	Final
High (DOC added)	3	10.700	0.398	
Two-tailed P-value	0.0481			
Student-t (4 df)	2.815			

Table 5.2 Average chlorophyll values by DOC treatment and surface ice conditions were not significantly different over the experimental period and there was no significant interaction between surface cover and DOC conditions. Two-way ANOVA was run using SigmaPlot 14 and used log transformed chlorophyll values to correct for normality.

DOC Treatment	Surface Treatment	N	Mean chlorophyll a (mg/l)	Std Dev
DOC added	Control	6	1.798	1.371
Control	Control	6	2.244	2.146
DOC added	Slush added	6	2.210	2.100
Control	Slush added	6	1.964	1.135
DOC added	Snow added	6	1.422	0.777
Control	Snow added	6	1.193	0.806
ANOVA – Run on log mean values				
Source of Variation	**DF**	**SS**	**F**	**P**
Surface Cover	2	0.141	0.482	0.622
DOC	1	0.0171	0.117	0.735
Surface Cover x DOC	2	0.0480	0.164	0.850

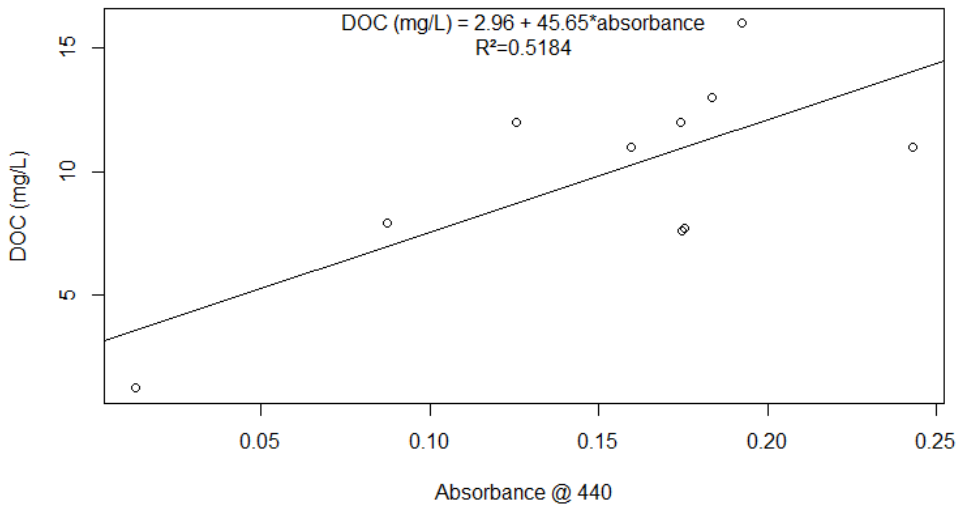

Figure 5.1 A linear regression model and resulting equation correlating colour measurements from spectrophotometric analysis (a440) and DOC (lab calculated). N=10

Figure 5.2 Visual comparison of DOC addition (lower 2 and 5th from the bottom) compared to control (3,4,6,7 from bottom) mesocosms

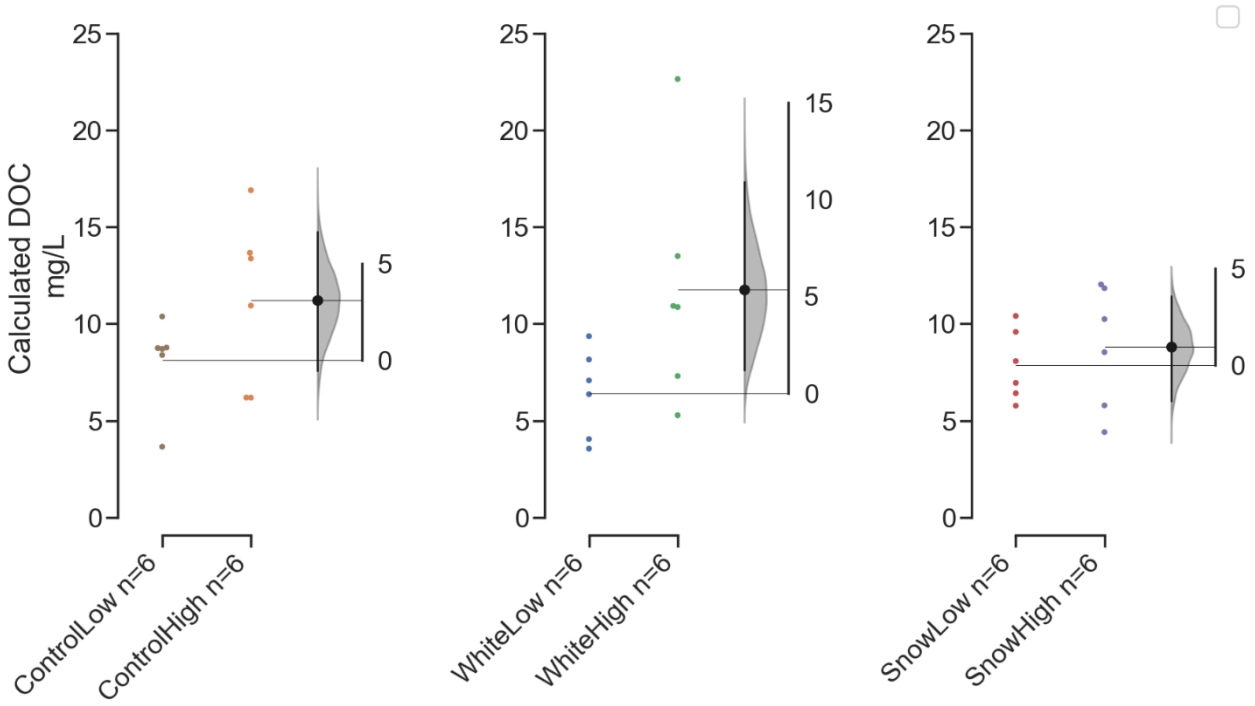

Figure 5.3 Mesocosm Dissolved Organic Carbon (DOC) differences using estimation statistics, showing differences in mean (horizontal lines) and distributions, where Control, White, Snow corresponds to surface cover type and Low/High correspond to DOC treatment.

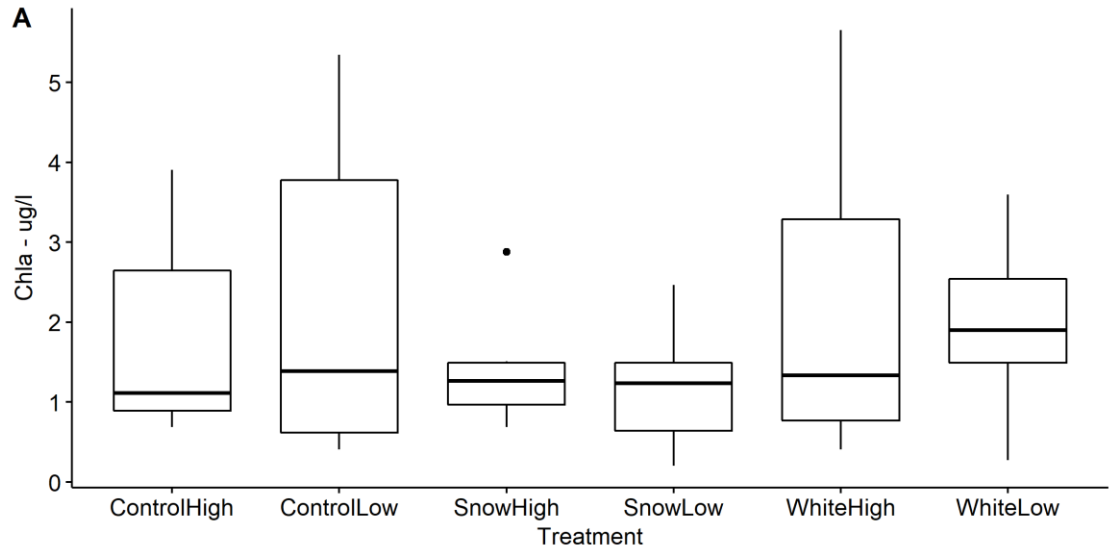

Figure 5.4 2016/17 Chlorophyll-α values from mesocosms. Mesocosms had both surface cover manipulation (control, snow added, white ice) and DOC manipulations (High or Low [control]) 2016/17 sampling period where black ice dominated the control systems Box plots line is median value, box spans the 25th and 75th percentiles, and whiskers are 1.5 x IQR (box).

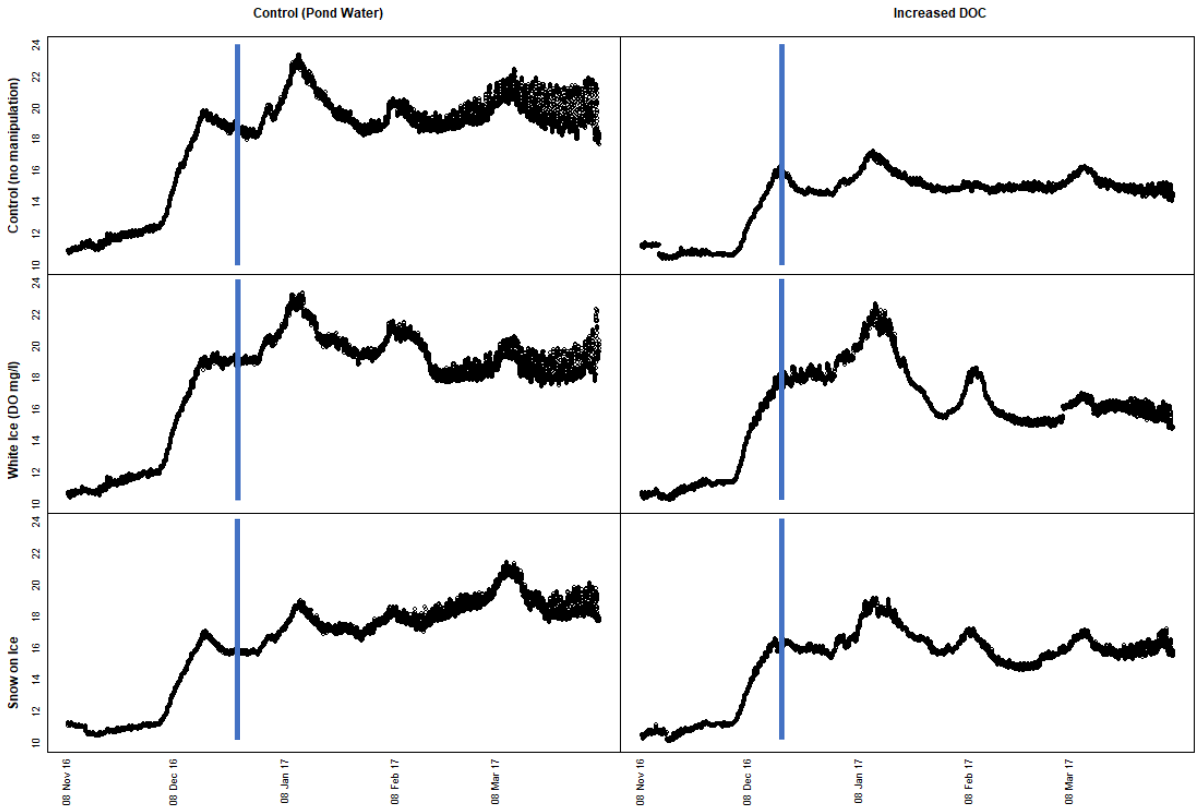

Figure 5.5 Continuously monitored and cumulative dissolved oxygen (DO) values in mesocosms under differing surface cover conditions (rows) and the addition of DOC (columns) over the winter 2016/17 field season. Vertical line designates winter solstice.

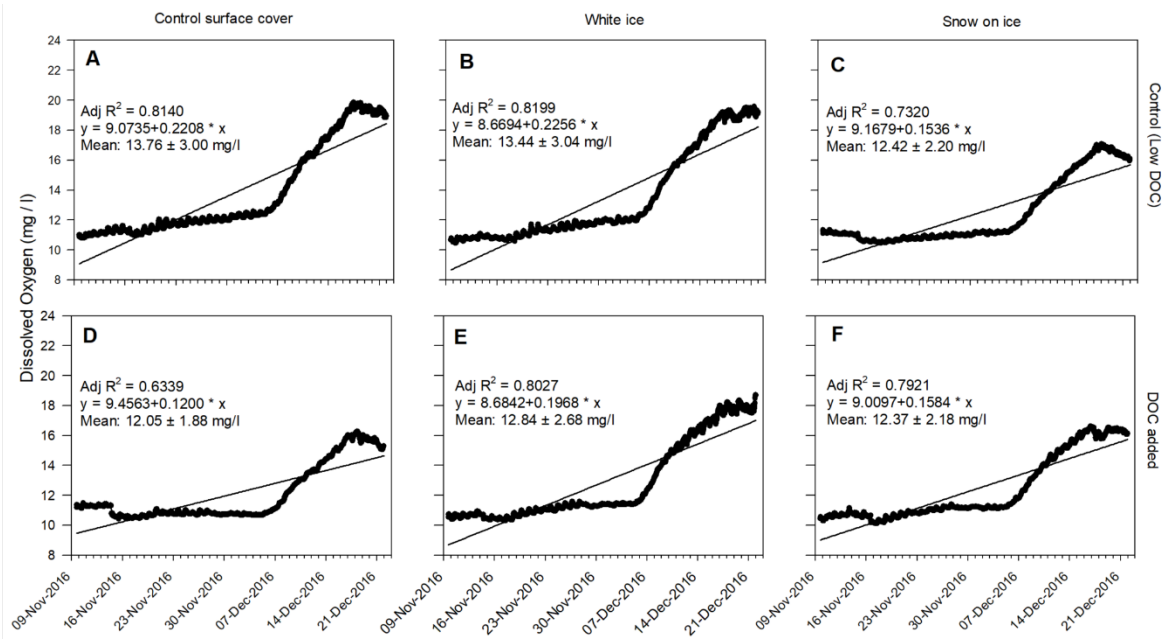

Figure 5.6 Dissolved oxygen trends from initial freeze-up until the winter solstice (21 December 2016) under different DOC and surface cover regimes. Linear regressions and corresponding slope equation calculated in SigmaPlot 14 and correspond to rates per day (x) of oxygen accumulation within the systems. Treatments as follows: A) Control (no surface cover manipulation-control (low DOC), B) Control – white ice, C) Control – snow on ice, D) High DOC (DOC added) – Control surface cover, E) High DOC – white ice, F) High DOC – snow on ice. N = 2032

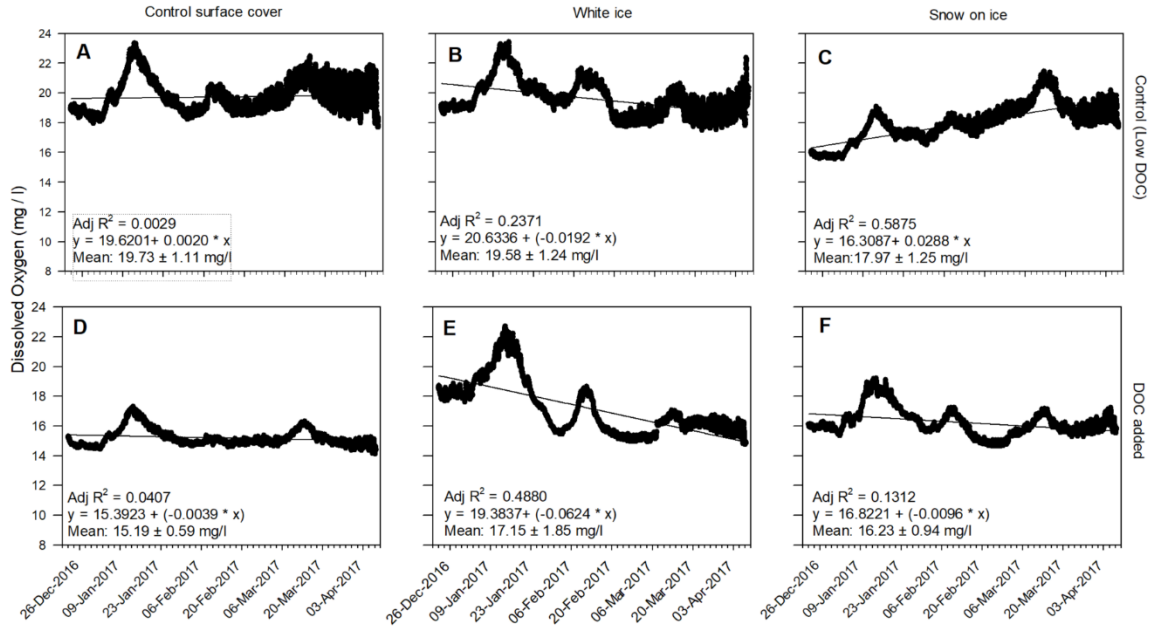

Figure 5.7 Dissolved oxygen trends from winter solstice (21 December 2017) to the end of the experimental period under different DOC and surface cover regimes. Linear regressions and corresponding slope equation calculated in SigmaPlot 14 and correspond to rates per day (x) of oxygen accumulation within the systems. Treatments as follows: A) Control (no surface cover manipulation-control (low DOC), B) Control – white ice, C) Control – snow on ice, D) High DOC (DOC added) – Control surface cover, E) High DOC – white ice, F) High DOC – snow on ice. N = 5107

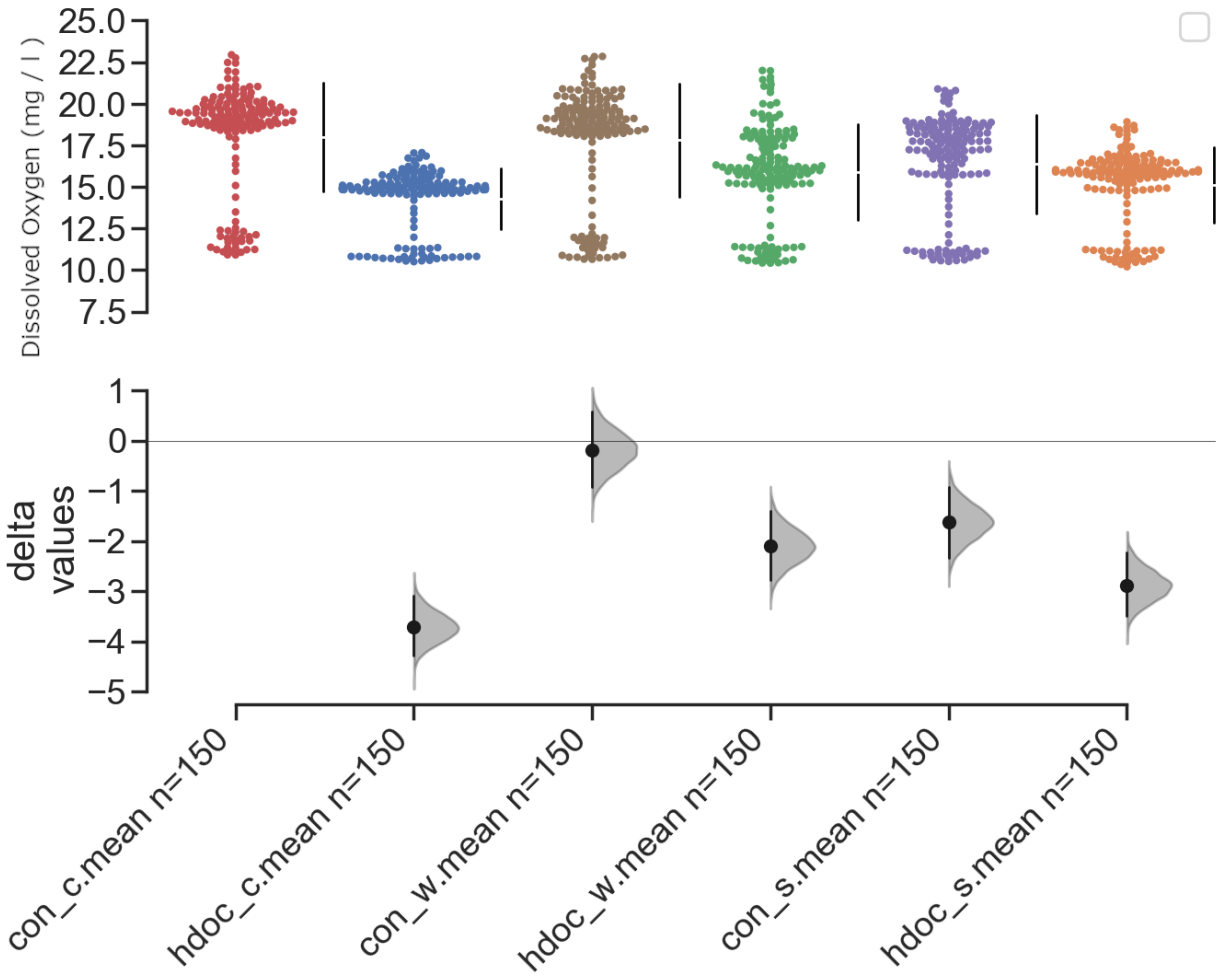

Figure 5.8 Mesocosm mean daily DO value differences using estimation statistics where: con=control DOC (no addition), hdoc = high DOC, w = white ice, s = snow on ice, c = control surface cover (no manipulation). Delta change values are relative to the control-control (DOC-Surface cover) condition.

6 Synthesis

It has been acknowledged that ongoing climate change will have a myriad of watershed-related effects, particularly for northern seasonally ice-covered lacustrine systems (Hampton et al. 2015, 2017; Prowse et al. 2015; Wrona et al. 2016). The effects of predicted shifts in surface cover quality (Dibike et al. 2012) on under-ice biological activity have been poorly quantified and were focused primarily on the physical aspects of surface cover (Prowse and Stephenson 1986; Bengtsson 1996; Vincent et al. 1998). This focus on physical conditions extends to modeling efforts, which have had limited biological components integrated (Saloranta and Andersen 2007; Sommer et al. 2012). More recently, there has been an increased awareness of, and acknowledgment that mid-latitude seasonally ice-covered systems are still active during the ice-on period, in contrast to the long-held belief that it was a period of low productivity and little interest (Jewson et al. 2009; Bertilsson et al. 2013; Pernica et al. 2017; Powers et al. 2017b). This relationship between ice-cover and under-ice processes will be of increasing importance as we continue to experience rapid environmental change which will directly drive shifts in observed hydrometeorological conditions during the ice-on period (Alexandra and Id 2019).

This study attempted to bridge the gap between changes in physical surface ice cover conditions and underlying chemical/biological conditions, using DO and chlorophyll-α as ecologically relevant endpoints. Through the use of controlled experiments, both in isolated above-ground mesocosms, as well as manipulated hydrologically disconnected shallow ponds, this research quantified the ecological biogeochemical and ecological impacts of various surface cover conditions over two

experimental winter periods. The use of small-scale, controlled, experimental systems is key to building a mechanistic understanding of the scaling-related relationship between surface cover conditions and under-ice environmental responses.

It was found that, as expected, snow-on-ice had a significant effect on under-ice optical transmission, when compared to black and white ice conditions. The high albedo, particularly of fresh snow, explains the effectiveness of this surface cover to limit light availability to negligible amounts (Warren 1982; Jewson et al. 2009; Garcia et al. 2019). This study found that the presence of snow-on-ice on experimental ponds limited light transmission to a mean seasonal value of just 4% of the incoming radiation in the PAR spectrum. This is in contrast to a seasonal mean transmission value of 33% for a black ice surface cover condition. The corresponding seasonal mean albedo values were 0.74 and 0.29, respectively. Similar albedo values were observed in controlled mesocosm conditions, aside from the snow-on-ice treatment, which was slightly decreased (albedo of 0.56), likely due to the modification of snow properties when moving and applying the treatment (i.e., compaction of snow).

Light availability for primary production has been identified as a key physical driver of affecting autotrophic food web structure and function in freshwater systems (Karlsson et al. 2009; Hrycik and Stockwell 2019). While the presence of snow on ice had the greatest effect on under-ice PAR, an effect was also noted under white-ice conditions, when compared to black ice. Light limitation, as a result of ice cover quality and the presence of snow, is vitally important under the predicted shifts in precipitation and temperature regimes expected in northern mid-latitudes (Dibike et al. 2012). A majority of the regions in which seasonally ice-covered lake systems exist in North America are

expected to either see an increase in precipitation and/or an increase in mean winter temperatures, and potentially temperature fluctuations. As a result, it is expected that an increase in white ice depth and snow-on-ice accumulation will occur, especially in smaller aquatic systems such as small lakes and ponds, where windblown redistribution of snow is minimal. If these conditions are realized, it is expected that there could be significant reductions in the availability of PAR during winter ice cover months. This will likely have direct implications on under-ice biological activity and productivity, as well as many physical processes that are radiation-driven, such as radiative or convective mixing (Pernica et al. 2017).

If PAR is reduced, due to surface cover conditions changing, it is expected that there will be a corresponding decrease in autotrophic productivity under-ice (Hampton et al. 2015). In shallow lentic systems, this reduction in related oxygen production will likely lead to an increased incidence of hypoxic or anoxic conditions once systems become completely ice-covered. Both dissolved oxygen levels and chlorophyll-α biomass are frequently used as water quality and biological endpoints for productivity as they are biologically relevant indicators and changes in their relative values can provide important information on primary production/respiration relationships in freshwater systems.

This study further quantified the relationships between surface cover conditions and under-ice chlorophyll-α values. The greatest impact was noted between surface covers that included snow, when compared to those that had no snow present. When snow was present on ice in the pond experimental systems, there was a significant decrease in autotrophic production as measured by chlorophyll-α values when compared to white

or black ice conditions, suggesting that the there is a direct impact of PAR availability, as controlled by surface cover, and overall pelagic productivity in these small systems.

Another variable that can directly influence under-ice pelagic light regimes is cDOC concentrations, with the observed trend generally being elevated cDOC concentrations resulting in decreased light transmission (Ask et al. 2009). While cDOC also provides nutrients, particularly for the heterotrophic/microbial food web, it is effective in significantly reducing light transmission and can therefore inhibit biological activity such as autotrophic production that is light dependant. The manipulation of cDOC was only conducted in the above-ground mesocosms and although differences in mean chlorophyll-α were present, they were not found to be significant ($p > 0.05$) and require additional replication to confirm if the trend is present. However, this may be due, in part, to the walls of the mesocosms not being opaque to lateral light penetration, and above ground, allowing for light to enter from multiple directions as opposed to exclusively through the manipulated ice cover.

Dissolved oxygen (DO) is a water quality endpoint that is frequently used to evaluate biological productivity and the ability of the system to sustain aerobic organisms. Though there are numerous factors that influence DO levels under-ice (Mathias and Barica 1980; Baird et al. 1987; White et al. 2008), from a biological perspective it can most simply be viewed as the difference between production and respiration of oxygen by aerobic organisms; additionally, the demand for oxygen in chemical processes, such as oxidation, can be substantial especially within benthic sediments and should be considered when evaluating entire lacustrine systems as opposed to the pelagic component of lakes (Coenen et al. 2019). While the use of DO

measurements is crude and does not give insight into specific production or respiration rates, it can be useful in determining the relative abundance of activity. It is also particularly of interest for oxygen-dependant organisms, such as fish (Barica and Mathias 1979; Shuter et al. 2012).

The hydrologically disconnected experimental pond systems used in this study reached anoxia extremely quickly after ice-on occurred, suggesting that there was more respiration occurring than production. Notably, when black ice was present, diurnal trends in mid-winter were sometimes visible, with DO levels rising above zero, suggesting a periodic increase in autotrophic production. It is expected that such responses may be of increased importance in larger lentic systems where DO is low, but not zero, and small variations in production may control whether hypoxic or anoxic conditions are present or not. As an increase in lower-transmittance surface covers (snow and white ice) is observed, it is logical, based on the results from these experiments, that there will be a net decrease in oxygen production during ice-on periods, assuming there is no change to events that atmospheric oxygen or oxygenated water (i.e., major freeze-thaw events producing cracks/openings, or increased streamflow introducing oxygenated water).

Complicating this relationship is the influence of cDOC, particularly on heterotrophic organisms, such as bacteria. When DOC was added to the mesocosm systems, following Lennon et al. (2013), there was a direct impact on DO levels, with elevated DOC correlating to a drop in dissolved oxygen. This is likely due to the increased availability of nutrients for heterotrophic organisms, thus promoting respiration and causing a net decrease in dissolved oxygen. However, it should be noted that

manipulations of DOC were only done in the mesocosm environments and would need additional larger-scale controlled experiments to be performed to further validate the generalization of the results.

With continued increases in human activity and ongoing climatic change, it is expected that a shift in DOC composition (Williams et al. 2016) and concentrations (Zhang et al. 2010) will be observed, with the general trend being increased delivery to freshwater systems. If DOC delivery does continue to increase, it would be logical the increased nutrients available for heterotrophic organisms, along with the corresponding light availability for primary producers, would drive an overall decrease in dissolved oxygen levels during ice on periods, though some freshwater systems may experience difference trends as a result of Arctic browning as a result of thawing permafrost and the delivery of sediments (i.e. Thompson et al. 2012; Moquin and Wrona 2015).

Controlled experiments are a vital research method to better understanding mechanistic relationships between variables, as they allow for more granular control, and a treatment-response method of testing hypotheses. However, the paired pond systems, and the mesocosm system approaches used in this study do have their respective limitations, as outlined in previous chapters. The main limitation of both of the systems used in this study is the lack of hydrologic connectivity; however, this is representative of the dominant prairie pothole topography found across the Canadian prairies. To better understand how the increased food web complexity and physical conditions of larger systems influence the relationship between surface cover and biological activity, larger-scale experiments and field validation is required. It would be advantageous to also utilize field experiments, using a gradient of both nutrient levels,

and hydroclimatic conditions, to evaluate whether the results found in this study are applicable in other lentic environments. This would also provide additional data, and aid in the development of biological components to winter lake modeling systems.

6.1 Implications

The applications of the findings here and any future work that builds on this research are numerous. Determining the role of winter surface cover conditions on biological activity helps to provide a more complete understanding of winter freshwater ecology in systems that are seasonally ice-covered. Suggested work around examining phytoplankton community composition and additional DOC research will further enhance our ability to link specific environmental drivers to under-ice, and corresponding ice-free conditions to potentially harmful algal blooms (HABs). These inter-seasonal connections, which require further research, would aid lake management decisions, and could provide advance warning around when deleterious conditions may occur. Additionally, the work here helps to elucidate how changes in surface cover quality and composition may influence the overwintering of phyto- and zooplankton communities. Additionally further studies in additional regions with different hydroclimatic conditions would be valuable to understand the applicability of these results in those areas.

The methods developed and executed in this project are novel and were developed for ease of sampling and low costs. This would make them ideal to be incorporated into regional lake monitoring programs, and therefore aid in the management decision making process. The findings and data from this project will be made available to government and academic research collaborators, with the intent that they will be

incorporated into relevant modeling efforts and future decision-making processes. This work more broadly fills a research gap addressed by numerous international science assessments (Wrona et al. 2013, 2016), and research syntheses (Hampton et al. 2015, 2017; Powers and Hampton 2016). This work directly advances the state of knowledge pertaining to the hydro-ecology of cold-regions aquatics systems.

The results from these studies suggest that surface ice cover plays an integral role in the function of under-ice lentic food webs. It also suggests that, in small hydrologically disconnected systems, surface cover conditions as opposed to time of ice-on sampling is the major driver of chlorophyll-α concentrations. As climatic change continues to occur, it is expected that there will be ongoing transformations of food web structure and function in northern mid-latitude seasonally ice-covered lentic systems. The importance of understanding the mechanistic relationships between the physical (surface cover) and biological (chl-α) is crucial to building a better understanding of winter activity under the ice (Powers and Hampton 2016). Current under-ice modeling efforts have been focused primarily on physical conditions (Saloranta and Andersen 2007), primarily due to the limited under-ice ecological research conducted. The results of this work may be helpful in refining process-based models to predict the influence of surface cover on under-ice biogeochemical conditions. Moving forward, it is imperative that we better integrate the physical-biological relationships that are known and continue to advance our knowledge of these relationships through controlled experiments and field validation.

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7 Appendices

7.1 Appendix A – SOP's

7.1.1 Acetone extraction of chlorophyll-α

UNIVERSITY OF CALGARY

Acetone Extraction of Water Column Chlorophyll
Standard Operating Procedure

Faculty/ Department or Institute/Centre	Biological Science	Date Created:	2 Nov 2015
		Created By:	C. Suzanne
Location	BI 372	Revision #:	3
		Revision Date:	26 Feb 2018
Supervisor	Dr. E. McCauley	Revised By:	C. Suzanne

Authorization

- Only trained personnel can perform acetone extraction of chlorophyll
- Do not perform acetone extraction of chlorophyll after-hours or alone

Hazards associated with equipment/machinery/materials/technique/process

- Exposure to acetone
- Use of tissue grinder

Personal protective equipment

- Lab coat
- Safety glasses
- Safety gloves

Environment where task is to be undertaken

- Only perform acetone extraction of chlorophyll in fumehood in BI 372

Before you start work

- Read the SDS before working with acetone
- Inform other lab personnel before performing acetone extraction of chlorophyll

Emergency procedures

- If exposed to acetone on skin, immediately rinse affected area with lots of water
- Use eye wash station immediately if acetone comes in contact with eye

Step by step procedures for task:
To Prepare Chlorophyll a samples (Total Chlorophyll >35um) for Acetone Extraction

- Take a well-mixed sample of the water column
- Set up 1000 mL vacuum flask and place a filter with stopper creating a seal
- Place single glass microfibre filter paper on filter and turn on vacuum.
    - Wet filter paper with DI
    - Clamp filter tower to filter
- In a graduated cylinder measure desired volume of sample and pour into filter tower
    - Rinse graduated cylinder well with DI and pour into filter tower
    - Rinse down filter tower with DI
- Once all liquid has been filtered, remove the clamped filter tower and gently remove filter paper without ripping or tearing using a clean pair of tweezers
    - This may be easier to do if the vacuum is turned off
- Place filter paper on a square of tin foil
    - Fold tin foil in half with filter paper inside without touching the filter paper
    - Fold the tin foil pack in half again so that the filter paper is now folded in quarters
    - Fold over the edges of the tin foil pack so that the filter is sealed in
- Label tinfoil pack with: Sample #, Date, Volume filtered, >35
- Place samples in a cooler on a freezer pack while processing the rest of the samples
- Repeat for the rest of the samples and transfer to freezer once all samples are processed

Acetone Extraction of Chlorophyll Samples

- Calibrate pipette to 3mL using distilled water and weigh scale
- Make 4L of 90% acetone and store in flammables cabinet
- Fill a beaker with 90% acetone and cover with tin foil in fume hood
- Tear filter in to 50mL grinding tube using tweezers and add 6mL of acetone to the grinding tube *never handle the filter with gloves, always use clean tweezers*
- Grind filter until contents resemble a well-mixed slurry *be sure to constantly move the tube while grinding to avoid heating the sample as this will degrade the chlorophyll*
- Scrape the slurry into a 15mL glass centrifuge tube
- Rinse down the tissue grinder with 3mL of 90% acetone and pour into glass centrifuge tube
- Rinse down the grinding tube using 3mL of 90% acetone and pour into glass centrifuge tube
- Ensure that filter is submerged in 90% acetone for complete extraction *total volume must be 12mL of acetone*
- Allow samples to sit for a minimum of 8hrs but for no more than 24hrs in the fridge
- Before reading on fluorometer, spin down centrifuge tubes at 1/2 speed for 5min, turn 1/2 way and spin again to remove filter from the supernatant

Reading Chlorophyll Samples on the Fluorometer

- Turn on fluorometer and allow for 15min warm up in the fume hood
- Select main menu and press enter
- Choose method, select raw fluorescence and press enter
- Screen will say Enter Concentration 000.00 <FIU> press enter *no not adjust*
- Insert known sample of 500ppb standard and press enter
- Insert blank select yes, fill cuvette with 90% acetone and press zero
- Fill cuvette with samples, wait for number to stabilize and record the before acid number
- If reading is too high and says "out of range", dilute sample by a factor of 10: Add 1 mL of sample and 9 mL of 90% acetone to a new centrifuge tube, fill cuvette again and read before acid number taking note of the dilution of data sheet
- Add two drops of HCl acid solution using eye-dropper into cuvette, wait one minute
- Record the after acid number
- Dispose filter in a waste jar in fume hood
- Rinse chlorophyll tubes well and acid bath before using again

Clean-up procedures

- Clean up area and fumehood after performing acetone extraction of chlorophyll
- Rinse centrifuge tubes with distilled water and dry
- Throw away latex gloves
- Acid bath all used glassware

Maintenance

- Make sure there is sufficient acetone before performing acetone extraction of chlorophyll
- Make sure the fluorometer has been calibrated recently (ask Lab Manager) and if not, then calibrate instrument
- Make sure there is sufficient known sample of 500 ppb before beginning procedure
- Make sure there is HCl acid solution prepared before beginning acetone extraction of chlorophyll (to make new HCl acid solution, add 8.5 mL of concentrated HCl to 500 mL of double distilled water and dilute to 1L and store in fridge)

Signed:_____Date:_____
Supervisor

7.1.2 Collecting under-ice samples

UNIVERSITY OF CALGARY

Water quality sample collection from ice covered lakes
Standard Operating Procedure

Faculty/ Department or Institute/Centre	Biological sciences	Date Created:	25 January 2016
		Created By:	D Barrett
Location	UofC Eco-reserve	Revision #:	
		Revision Date:	
Supervisor	Dr Ed McCauley, Dr Fred Wrona, Christina Suzanne	Revised By:	

Authorization

- Obtain water samples often requires licensing from regional or water authorities
- Invasive sampling must be allowed
- Only trained personnel can collect water quality samples from ice covered lakes

Hazards associated with equipment/machinery/materials/technique/process

- Immersion in cold water due to ice failure
- Immersion of body parts in cold water to facilitate sample collection
- Potential for dangerous preservatives (formaldehyde etc) to be in sample collection bottles
- General cold-weather environmental concerns
- Slipping on ice

Personal protective equipment

- Warm clothing
- Safety glasses
- Safety gloves
- Anti-slip footwear
- Elbow length glove

Environment where task is to be undertaken

- Field location (ex. University of Calgary eco-reserve ponds)
- Due to working on ice, recommended that persons never operate alone
- Possibility of wildlife such as coyotes and deer

Before you start work

- Review specific sample collection (ex. rinsing out of preservative, pre-filtering etc)
- For phytoplankton and chlorophyll α collection, see relevant SOP's
- Preparation of area, tools required, notifications or postings
- Bring cooler and ice pack to keep samples cool after collection
- Drill hole in ice to access underlying freshwater – see relevant SOP
- Read the SDS before working with preservatives

Emergency procedures

- Arrange emergency contact prior to departure
- Arrange will-contact-by time at which if employee has not contacted previously decided upon contact, contact will probe further
- Always have field first aid kit on hand
- If exposed to preservative on skin, immediately rinse affected area with lots of water

Step by step procedures for task

- Follow processes required for each individual sample collection
- Label bottle and note time of sample, recording both on bottle and in field book
- Attach sample bottle to rod/dowling/meter stick using hose clamp or zip-tie
- Quickly lower sample bottle through bore hole to desired depth
- Raise filled sample bottle up to surface and attach lid while below water with gloved hand?

- Be sure to do any post-collection steps as required by the specific SOP (ex. Addition of preservative)
- Keep samples cool (~4°C is ideal) while transporting back to laboratory for further analysis or processing
- Complete COC if submitting to analytical laboratory for analysis

Clean-up procedures

- None
- Dispose of safety gloves

Waste disposal procedures

- None
- If rinsing out preservative from sample bottle, dispose of preservative waste away from field site and in a safe manner

Maintenance

- None
- Make sure to have sufficient and appropriate sample bottles prior to entering the field

Signed:_____Date:_____
Supervisor

7.1.3 Coloured DOC quantification

UNIVERSITY OF CALGARY

Sampling and Analysis Procedure for Coloured Dissolved Organic Carbon (CDOC) through photometric methods
Standard Operating Procedure

Faculty/ Department or Institute/Centre	Biological Sciences	Date Created:	28 November 2016
		Created By:	C. Tomaszewski
Location	BI 372	Revision #:	
		Revision Date:	
Supervisor	Dr. Ed McCauley	Revised By:	

Authorization

- Only trained personnel can perform CDOC quantification

Hazards associated with equipment/machinery/materials/technique/process

- None

Personal protective equipment

- Safety gloves

Environment where task is to be undertaken

- Only to be performed in BI372
- Filtering takes place in BI 354

Before you start work

- Turn on spectrophotometer machine before beginning work
- Ensure filtering apparatus is set up
- Make sure you have enough DDW before beginning

Emergency procedures

- None

Step by step procedures for task

- **Filtering/processing**
  - Take a well-mixed sample of the water column
  - Set up 500 mL vacuum flask and place a filter with stopper creating a seal
  - Place single glass microfiber (GFC) filter paper on filter and turn on vacuum.
    - DO NOT wet filter paper with DI (pour sample directly on filter)
    - Clamp filter tower to filter
  - In a graduated cylinder measure 50 mL of sample and filter through the vacuum filtering apparatus, and rinse the collection flask out with the filtrate
  - Filter approximately 100mL of sample through the filter
  - Once all liquid has been filtered, remove clamped filter tower and pour filtered water into clean graduated cylinder/beaker
  - Place processed sample into fridge (keep on ice and in dark until reading on machine)
  - Discard filter paper into garbage
  - Rinse collection flask with DI water in between each sample
  - Repeat for all samples
- **Reading colour on spectrophotometer**
  - Turn on spectrophotometer and allow 20 minutes for warm up
  - Press 'return'
  - Select photometric (#1)
  - Select one wavelength (lambda) (#1)
  - Make sure wavelength is set to 440nm
    - If not, press go to WL and change to 440
  - Do not enter anything for data or k data fields

- Rinse and fill both cuvettes with DDW, making sure there are no air bubbles in the cuvette. Use a kimwipe to dry off cuvette, particularly the ends, and remove any fingerprints/streaks
- Insert two cuvettes into slots
- Press autozero button
- Remove cuvette closest to you and dump DDW, triple rinse with samples then fill cuvette and wipe with kimwipe (ensuring no air bubbles)
- Place first unknown sample in the front cuvette leaving the blank cuvette for all readings
- Press start
- Record absorbance measure
- Triple rinse with DDW between samples, rinse with sample below filling. Wipe down cuvettes before readings
- Repeat for all unknown samples
- When finished all samples, empty both cuvettes, rinse with DDW, then fill with DDW and leave in spectrophotometer
- Wipe down machine with a damp kimwipe to clean any spills or splashes
- Turn off machine when finished

Clean-up procedures

- Discard GFC filter in garbage
- Discard sample water down sink
- Fill cuvettes with DDW and leave in Spectrophotometer
- Rinse all glassware and put on acid bath cart

Maintenance

- Make sure spectrophotometer has been calibrated and is in working order (ask Lab Manager)

Signed: _____ Date: _____
Supervisor

7.1.4 Obtaining ice cores/augering from ice-covered ponds

UNIVERSITY OF CALGARY

Obtaining ice cores from ice-covered lakes
Standard Operating Procedure

Faculty/ Department or Institute/Centre	Biological sciences	Date Created:	25 January 2016
		Created By:	D Barrett
Location	UofC Eco-reserve	Revision #:	
		Revision Date:	
Supervisor	Dr. McCauley, Fred Wrona, Christina Suzanne	Revised By:	

Authorization

- Access and permission to drill through ice required
- Ability to operate machinery (gas or electric power) must be allowed

Hazards associated with equipment/machinery/materials/technique/process

- Immersion in cold water due to ice failure
- General risks associated with moving machinery
- Sharp blades on ice auger
- General cold-weather environmental concerns
- Slipping on ice

Personal protective equipment

- Warm clothing
- Safety glasses
- Anti-slip footwear
- Work gloves

Environment where task is to be undertaken

- Field location (ex. University of Calgary eco-reserve ponds)
- Due to working on ice, recommended that persons never operate alone
- Possibility of wildlife such as coyotes and deer

Before you start work

- Be sure to understand components and installation of ice auger parts before use (see pictures and/or operators manual: http://www.icedrill.org/library/printsection.shtml?ID=195)
- Be sure ice is of sufficient thickness (10cm minimum for holding 2 persons)
- Assemble auger components
- Transport auger with battery power disconnected from auger motor
- Make sure battery is fully charged before operating

Emergency procedures

- Arrange emergency contact prior to departure
- Arrange will-contact-by time at which if employee has not contacted previously decided upon contact, contact will probe further
- Always have field first aid kit on hand

Step by step procedures for task

- Transport auger body and motor to site
- Determine appropriate location for drilling
- If collecting ice core after drilling, ensure catching device (tube etc) present close to drill location (Figure 1)
- Ensure battery disconnected from auger motor then attach auger to auger motor by first attaching sleeve to motor with bolt and nut (Figure 2)
- Connect auger tube to sleeve using pin (Figure 3)

- Make sure bolt and pin are securely in place
- Reconnect batter lead to auger motor
- Place auger on location desired for drilling making sure the auger is level, straight up and down (Figure 4)
- When ready press button labelled "F" on the auger head to initiate drill
- Drill until through ice at which time remove quickly from hole to prevent freezing or sticking inside recently bored hole
- Upon successfully drilling through the ice and removing the auger, position the core tube at one end of the receiving catching device (Figure 5)
- Disconnect auger battery from auger motor
- Remove auger tube by moving switch on auger tube head (Figure 6)
- Remove ice core from auger tube to catching device, resulting in something similar to Figure 1
- Re-assemble auger core tube to auger head/motor
- Place cores carefully in labelled Ziploc bag or other storage device
- Place Ziploc bag in cooler to be transported back to lab and stored in freezer

Clean-up procedures

- Once back in lab, rinse auger with tap water to prevent growth of organisms or rusting. Allow to air dry before returning to case

Figures

Figure 1 Ice core catching device example set-up

Figure 2 Attach sleeve to auger motor with bolt and nut

Figure 3 Attach auger tube to sleeve and connect using pin

Figure 4 Place auger over location for drilling ensuring it is level

Figure 5 After removing auger from bore-hole, place near receiving device

Figure 6 Remove motor and auger head by moving switch, shown in red circle

Waste disposal procedures

- None
- Dispose of used coring blades in sharps containers back in lab

Maintenance

- Ensure auger battery is charged prior to departing for field
- Check coring blades at bottom of auger, replace as required using supplied spares and supplied alan/hex key
- Identify who is required to do it and what documentation must be in place.

Signed:_____Date:_____
Supervisor

- Make sure bolt and pin are securely in place
- Reconnect batter lead to auger motor
- Place auger on location desired for drilling making sure the auger is level, straight up and down (Figure 4)
- When ready press button labelled "F" on the auger head to initiate drill
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- Remove ice core from auger tube to catching device, resulting in something similar to Figure 1
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Signed:_____Date:_____
Supervisor