The Bioburden and Ionic Composition of Hypersaline Lake Ices: Novel Habitats on Earth and Their Astrobiological Implications

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Abstract

We present thermophysical, biological, and chemical observations of ice and brine samples from five compositionally diverse hypersaline lakes in British Columbia's interior plateau. Possessing a spectrum of magnesium, sodium, sulfate, carbonate and chloride salts, these low-temperature high-salinity lakes are analogs for planetary ice-brine environments, including the ice shells of Europa and Enceladus, and ice-brine systems on Mars. As such, understanding the thermodynamics and biogeochemistry of these systems can provide insight into the evolution, habitability, and detectability of high priority astrobiology targets. We show that biomass is typically concentrated in a layer near the base of the ice cover, but that chemical and biological impurities are present throughout the ice. Coupling bioburden, ionic concentration and seasonal temperature measurements, we demonstrate that impurity entrainment in the ice is directly correlated to ice formation rate and parent fluid composition. We highlight unique phenomena including brine supercooling, salt hydrate precipitation, and internal brine layers in the ice cover, important processes to consider for planetary ice-brine environments. These systems can be leveraged to constrain the distribution, longevity, and habitability of low-temperature solar system brines – relevant to interpreting spacecraft data and planning future missions in the lens of both planetary exploration and planetary protection.

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Abstract

We present thermophysical, biological, and chemical observations of ice and brine samples from five compositionally diverse hypersaline lakes in British Columbia's interior plateau. Possessing a spectrum of magnesium, sodium, sulfate, carbonate and chloride salts, these low-temperature high-salinity lakes are analogs for planetary ice-brine environments, including the ice shells of Europa and Enceladus, and ice-brine systems on Mars. As such, understanding the thermodynamics and biogeochemistry of these systems can provide insight into the evolution, habitability, and detectability of high priority astrobiology targets. We show that biomass is typically concentrated in a layer near the base of the ice cover, but that chemical and biological impurities are present throughout the ice. Coupling bioburden, ionic concentration and seasonal temperature measurements, we demonstrate that impurity entrainment in the ice is directly correlated to ice formation rate and parent fluid composition. We highlight unique phenomena including brine supercooling, salt hydrate precipitation, and internal brine layers in the ice cover, important processes to consider for planetary ice-brine environments. These systems can be leveraged to constrain the distribution, longevity, and habitability of low-temperature solar system brines – relevant to interpreting spacecraft data and planning future missions in the lens of both planetary exploration and planetary protection.

1. Introduction

1.1 *Field Site Overview*

The Interior Plateau of central British Columbia houses a diverse array of endorheic (closed basin) hypersaline lakes. Seasonal snowmelt and groundwater flow leaches salts and minerals from the local geology before collecting in the low-lying perennial and ephemeral lakes and playas. The hot and arid summer climate of the region leads to substantial evaporation, and with minimal rainfall to refresh the lakes their salinities dramatically increase, reaching concentrations of 30-40% salt by weight. In some cases, the lakes reach their saturation point and pure hydrated salts (e.g. epsomite, mirabilite, meridianiite, natron, trona) begin to precipitate in the lake waters and underlying sediments [*Renaut and Long*, 1989]. This cyclic concentrative process can lead to the formation of thick (~5m), solid salt basements and columns beneath the lakes [*Jenkins*, 1918; *Renaut and Long*, 1989] (See Figure 1). Many lakes in this region exhibit a unique 'spotted' morphology, where tens to hundreds of individual brine pools subdivide the lake (Figure 1). The formation process of the individual brine pools is unknown. Proposed formation mechanisms include density driven subsistence caused by annual salt precipitation [*Renaut and Long*, 1989], bottom up dendritic growth of sub-pool salt structures from the salt basement [*Jenkins*, 1918], and freeze-thaw processes in the shallow subsurface [*Renaut and Long*, 1989], akin to frost heave driven patterned ground formation [*Peterson and Krantz*, 2008]. Regardless of their origin, the brine pools are stable structures whose perimeters do not vary seasonally and have likely remained relatively unchanged over much longer timescales (~10s to 100s of years) [*Renaut and Long*, 1989].

While these extremely high salinity environments are toxic to many organisms, there exists a rich and unique halophilic ecosystem within each of the lakes [*Pontefract et al.*, 2019; *Pontefract et al.*, 2017]. As autumn ends the resident organisms are subjected to an additional environmental stressor – extreme cold. With mean January temperatures ranging from -9 to -12°C and nighttime lows that can exceed -45°C the lakes form a substantial ice cover [*Brown et al.*, 2020a; *Renaut and Long*, 1989]. The ice that forms from the hypersaline brines of these lakes is highly porous and contains both salts and organisms entrained during ice formation. This process further concentrates the underlying brine, depressing its freezing point by as much as ~9°C, depending on the composition of the lake [*Buffo et al.*, 2019]. The interstitial brine of the ice and the underlying brine reservoir constitute novel extreme environments that support a community of halophilic psychrophiles [*Buffo et al.*, 2019; *Pontefract et al.*, 2019; *Pontefract et al.*, 2017].

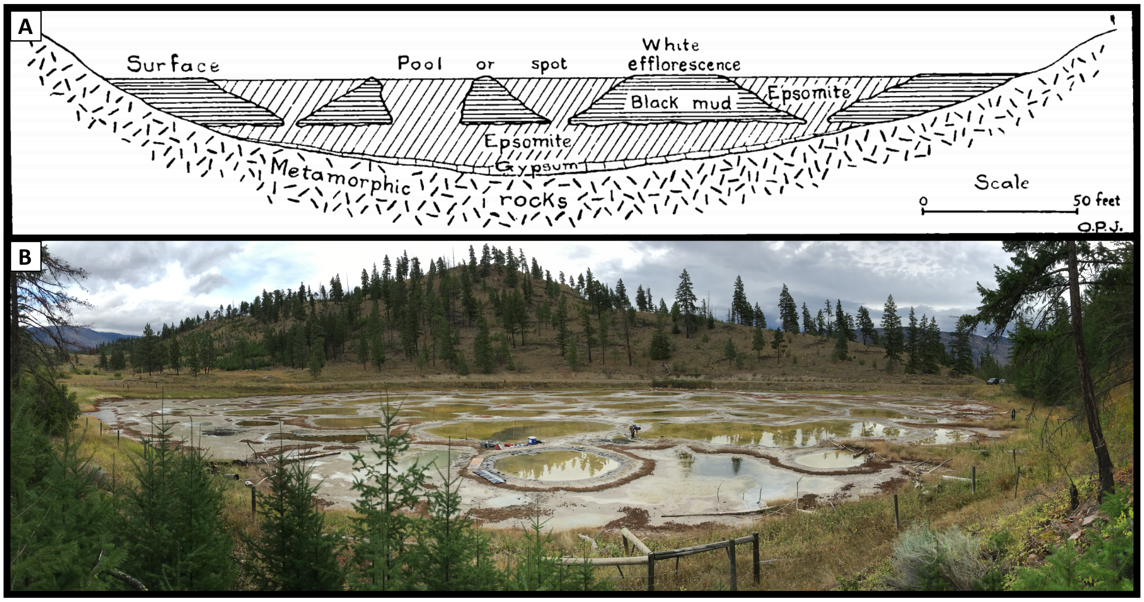

Figure 1: Structure of the hypersaline lakes. A) The subsurface structure of the spotted lakes, depicting the epsomite basement and columns/inverted cones that underly the surficial pools, modified from [*Jenkins*, 1918]. **B)** Photograph of Basque Lake 2, taken in September 2019.

The wintertime abundance and productivity of microfauna in this porous system is likely limited by physicochemical properties of the ice and brine, such as pore connectivity, water activity (a measure of the amount of water available for microbial processes), and chaotropicity/kosmotropicity (a measure of the tendency for solutes to disrupt or stabilize biological structures, respectively) [*Loose et al.*, 2011; *Murray et al.*, 2012; *Stevens and Cockell*, 2020; *Weeks and Ackley*, 1986]. The osmotic stress and potential destabilizing properties (chaotropicity) of hypersaline environments is well documented in the literature [*Fisher et al.*, 2021; *Oren*, 2013] and has even been discussed in the context of Martian environments [*Fox-Powell et al.*, 2016; *Pontefract et al.*, 2017; *Tosca et al.*, 2008]. These works emphasize the importance of the concentration and chemical composition of the brine, as both water activity and chao-/kosmotropicity (strongly limiting factors for life as we know it [*Fox-Powell and Cockell*, 2018; *Pontefract et al.*, 2017]) are heavily dependent on the ionic species present. Conversely, both ice and hydrated salts are capable of entraining and preserving biomaterials [*Buffo et al.*, 2019; *Pontefract et al.*, 2017; *Srivastava et al.*, 2021], potentially providing a mechanism for long term stasis of organisms when conditions become less favorable [*Cosciotti et al.*, 2019; *Srivastava et al.*, 2021].

Cumulatively, observations of the thermal, structural, and biogeochemical profiles of British Columbia's diverse hypersaline lakes provide a method to assess the spatiotemporal habitability and biosignature distribution of unique ice-brine systems. This not only has far-reaching implications for extremophilic adaptation and the limits of life on Earth but provides an ideal system to investigate the thermophysical evolution and hypothetical biogeochemical dynamics of analogous planetary ice-brine environments.

1.2 *Scientific Motivation*

The most promising worlds within our solar system that may harbor environments suitable for life all lie beyond the sun's habitable zone (e.g. Europa, Enceladus, Mars), precluding the long-term stability of liquid water on their surfaces [*Fox-Powell et al.*, 2016; *Marion et al.*, 2003; *Parkinson et al.*, 2008; *Priscu and Hand*, 2012; *Tosca et al.*, 2008]. As such, most high priority astrobiology targets lie beneath icy outer shells/regolith or occur as ephemeral/relict surface expressions of subsurface fluids. In the case of Europa and Enceladus this manifests as global ice shells overlying regional or global subsurface oceans [*Čadek et al.*, 2016; *Sotin and Tobie*, 2004], with possible endogenic expression of ocean material through ocean-surface geophysical processes (e.g. diapirism [*Nimmo and Pappalardo*, 2006; *Pappalardo and Barr*, 2004; *Schmidt et al.*, 2011], extensional band formation [*Howell and Pappalardo*, 2018], solid state convection [*Barr and McKinnon*, 2007; *McKinnon*, 1999; *Weller et al.*, 2019], fractures [*Craft et al.*, 2016; *Nathan et al.*, 2019; *Nimmo and Schenk*, 2006; *Walker et al.*, 2014], and plumes [*Bauer et al.*, 2010; *Glein et al.*, 2015; *Sparks et al.*, 2016] – see Figure 2). For Mars it has been suggested that both historical and contemporary shallow subsurface brine systems and episodic surface expression of brine are responsible for the formation of fluvial and lacustrine geomorphological features (e.g. Gale Crater – a potentially remnant analog to the Canadian lake systems investigated here – Figure 2) and continue to interact with and be sourced from observed ground ice [*Carr*, 1987; *Ojha et al.*, *2020; Ojha et al.*, 2015; *Rapin et al.*, 2019; *Toner et al.*, 2014; *Vaniman et al.*, 2004; *Wray et al.*, 2011; *Zorzano et al.*, 2009]. Furthermore, subglacial hydrology in the Noachian era [*Fastook and Head*, 2015; *Ojha et al.*, *2020*] and potential saline lakes beneath the present day south polar layered deposit [*Grau Galofre et al.*, 2020; *Lauro et al.*, 2020; *Orosei et al.*, 2018; *Sori and Bramson*, 2019] may constitute additional habitable aqueous environments, suggesting such systems could have existed in a number of regions in both Mars' past and present. With the unique link between water and life as we know it, identifying and improving our understanding of these ice-ocean/brine systems is imperative to future mission design in the lens of both planetary exploration and planetary protection.

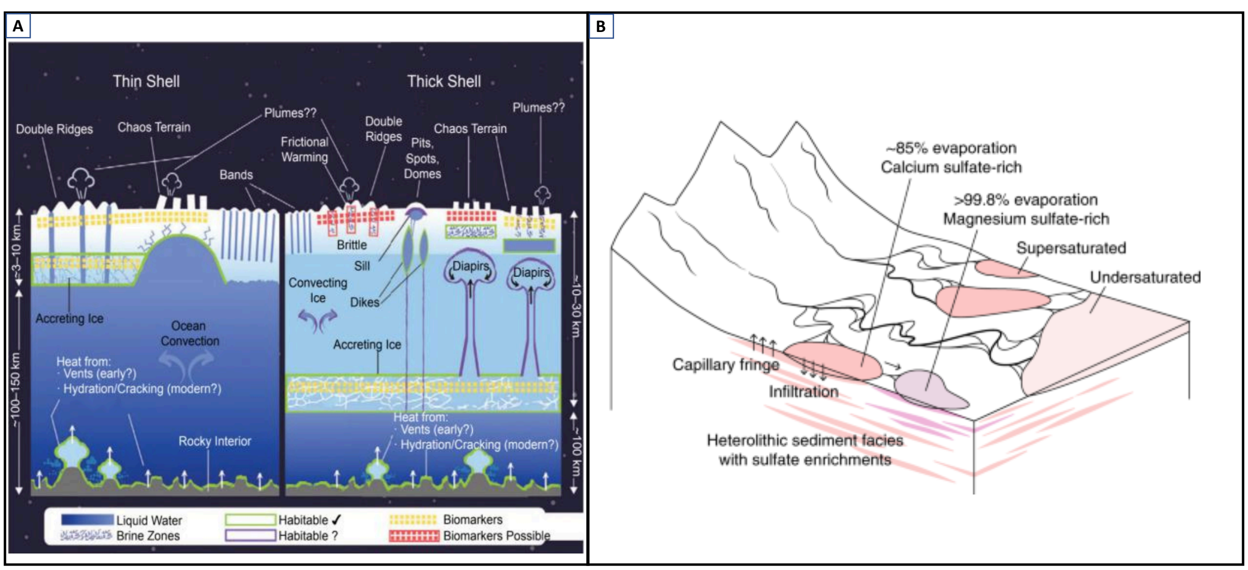

Figure 2 – Putative ice-brine environments on Mars and ice-ocean worlds. A) The ice-ocean-seafloor system of Europa, highlighting the potential for ice shell hydrology driven by regional geodynamics. In addition to the global subsurface ocean, concentrated brine could facilitate stable hydrological feature within the shell. Understanding their

biogeochemical evolution is crucial to predicting their distribution, longevity and habitability as well as linking shallow subsurface characteristics to underlying ocean properties. **B)** Predicted fluvial and lacustrine dynamics in Mars' ancient Gale crater [*Rapin et al.*, 2019]. Cyclic filling and evaporation of endorheic pools coupled with freezing surface temperatures could create environments strikingly similar to those found in the Canadian ice-brine systems investigated here (particularly their geochemical evolution and habitability). (Photo Credit: A) [*Schmidt*, 2020] B) [*Rapin et al.*, 2019])

The remaining hurdle is accessing and observing the underlying ocean/brine reservoir, as it frequently lies beneath meters or kilometers of ice. In the absence of *in situ* measurements, we rely on remote sensing techniques (e.g., spectrographs, ice penetrating radar) to observe ice characteristics and relate these measurements to properties of the underlying liquid [*Di Paolo et al.*, 2016; *Fanale et al.*, 1999; *Kalousova et al.*, 2017; *Ojha et al.*, 2015; *Orosei et al.*, 2018]. Fortunately, when ice forms it entrains biogeochemical signatures of its parent water reservoir and formation history [*Buffo et al.*, *2020; Buffo et al.*, 2019; *Buffo et al.*, 2018; *Kargel et al.*, 2000]. On Earth, this has been observed in the salinity and bioburden profiles of sea ice [*Cottier et al.*, 1999; *Cox and Weeks*, 1974; *Eicken*, 1992; *Loose et al.*, 2011; *Nakawo and Sinha*, 1981; *Thomas and Dieckmann*, 2003] and lake ice [*Murray et al.*, 2012; *Priscu et al.*, 1998; *Santibáñez et al.*, 2019]. While the thermodynamics and biogeochemistry of ice formed from Earth's sodium chloride (NaCl) rich ocean has been studied for nearly a century (e.g., [*Malmgren*, 1927]), the analogous processes in ices formed from more exotic ocean/brine compositions remain less constrained (exceptions being the ice covers of Antarctic dry valley lakes – e.g., [*Doran et al.*, 2003; *Murray et al.*, 2012; *Priscu et al.*, 1998]). Exotic salt assemblages including magnesium, sulfate, and acid-bearing salts have been detected on Europa [*Fanale et al.*, 1999] and magnesium, sulfate, chlorate, and perchlorate salts are ubiquitous components of Mars' geology [*Ojha et al.*, 2015; *Pontefract et al.*, 2017; *Tosca et al.*, 2008; *Vaniman et al.*, 2004]. This is important as it suggests the oceans and brines of other solar system bodies may be quite different than Earth's ocean [*Kargel et al.*, 2000; *Pontefract et al.*, 2017; *Toner et al.*, 2014; *Zolotov*, 2007; *Zolotov and Shock*, 2001]. Likewise, the ice that forms in these planetary environments may have substantially varied characteristics (e.g., microstructure, biochemistry, strength, viscosity). Differing chemistries could drastically impact the habitability and geophysics of ice-ocean/brine worlds on both local and global scales and affect the relationship between ice characteristics and interior reservoir properties and dynamics – an imperative for the interpretation of spacecraft observations. Fortuitously, the hypersaline lakes of British Columbia provide a natural laboratory in which to observe the thermophysical and biogeochemical evolution of ices formed from compositionally diverse analog brines (e.g., [*Fox-Powell and Cockell*, 2018; *Renaut and Long*, 1989]). Furthermore, the $MgSO_4$ and Na_2SO_4 dominated chemistries of these lakes mirror the predicted ocean/brine compositions of both icy satellites [*Zolotov*, 2007; *Zolotov and Shock*, 2001] and Mars [*Pontefract et al.*, 2017; *Toner et al.*, 2014; *Vaniman et al.*, 2004]. Placing empirical constraints on the relationships between ice biogeochemical properties, system thermodynamics, and brine biogeochemistry will provide a novel data set of an understudied extremophilic environment on Earth, improve our understanding of the limits of life in low-temperature saline analog environments relevant to both Mars and icy worlds and act as a benchmark to validate models designed to simulate these planetary environments [*Brown et al.*, 2020b; *Buffo et al.*, 2019].

2. Methods

Five lakes were visited during two field campaigns (February 2019 and February 2020): Basque Lake 1 [50.60012°, -121.35967°], Basque Lake 2 [50.59336°, -121.34974°], Basque Lake

4 [50.58867°, -121.34317°], Last Chance Lake [51.32775°, -121.63576°], and Salt Lake [51.07298°, -121.58441°] (Figure 3). Basque Lakes 1, 2, and 4 as well as Salt Lake are magnesium sulfate ($MgSO_4$) dominated systems, while Last Chance Lake is a sodium sulfate (Na_2SO_4) and sodium carbonate (Na_2CO_3) dominated system.

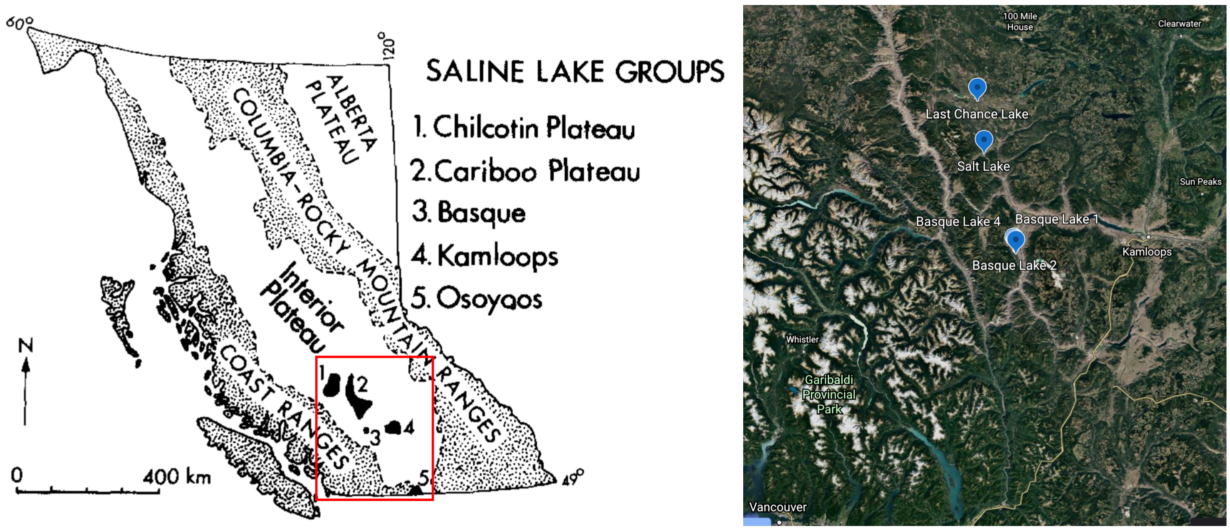

Figure 3 – Hypersaline lake locations. Left) Map of the hypersaline lake groups of central British Columbia (modified from [*Renaut and Long*, 1989]) Last Chance Lake and Salt Lake are members of the Cariboo Plateau group while Basque Lakes 1, 2 and 5 are members of the Basque group. **Right)** Enlarged view of the region outlined in red showing the lake locations visited during February 2020.

At each of the five lakes, sample sites (n=1-3) were selected to represent diverse locations within the lake, either discrete brine pools, or edge to center transects when pools could not be identified due to high lake levels (leading to subaqueous pools). At each site the thickness of the ice and underlying brine layer was measured (Section 3.2). When present, precipitated salt at the base of the brine layer was also measured. Ice cores were extracted from descending sections of the ice column (Figure 4) and stored in sterile amber Nalgene bottles. A temperature profile was taken using a probe thermometer with an accuracy of 0.01 °C as the cores were removed and brine was extracted from the underlying pool/lake if the ice was not frozen to the underlying sediments (Figure 4 and Section 3.3.1). Additionally, we deployed long-term temperature loggers in September 2019 and recovered them in February 2020 to observe seasonal variations in brine and air temperature (Section 3.3.2). The HOBO temperature loggers recorded data hourly and were placed at Basque Lake 2 and Last Chance Lake, with one logger positioned directly above the sediment layer in a chosen pool, another in the middle of the water column, and a third logger placed around the lake to gather surrounding air temperature. Lastly, brine infill experiments were conducted; ‘sackholes’ were drilled to different depths within the ice cover and allowed to fill with brine via percolation through the underlying porous ice. By measuring the rate at which the brine infills the hole, the permeability of the ice beneath the hole can be estimated (Section 3.5) [*Freitag and Eicken*, 2003].

Figure 4 – Field work at Basque Lake 2. A) Drone image of Basque Lake 2 taken in February 2019. The segregated brine pools, more evident in (D), can still be seen under the snow cover. **B)** Ground photo of a sample site as we begin to drill the borehole array seen in the next panel. **C)** The typical sampling procedure where ice thickness and temperature are recorded, ice cores are gathered, and brine is extracted from the underlying lake. **D)** The lake without an ice cover, photographed in September 2019 (taken from hillside at top left of image A).

To prepare the samples for biological and chemical analysis, the ice and brine samples were melted in a hot water bath within 18 hours of collection before being split into two subsamples. One split involved aliquoting 45 ml of the unfiltered sample to a 50 ml Falcon tube and adding 4.5 ml of 2.5% glutaraldehyde solution to fix the cells for bioburden analysis (final glutaraldehyde concentration of 0.25%). Next, 2 ml of the fixed solution was aliquoted into a cryotube. Samples were then frozen in a -20 °C freezer and shipped frozen to Scripps Institution of Oceanography (cryotubes) and Georgia Institute of Technology (Falcon tubes). Cell counts were acquired using two techniques: **1)** staining the cells with 4′,6-diamidino-2-phenylindole (DAPI) and imaging them using a Zeiss Epifluorescent Microscope (Supplementary Section S1) and **2)** flow cytometry (Section 3.4) following the methods of [*Klempay et al.*, 2021]. Fixed samples were stained with SYBR Green I nucleic acid dye (Molecular Probes) and spiked with a known quantity of 123count eBeads counting beads (Thermo Fisher). Samples were then run on a Guava easyCyte HT flow cytometer (Luminex). Flow cytometry outputs were analyzed in R using the flowCore library [*Ellis et al.*, 2009] and custom scripts. For the second split we filtered the remaining sample through a 0.2 μm polyethersulfone (PES) membrane Sterivex filter. These splits were frozen in a -20 °C freezer and cold shipped to ALS Environmental in Tucson, Arizona for chemical analysis via ion chromatography (IC) and ion coupled plasma mass spectrometry (ICP-MS) methods. Samples were analyzed for the following ions: Br, Cl, F, NO_3, SO_4, CO_3 (alkalinity), Ca, Mg, K, and Na (Section 3.4) as well as total dissolved solids (TDS - fixed and volatile), pH, density, and conductivity.

3. Results

3.1 *Physical Properties of the Ice*

The ice cover of the lakes visited in both field campaigns was characterized by porous, soft, cloudy/bubbly ice that was frequently damp with brine in all but the coldest ambient sampling temperatures. The ice crystals had a lamellar structure similar to columnar sea ice (Figure 5a). The random orientation of the crystals is indicative of growth under quiescent conditions, as expected for endorheic basins with limited flow. Upon drilling, the ice was noticeably odorous (sulfur scented), and this increased with depth, consistent with measurements indicating substantial amounts of volatiles are entrained within the ice cover (Supplementary Figure S3). The basal portion of the ice cover (~5 cm) possessed visible brine channels (Figure 5b) similar to those seen in sea ice.

These high porosity dendritic conduits are the byproduct of density driven convective overturn in the lower layers of the ice [*Notz and Worster*, 2009]. In other systems where brine channels are observed, this convective overturn proceeds as follows: As an ocean/brine freezes, salts are rejected from the forming ice lattice, producing a porous ice matrix saturated with hypersaline interstitial brine (a 'mushy layer' [*Feltham et al.*, 2006]). Where the porous ice matrix is permeable enough to sustain Darcy flow (fluid flow in a porous media [*Bear*, 2013]) the cold, saline, dense fluid at the top of the mushy layer downwells into the underlying fluid reservoir, forming (dissolving) brine channels. The resultant porous basal layer of ocean/brine-derived ices is capable of sustaining substantial thermochemical gradients that can provide metabolic energy sources to any resident organisms [*Loose et al.*, 2011]. In sea ice this layer supports a substantial biological community, including primary producers essential to the polar oceans (e.g., ice algae), grazers, bacteria, and macrofauna [*Loose et al.*, 2011; *Tedesco and Vichi*, 2014]. Similarly, upon ultraviolet irradiation, an ice core extracted from Salt Lake during the February 2019 season exhibited visibly enhanced autofluorescence in the region adjacent to the ice-brine interface (Figure 5c-d), suggesting an amplified presence of microbiota when compared to the upper portion of the ice cover. This is corroborated by the quantitative biological profiles of Section 3.4.

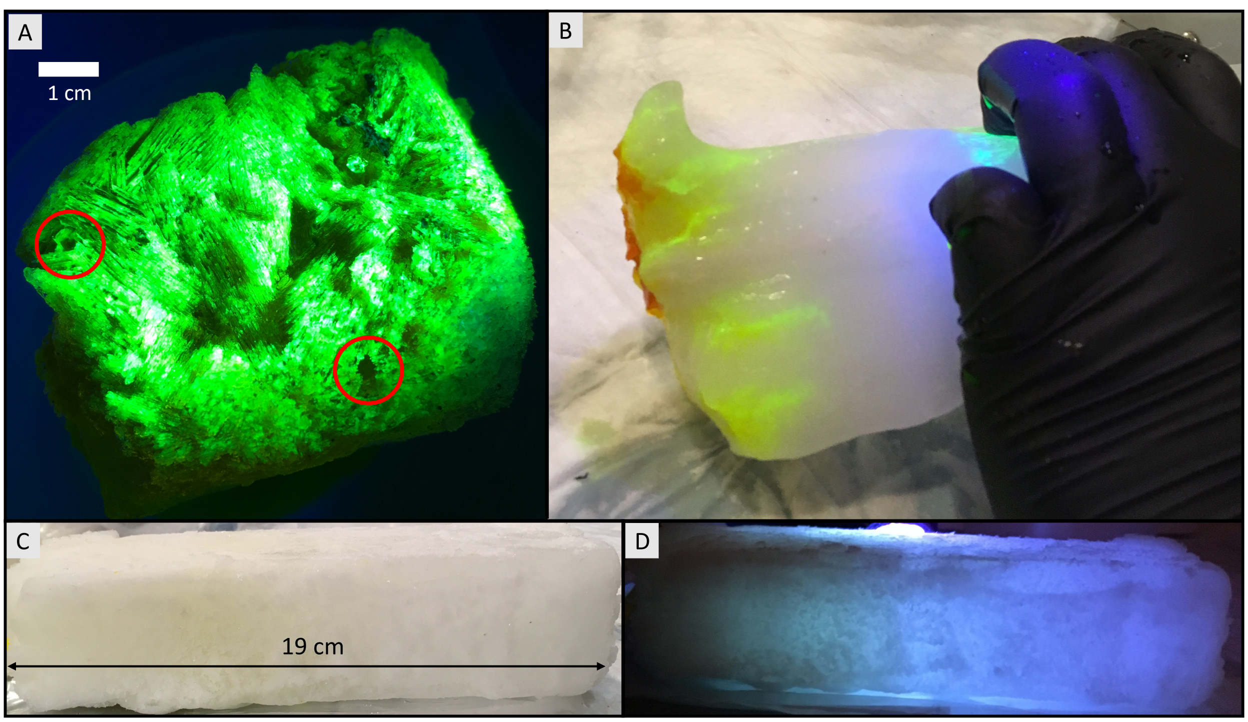

Figure 5 – Structural properties of Cariboo Plateau lake ices. A) Lamellar crystal structures at the base of an ice core from Salt Lake (February 2019). A fluorescein solution was poured onto the base of the inverted ice core and it was illuminated with an ultraviolet (UV) flashlight. The openings of two brine channels can are circled in red. **B)** Brine channels in the basal layer of the Salt Like ice core pictured in frame A. We melted the edges of the ice core away by running it under water, revealing locations where fluorescein had percolated into the ice core via brine

channels (again, illuminated by a UV flashlight). **C)** Salt Lake ice core before fluorescein was added (visible light). Bottom of the core is to the left of the image. **D)** Same as panel D under UV illumination. Autofluorescence is evident near the base of the ice core, indicating the presence of microorganisms.

3.2 *Stratigraphy of the Ice-Brine Lake Systems*

During the winter most of the lakes possess four primary layers; (1) an ice cover, (2) a shallow (<1 m thick) brine layer, (3) solid salt hydrates (See Supplementary Figure S2), and (4) underlying sediments. Stratigraphic profiles of 12 sample sites visited during February 2020 can be seen in Figure 6. Most sites have the expected ice→brine→salt hydrate→sediment vertical layering that occurs when salts are rejected from the forming ice cover, saturate the underlying brine, and precipitate out of solution, forming a salt hydrate layer atop the underlying sediments. However, there are a number of interesting exceptions as well as additional layering features that may provide further insight into the physical and geochemical evolution of these systems. Typically, deeper lakes are fresher and thus may not reach their saturation points during winter (e.g., Salt Lake – note the limited freezing point depression of the sub-ice brine [Figure 7]). An exception is Basque Lake 1 sample Site 3, which possessed a salt hydrate layer suggesting the lake brine is saturated; this is reasonable given the extremely low temperature of the brine [Figure 7]. Other interesting features include: **i)** an intra-ice brine layer observed in the ice cover of Basque Lake 2 sample Site 2 that may be indicative of eutectic melting in the ice or over pressurization of the underlying brine (if progressive freezing is not compensated by ice uplift); **ii)** the alternating layers of salt and sediment at Basque Lake 4 sample Site 2 indicating localized salt precipitation features (note the lack of a salt layer for Basque Lake 4 sample Site 1) that are likely the remnant 'crystal bowls' [*Renaut and Long*, 1989] of previously existing brine pools (see Figure 2 of [*Foster et al.*, 2010]); **iii)** the confirmed existence of a substantial salt basement beneath the lakes (e.g. Basque Lake 4 sample Site 2 – in many cases there likely exists a salt hydrate basement beneath the lowest sediment layer measured [*Jenkins*, 1918; *Renaut and Long*, 1989]); **iv)** two visibly distinct salt hydrate layers at the base of Last Chance Lake (a 1 cm layer overlying a 5 cm layer – Supplementary Figure S2). The geophysical and biogeochemical implications for both terrestrial and planetary environments are discussed in Section 4.

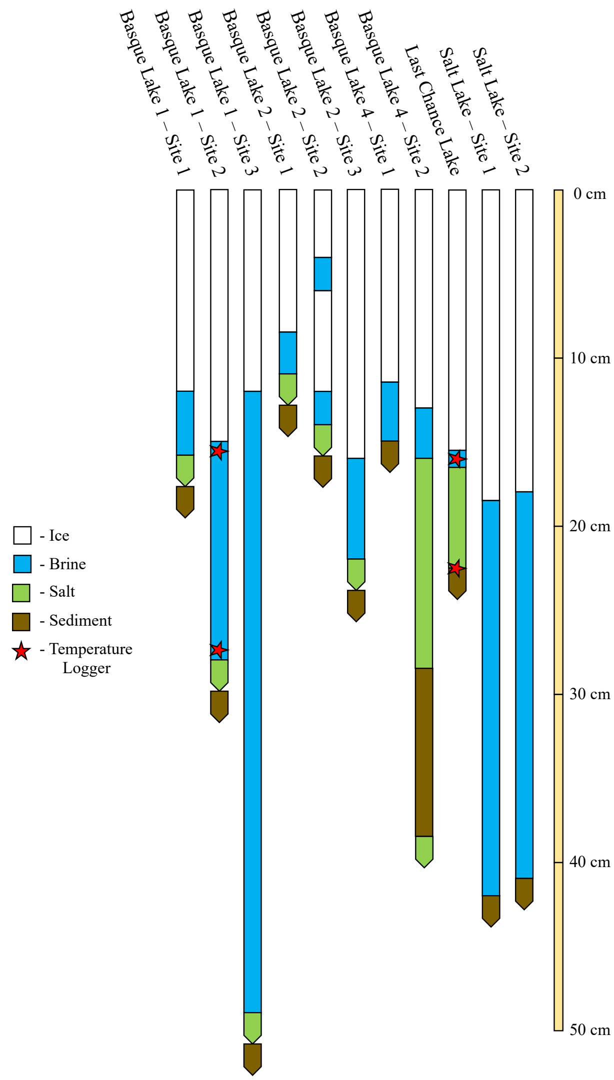

Figure 6 – Stratigraphic profiles of select February 2020 sample sites. Portions of the profiles represented by bars with squared ends have known thicknesses. Portions of the profile with pointed ends have unknown thicknesses (either they were too thin to measure, too thick to extract, or were the lowest layer accessed).

3.3 *Temperature Profiles*

We acquired two distinct temperature datasets during our 2019 and 2020 February field campaigns. The first consists of vertical temperature profiles for all extracted ice cores. The second consists of hourly air and brine temperature records from September 2019 to February 2020, obtained using HOBO temperature sensors.

3.3.1 *Ice Core Temperatures*

Ice and brine temperature profiles for all sites visited in 2019 and 2020 can be seen in Figure 7. Due to consistently colder ambient air temperatures (Figure 7 values at 0 cm), the temperature profiles for the 2019 ice covers are much more monotonic, with cold temperatures near the surface that increase with depth. Conversely, high daytime temperatures during February 2020 can be seen warming the near surface ice, in many cases to values that exceed the temperatures deeper in the ice column. Profiles without brine measurements are frozen to the sediment bed. Brine temperatures range from -5.31°C to -0.53°C and are significantly below the freezing point of fresh water due to the presence of dissolved salts (Section 3.4). An inter-ice brine layer (~0.5-2 cm thick) was observed at Basque Lake 2 sample Site 2 in February of 2020 (filled red square at ~5 cm depth in Figure 7). This unique feature has important implications for the potential generation of brines in the icy regolith of Mars and ice shells of ice-ocean worlds. We discuss potential formation mechanisms for the layer as well as its astrobiological relevance in Section 4. It is not uncommon for salt hydrate layers to occur at the base of the lakes (Section 3.2), however extraction of these layers through holes in the ice cover is far from straightforward. With strengths and hardnesses that exceed those of ice [*Durham et al.*, 2005] even thin layers (~6 cm) must be chiseled through. In 2020 at Last Chance Lake sample Site 1, the wooden stake holding the HOBO temperature sensors was firmly encased by the salt hydrate layer at the base of the lake, with one temperature sensor attached to the stake below the hydrate layer (See Figure 4 and Section 3.3.2). To extract the temperature sensor, the hydrate layer was chiseled through with a hammer and metal rod. Exceptionally warm temperatures were measured in both of the distinct hydrate layers described in Section 3.2 (green circles of Figure 7). The potential importance of salt hydrates in these and analogous planetary ice-brine systems is discussed in Section 4.

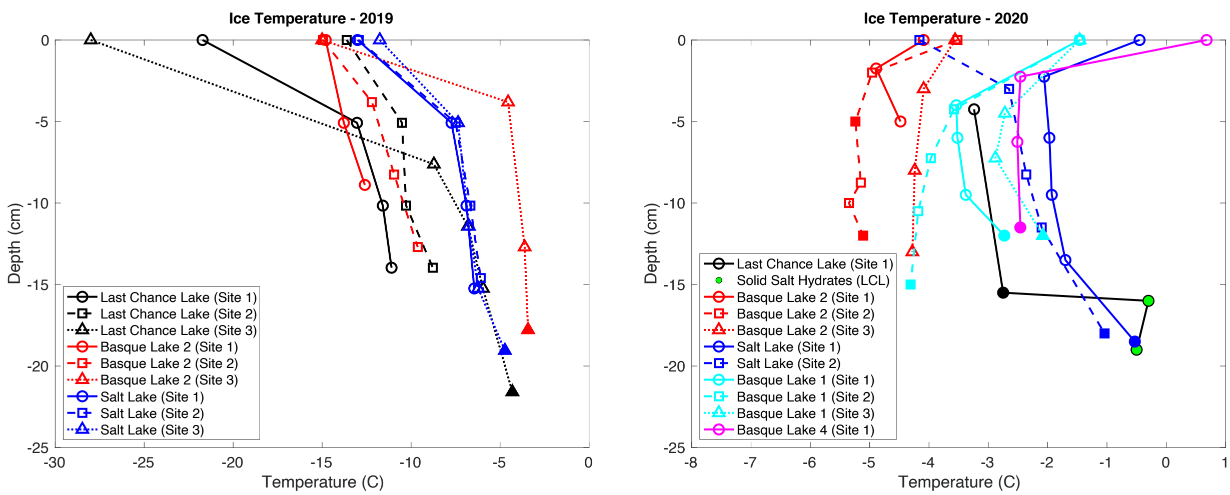

Figure 7 – Ice cover temperature profiles. Left) Vertical ice core temperature profiles taken during February 2019. Filled symbols signify a brine temperature. **Right)** Vertical ice core temperature profiles taken during February 2020. At Last Chance Lake two distinct salt hydrate layers had precipitated just above the lake sediments. Note the unique interior brine layer at the Basque Lake 2 Site 2 location. (Note: probe thermometer accuracy is 0.01 °C)

3.3.2 *Seasonal Temperatures*

During September 2019 six HOBO temperature loggers were installed at the hypersaline lakes (three at Last Chance Lake, three at Basque Lake 2). At each lake one temperature logger was attached to a nearby tree to measure air temperature. The other two loggers were affixed to a wooden stake with zip ties and driven into the sediments of a brine pool. One logger was placed near the brine-sediment interface, the other was placed in the middle of the water column. Between September 2019 and February 2020, the loggers recorded hourly temperature readings, the results of which can be seen in Figure 8. At the time of extraction both temperature loggers were in the sub-ice brine layer of Basque lake 2. At Last Chance Lake one temperature logger was in the sub-ice brine layer, the other was beneath a 6 cm layer of precipitated salt hydrates (See Figure 6 for exact locations). During the fall large diurnal variations in temperature occur in both the air and brine. Upon the formation of an ice cover in late November the brine becomes extremely well insulated and no longer varies appreciably on the diurnal scale. The minimum brine temperatures achieved are -6.37°C (December 1) and -8.45°C (November 30) for Basque Lake 2 and Last Chance Lake, respectively. The subsequent warming of the brine and a lack of any appreciable temperature variations after the brine reaches its minimum temperatures (even under substantial cooling that occurred mid-January), suggests significant insulation by the overlying ice, a substantial heat source due to salt hydrate precipitation in the underlying brine, or both. An early season minimum brine temperature favors the presence of the latter, as it appears the brine becomes supercooled, precipitates salt hydrates, and then remains at or near its eutectic temperature (no longer requiring supercooling/supersaturation to nucleate salt hydrates as a substrate already exists for continued precipitation [*Toner et al.*, 2014]). Further discussion of this process and its relevance to both terrestrial and planetary ice-brine systems is included in Section 4.

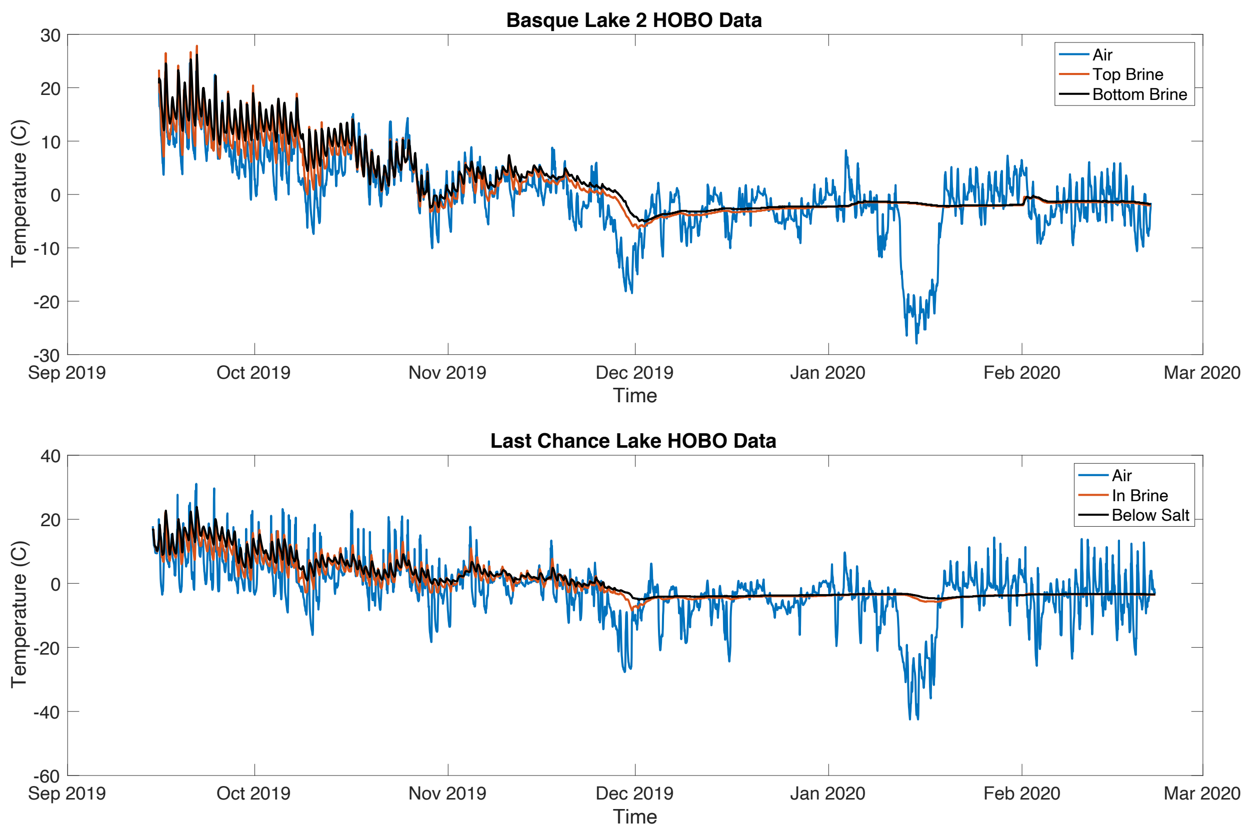

Figure 8 – Seasonal variations in air and brine temperatures. Top) Hourly temperature data for Basque Lake 2. **Bottom)** Hourly temperature data for Last Chance Lake.

3.4 *Major Ion & Biological Profiles*

Biological and chemical profiles from each lake were derived using ion chromatography (IC), ion coupled plasma mass spectrometry (ICP-MS), fluorescence microscopy, and flow cytometry (FCM). The vertical distribution of major ions in each lake (ice cover and underlying brine) are shown in Figure 9. A number of the ion profiles have 'c-shaped' distributions, with high ion content in the near surface ice, lower ion content in the middle portion of the ice column, and increasing ion content approaching the base of the ice column and underlying brine, similar to the characteristic bulk salinity profile of first year sea ice [*Eicken*, 1992; *Malmgren*, 1927]. The amplifications near the surface are due to rapid freezing as heat is lost to the colder atmosphere before salt can be rejected into the underlying fluid. Amplifications near the base of the ice are due to the ice-brine interface existing as a porous two-phase layer, composed of a solid ice matrix bathed in concentrated interstitial brine – a ubiquitous feature of ice formed from saline solutions (e.g. [*Buffo*, 2019; *Feltham et al.*, 2006]). A number of exceptions to the 'c-shaped' trend exist in our observations: **i)** exceptionally low ion contents in the shallow ice of Salt Lake (Figure 9a) which we have reason to believe is due to analysis error, as the reported ion concentrations are not charge balanced, **ii)** only one ice sample from Last Chance Lake was acquired due to exceptionally warm temperatures (deeper ice had excessive brine infill and could not be adequately cored without contamination from the infilling brine), **iii)** an internal brine layer existed in the ice cover of Basque Lake 2 (see Figure 6), which exhibited exceptionally high ion concentrations (and cell densities).

Vertical microbial cell density distributions for all of the lakes are shown in Figure 9. Cell densities generally tend to increase with depth – with the notable exception of Basque Lake 2 – which features an inter-ice brine layer. This interesting layer exists 5 cm below the surface and has almost twice the number of cells per milliliter (2.503×10^6 cell ml^{-1}) as the three other depths measured in the ice cover (1.449×10^6, 1.229×10^6, and 1.358×10^6 cell ml^{-1}). All three of the Basque Lakes sampled (1, 2 and 4) are dominated by sulfate with the second most abundant ion being magnesium, although Basque Lake 2 is more concentrated than its neighbors. Another $MgSO_4$ dominated lake sampled, Salt Lake, has a noticeable peak of cell density at a depth of 13.5cm which then decreases at the subsequent brine layer. Last Chance Lake is dominated by carbonate, sodium, chloride, and sulfate with a negligible concentration of magnesium; it also has the lowest cell density in its basal brine. The geophysical and astrobiological implications of the observed cell densities and major ion distributions are discussed in Section 4.

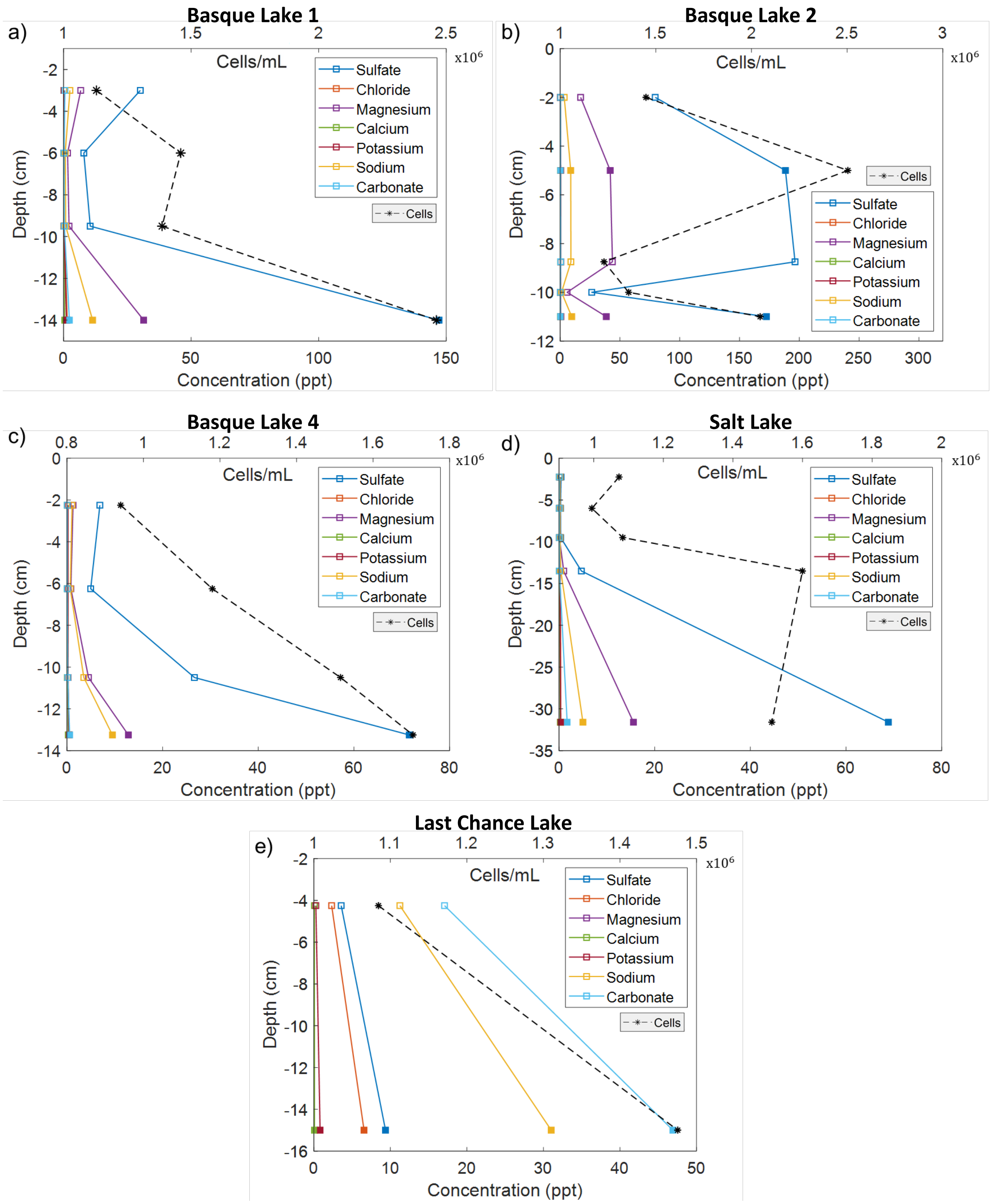

Figure 9 – Major ion and cell density profiles of selected lake sites. a) Basque Lake 1 – exhibiting a 'c-shaped' ionic profile and a general increase in cell density as the ice-brine interface is approached. **b)** Basque Lake 2 – with amplified salt and cell concentrations in the internal brine layer ~5 cm below the surface, even exceeding those of the underlying brine. Basque Lake 2 is the most concentrated lake of the Basque Lakes sampled and contains a higher cell density in the top ice layer than the other lakes. **c)** Basque Lake 4 – similar to Basque Lake 1, Basque Lake 4 exhibits

a 'c-shaped' ionic profile and a trend of increasing cell density with depth. Basque Lake 4 has a lower $MgSO_4$ concentration and lower cell density in its underlying brine than the other Basque Lakes. **d)** Salt Lake – there exists a spike in cell density in the bottom ice sample, around -13.5cm, exceeding that of the brine sample at -32cm. **e)** Last Chance Lake – only one ice sample was obtained during February 2020 due to warm temperatures. Last Chance Lake is the only Na-CO_3 rich lake sampled and has the lowest cell density in its underlying brine. All ionic concentration and cell density values can be found in Supplementary Table S1 and Table S2, respectively. (Note: filled symbols represent brine samples).

3.5 *Permeability*

One of the primary controls of fluid and solute transport in ocean and brine derived ices (e.g., sea ice) is the permeability of the ice cover. Desalination of the ice, through both gravity drainage and surface flushing, depends critically on the flow of brine and meltwater through the ice, and in turn governs the resultant salinity distribution in the ice [*Notz and Worster*, 2009]. In high porosity (generally highly permeable) regions near the ice-ocean interface, nutrient and detritus transport is a crucial process governing the productivity and sustainability of biological communities that thrive in the interstitial brine [*Loose et al.*, 2011; *Thomas and Dieckmann*, 2003]. It stands to reason that fluid transport, and thus permeability, likely plays an equally important role in the biogeochemical evolution of the hypersaline ice-brine systems investigated here.

In February 2020, it was relatively warm (air temperatures exceeded 0 °C by mid-morning) the day Last Chance Lake was sampled. Ice cores were extracted at one site, however by the time a second site was selected, brine infill into boreholes of all depths precluded coring as inflowing brine from the underlying reservoir would contaminate ion and bioburden estimates. Taking advantage of the highly porous ice we conducted three slug test experiments (borehole depths of 6cm, 8cm, and 10cm) to investigate the permeability of the ice cover, the results of which can be seen in Figure 10. These recovery curves track the hydraulic head of the brine infilling the borehole over time. Here hydraulic head is equivalent to the difference between the height of the infilling brine and the freeboard level. This is a common technique used in sea ice research, and it can be shown that the temporal evolution of the hydraulic head is related to permeability via *Freitag and Eicken* [2003]:

$$h(t) = h(t_0) \exp\left(-k \frac{g\rho t}{\eta L}\right) \qquad (1)$$

where $h(t)$ is the hydraulic head at time t, $h(t_0)$ is the hydraulic head at the onset of the slug test, k is permeability, g is gravity, ρ is brine density, η is the dynamic viscosity of the brine, and L is the distance between the bottom of the borehole and the base of the ice cover. Best fit curves of this functional form have been applied to all three slug test experiments and can be seen plotted against the data in Figure 10. Our measurements are well represented by the decaying exponential of Equation 1. As borehole depth increases the quality of the fits decreases. This is likely due to increased lateral brine infiltration in deeper boreholes. Equation 1 assumes only vertical brine infiltration from the base of the borehole. While this is likely the main source of brine infiltration, the sides of the borehole will also experience a nonzero amount of lateral infiltration. Deeper boreholes have larger sidewall surface areas and thus will be more affected by this process. Typically, studies implementing the slug test technique will deploy a hollow cylindrical casing to minimize the effects of lateral brine infiltration. The impromptu nature of our study left us wanting in this regard and would have likely reduced the amount of misfit in our deeper borehole measurements. Nevertheless, we can use the exponential coefficients of our fit lines and Equation 1 to acquire upper bound estimates of the ice permeability. The permeabilities of the 6cm, 8cm, and 10cm boreholes are 1.5840e-10 m^2, 1.1785e-10 m^2, and 6.2964e-11 m^2, respectively. There is a slightly decreasing trend in permeability with depth, which could correlate to enhanced porosities in the rapidly cooled, near surface ice (e.g. [*Buffo et al.*, 2018]). As the ice-brine interface is approached permeabilities increase dramatically, evidenced by boreholes drilled below 10cm infilling with brine too rapidly to accurately measure the change in hydraulic head. The estimated permeabilities of the Last Chance Lake ice fall in the center of permeabilities observed in natural and laboratory grown sea ice, which range from 10e-13 m^2 to 10e-7 m^2 (see Figure 7a of *Freitag and Eicken* [2003]).

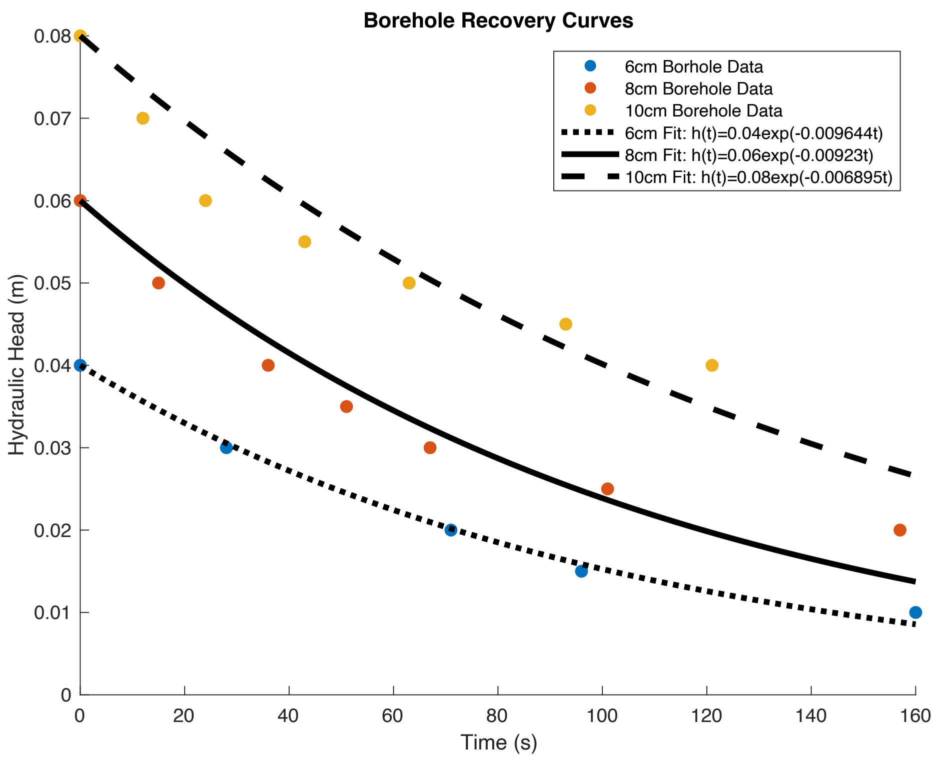

Figure 10: Slug test recovery curves used to estimate ice permeability. Temporal variations in hydraulic head are measured as brine infills three boreholes of different depth (6cm, 8cm, and 10cm from the ice surface). Exponential decay curves (Equation 1) are fit to the hydraulic head measurements (based on the method presented in [*Freitag and Eicken*, 2003]).

3.6 *Porosity*

Permeability is largely driven by porosity (ϕ), which plays an important role for both thermophysical and biogeochemical processes in ice-brine systems. Porosity, or liquid fraction, aids in facilitating solute (e.g. salts, nutrients, waste) transport throughout the ice via connected brine networks. Similar to sea ice, higher porosity regions will likely be associated with amplified fluid flow, while low porosity regions should limit fluid flow and trap brines in discrete brine pockets as flow networks become more disconnected upon continued solidification [*Golden et al.*, 1998; *Golden et al.*, 2007]. The complex microstructure of saline ices, coupled with their physical and thermal fragility, makes direct measurements of ice porosity quite challenging in the field. Even in laboratory settings the utilization of micro computed tomography (micro-CT) methods to measure ice porosity must carefully control the temperature of the sample to avoid melting, freezing, and redistribution of the fluid phase (e.g. [*Golden et al.*, 1998; *Golden et al.*, 2007; *Maus* *et al.*, 2020]). Fortunately, there exists a link between the temperature, ionic composition, and liquid fraction of a solution. Conceptually similar to the eutectic phase diagrams of simpler binary or ternary solutions, equilibrium and fractionation chemistry models (e.g. FREZCHEM [*Marion et al.*, 1999], PHREEQC [*Parkhurst and Appelo*, 1999; 2013], SeaFreeze [*Journaux et al.*, 2020]) are able to utilize Pitzer equations or Gibbs free energy approaches to predict the composition and phase evolution of a solution as it is frozen.

Here, we use PHREEQC, our *in situ* ice temperature measurements (Section 3.3.1), and our ionic concentration measurements (Section 3.4) to estimate the porosity (liquid fraction) of the ice covers (Figure 11). The colder atmospheric temperatures present during February 2019 result in low porosities and porosity profiles which generally increase with depth. Conversely, the warm surface temperatures during the February 2020 campaign result in higher overall porosities, particularly in the warm shallow ice layers, likely due to melting/dissolution of the ice. This link between warm surface temperature and high porosities may provide an explanation for the brine layer observed at Basque Lake 2. If the shallow ice was warmed to a point where solutes could be mobilized in the pore space of the ice (e.g. by air temperatures >0 °C [Figure 8]), they would percolate downward until they reached an impermeable layer (e.g. colder ice [Figure 7]), potentially producing a highly saline region within the ice. This high salinity region could lead to the dissolution of the surrounding ice, ultimately producing the observed internal brine layer. It is interesting that many of the estimated porosities are near the sea ice percolation threshold (ϕ_c) of ~0.05, predicted by *Golden et al.* [2007]. This suggests that solutes are mobile in the ice cover when $\phi > \phi_c$, and when freezing occurs salination of the interstitial fluid leads to gravity drainage [*Notz and Worster*, 2009] and freshening of the pore fluid. This allows for continued freezing of the ice layer. However, once the porosity is ~0.05 ($\phi \approx \phi_c$) the percolation limit is reached and fluid flow within the ice is drastically reduced, preventing freshening of the pore fluid via gravity drainage and inducing a slower continued freezing rate of the residual brine as it is further concentrated (i.e. its freezing point is further reduced), resulting in the clustering of porosities around 0.05. Porosities ~0.05 are in the range observed for seawater-derived ices at similar temperatures (e.g. [*Golden et al.*, 2007]). This is expected even for the amplified ion concentrations in the magnesium sulfate dominated ices as $MgSO_4$ has less of an impact on freezing point depression than does NaCl. This chemistry-dependent variability in freezing point depression is also evidenced by the amplified porosities (and lower minimum brine temperatures) seen at Last Chance Lake, which has a higher concentration of sodium chloride. The implications of porosity for material transport, astrobiology, and the geophysics of planetary ices is discussed in Section 4.

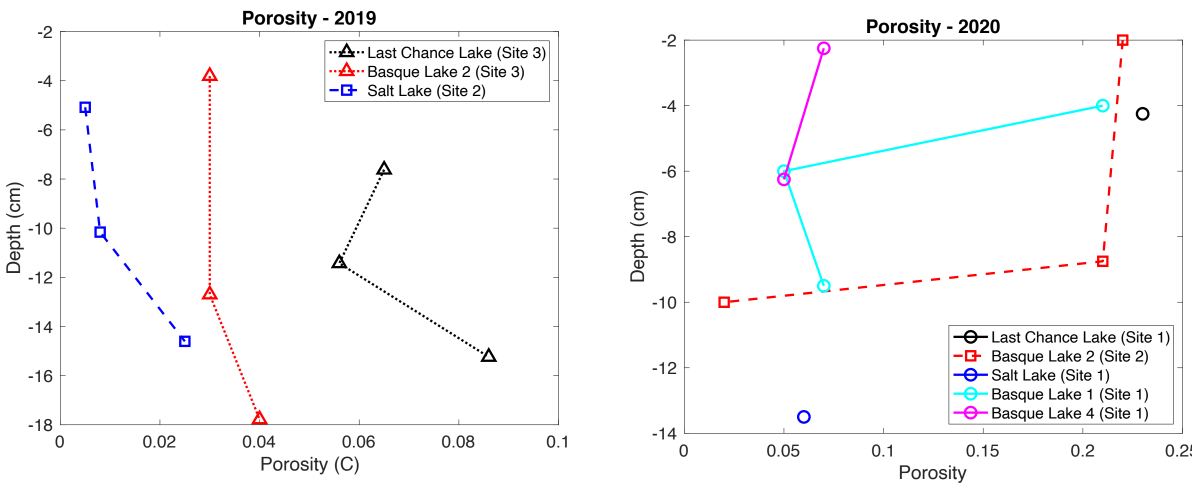

Figure 11: Porosity profiles within the lake ice covers. Left) Porosity of the lake ice covers during February 2019. **Right)** Porosity of the lake ice covers during February 2020. Symbols and colors correspond to those presented in the temperature profile plots of Figure 7.

4. Discussion

Our investigations provide insight into the relationships between both the bioburden and ionic composition of chemically diverse ices, their formation history, and their underlying parent fluid properties. This is most evident in the depth dependent trends of biological entrainment, ion entrainment, and permeability. Our results show that the characteristics of these salt rich lake ices follow similar trends to those observed in sea ice, which suggests that material entrainment is **1)** inversely proportional to thermal gradients at the ice-ocean/brine interface (equivalently ice thickness), **2)** proportional to the concentration of the parent ocean/brine reservoir and **3)** depends critically on the dynamics occurring in the gradient-rich multiphase layer near the base of the ice cover [*Buffo et al.*, 2018; *Eicken*, 1992; *Golden et al.*, 2007; *Hunke et al.*, 2011; *Nakawo and Sinha*, 1981; *Notz and Worster*, 2008]. Effectively, this work suggests that the physical mechanisms that govern the fractionation of liquids, solids, and biota within ice are similar yet dependent on composition. Such a result implies that infusion of these dynamics into models of planetary ice processes and analysis of planetary data sets is well founded. Additionally, we observed a number of novel features in naturally occurring ice-brine environments that have important geophysical and astrobiological implications for both terrestrial and planetary cryohydrologic systems; these include internal brine layers in the ice cover of Basque Lake 2, widespread and heterogeneous salt hydrate precipitation and the associated effects of their latent heat of fusion on brine longevity and resistance to solidification, and the likely supercooling of brines in a natural setting.

The general trends in major ion content, cell density (bioburden), porosity, and permeability suggest a dynamic ice cover capable of fluid, solute, and biological transport. As the ice solidifies, rejects salts into interstitial brine veins and pockets, and the brine concentrates, the system becomes gravitationally unstable and the interstitial brine convects downward into the underlying brine reservoir, forming brine channels in the ice (Figure 5). The freshened ice can thus continue freezing, leading to a trend of increasing porosity and permeability with depth in the ice cover. While the upper layers of the ice cover typically possess lower bulk ion concentrations than the underlying fluid, the remaining salts are likely concentrated in residual brine pockets, making any liquid regions in the upper ice column highly saline and potentially less hospitable to any resident organisms. The lower liquid content, generally colder temperature, and potentially chaotropic brine in the upper layers of the ice suggest that lower portions of the ice cover are likely to provide greener pastures for any biology present in the system (a trend mirrored in fresh lake ice [*Santibáñez et al.*, 2019] and sea ice [*Loose et al.*, 2011; *Tedesco and Vichi*, 2014; *Thomas and Dieckmann*, 2003]). Indeed, the bioburden profiles of all the lake ice covers show amplified cell densities in the basal ice and underlying brine. In the case of Salt Lake, the highest cell density in the ice-brine system was observed within the basal ice layer. This is consistent with observations of sea ice, where volumetric ice algae densities in the lower layer of the ice column typically far exceed those of the underlying ocean [*Ackley and Sullivan*, 1994; *Spindler*, 1994]. This feature may have been most prominent at Salt Lake due to the deeper underlying water column, as the benefits of colonizing the porous basal ice layer may not have been as prevalent in the shallower lakes due to the proximity of the brine-sediment interface – another advantageous substrate for biology to thrive within. It is important to note that there was appreciable biology present in all samples, including the shallow ice regions. We have not conducted any culture studies to explore the viability of cells in different regions of the ice cover, however our results suggest that substantial bioburden (viable or not) is entrained in diverse saline ices and that the density of the entrained bioburden is related to the ice formation history and underlying brine properties (e.g. brine chemistry, brine bioburden, ice liquid fraction). The similar quantitative relationship between salt entrainment and ice formation history has been shown to be important in describing the geochemistry of planetary ice shells [*Buffo et al.*, *2020; Chivers et al.*, 2021; *Hammond et al.*, 2018; *Vance et al.*, 2020]. Constraining the rates of impurity entrainment in saline ices has important implications for our understanding of ice-ocean worlds, where ice shell geophysics depend critically on material properties of the ice [*Durham et al.*, 2005; *Han and Showman*, 2005; *McCarthy et al.*, 2011; *Pappalardo and Barr*, 2004], biosignature expression is dependent on the entrainment and transport dynamics of the ice shell [*Schmidt*, 2020; *Schmidt et al.*, 2017] and constraining the habitability of the underlying ocean will lean heavily on the interpretation of spacecraft measurements of the ice shell [*Howell and Pappalardo*, 2020].

The internal brine layer observed at Basque Lake 2 sample Site 2 is of substantial interest as it supports the idea that high porosity water rich regions may persist in the upper layers of an ice column. While similar environments have been repeatedly observed in meteoric ice (e.g., firn aquifers, brine infiltration into the McMurdo Ice Shelf) the occurrence of discrete internal brine layers in ocean/brine derived ices is much more limited. Brine layers have been observed in cryopegs [*Gilichinsky et al.*, 2005; *Gilichinsky et al.*, 2003; *Iwahana et al.*, 2021; *Shimanov et al.*, 2020], marine permafrost [*Colangelo-Lillis et al.*, 2016], and a limited number of Antarctic Dry Valley lake ice covers [*Priscu et al.*, 1998]. In the case of Basque Lake 2 a solid ice layer overlayed a discrete brine pocket which we were able to drain by syringing brine out (<1 L total volume). The brine layer thickness varied locally from 0.5-2 cm in thickness. The brine layer was not present at drill sites < 1 m away, thus the brine layer was highly localized and not substantially connected to the basal brine layer (as there was no inflow into the voided pocket). We do not know the exact physical processes which would facilitate the generation of such an internal brine layer but propose that eutectic melting of a high salinity region due to surface warming or pressurization of the underlying brine pool as potential mechanisms. If there existed a region of enhanced salinity within the ice cover warming from the surface could raise the internal ice temperature above its eutectic melting point, creating a localized melt pocket/sheet which would grow in volume through dissolution of ice until the concentration of the melt pocket was such that its freezing point matched the local temperature. Such high salinity regions could form due to localized interstitial hydrate precipitation during a period of rapid ice formation, or from the collection of downwelling melt created by warm surface temperatures when it reached an impermeable layer. The low temperature of the internal brine (-5.24°C), its similarity to the basal brine temperature (-5.11°C, which is likely at its saturation point), and their separation by a colder ice layer (-5.35°C) would support the latter theory. Conversely, over pressurization of the underlying brine pool could drive upward brine transport into porous regions of the ice cover. The relatively small size of the brine pools, along with the low permeability of the surrounding sediments and underlying salt hydrate basements (Figure 1), could facilitate over pressurization in the basal brine and force brine upwards into the ice cover, where it would preferentially infiltrate porous regions/layers of the ice (akin to sill intrusion in magmatic systems). If the conduit sourcing the brine layer refroze it could isolate the intra-ice brine, forming the observed feature.

While occurring on a smaller scale in the lake ice cover, these exact processes have been theorized as mechanisms that could induce analogous cryovolcanic/cryohydrologic processes in the ice shells of ice-ocean world (e.g. lens formation via eutectic melting [*Schmidt et al.*, 2011], sill and dike formation via intrusion of underlying ocean fluid [*Craft et al.*, 2016; *Michaut and Manga*, 2014]). Such near surface liquid environments would provide accessible targets for upcoming missions, important for both planetary exploration and planetary protection. As such, understanding the generation, distribution, longevity, and potential habitability of such features is imperative to constraining the spatiotemporal habitability of such worlds and will inform mission design and data synthesis. Similarly, salt rich aqueous environments on both ancient (e.g. [*Fastook and Head*, 2015; *Fastook et al.*, 2012; *Ojha et al.*, *2020*]) and present day (e.g. [*Lauro et al.*, 2020; *Ojha et al.*, 2015; *Orosei et al.*, 2018]) Mars would likely be characterized by cryohydric processes, some exceptionally similar to the Canadian lakes we visited [*Fox-Powell and Cockell*, 2018; *Fox-Powell et al.*, 2016; *Pontefract et al.*, 2019; *Pontefract et al.*, 2017]. These unique analog systems can be leveraged to improve habitability classifications for Martian regions, quantify putative Martian hydrological processes, and aid in the search for remnant, relict, and contemporary habitable environments.

Another interesting process observed at the lakes is supercooling, the depression of a liquid below its freezing point. Evidence for supercooling can be seen in the seasonal evolution of brine temperatures at Basque Lake 2 and Last Chance Lake (Figure 8). The minimum brine temperatures occur early in the season, likely before any basal salt hydrate layer is present (e.g. in September 2019 there were no basal hydrates present at Last Chance Lake – compare to Supplementary Figure S2). The lack of nucleation sites for precipitation to occur requires the brine to supersaturate and supercool until the energy barrier for initial salt hydrate nucleation is overcome [*Toner et al.*, 2014]. Once this minimum temperature is reached and an initial basal salt layer is established, further precipitation occurs at the eutectic temperature and concentration of the sub-ice brine (no supercooling is needed to facilitate further nucleation as there now exists nucleation sites). This leads to stable brine temperatures, evident in both lakes for the remainder of the season (Figure 8). Only minimal deflections in brine temperature occur, even under extreme surface temperature variability (e.g., multiple days of surface temperatures ≤-15°C during mid-January and multiple days of surface temperatures above the brine eutectic temperatures in early February). A principal explanation for the exceptional stability of the sub-ice brine temperatures is the large latent heat of fusion associated with precipitation of both ice and salt hydrates, which can have latent heats of formation that exceed that of the ice-water phase transition by orders of magnitude [*Grevel et al.*, 2012]. A substantial heat source and sink, the energy associated with the phase change of these materials will buffer any drastic and rapid changes in brine temperature. Additionally, the precipitation of salt hydrates upon saturation of the sub-ice brine substantially increases the longevity of the fluid reservoir, as all of the heat associated with the latent heat of formation of the hydrates must also be lost from the system before solidification of the brine can continue. This is supported by ice thickness measurements of Little Salt Pond, a substantially less saline spring-fed pool adjacent to Salt Lake (~100 m), which exhibited an ice thickness of 31 cm compared to Salt Lake's measured ice thicknesses of 18 cm and 18.5 cm.

Supercooling and salt hydrate precipitation have important implications for low temperature saline systems on Earth, Mars, and ice-ocean worlds. The observation of supercooling in naturally occurring brines on Earth gives credence to the possibility for analogous supercooled environments on other bodies (e.g. [*Toner et al.*, 2014]) and is a unique extremophilic habitat in its own right. If these supercooled environments can persist for geologically significant time scales, they could broaden the thermal limits where liquid water is stable, and thus the window of the limits of life. We note that the time scale over which the brines observed in this study remain supercooled is on the order of days, however the Canadian lake systems are minimally buffered from the local extreme surface temperatures. If a brine system on Mars or Europa was buried in the subsurface regolith or ice shell it would likely be subject to more gradual thermal variations and could potentially remain in a supercooled state for much longer. The antifreeze potential associated with salt hydrate precipitation could be even more impactful. In magnesium sulfate systems, one of the first stable hydrate phases is meridianiite ($MgSO_4{\cdot}11H_2O$). With a latent heat of formation of $1.44e^7$ J/kg [*Grevel et al.*, 2012] (~43 times that of ice) its precipitation releases an immense amount of heat into saturated magnesium sulfate systems (i.e. the lakes observed in this study). This suggests that if ice-brine systems reach saturation and begin to precipitate out salt hydrate phases, their continued solidification could be drastically impeded, significantly extending their lifetimes. These salt rich niches could provide stable (or quasi-stable) aqueous environments for halophilic psychrophiles, water activity and chaotropicity permitting. Potential regions where this phenomenon could (have) occur(ed) include surface and shallow subsurface saline lakes and sediments on Mars [*Rapin et al.*, 2019; *Toner et al.*, 2014], subglacial Martian lakes [*Lauro et al.*, 2020; *Orosei et al.*, 2018; *Sori and Bramson*, 2019] similar to some terrestrial hypersaline subglacial systems [*Rutishauser et al.*, 2018], and hydrological brine features in the ice shells of ice-ocean worlds (e.g. lenses and lenticulae [*Chivers et al.*, 2021; *Schmidt et al.*, 2011], sills, dikes, porous regions [*Buffo et al.*, *2020*]). Constraining the physical, biological, and chemical dynamics of these understudied analog environments will feed directly into forward models of planetary ice-brine environments aimed at quantifying their spatiotemporal evolution, habitability, and dynamic processes.

The presence of salt hydrates in ice-brine systems can add additional geochemical complexities. When salt hydrates precipitate out of solution, they introduce new phases to the system and alter the chemistry of the remaining brine. Much like fractional crystallization in magmatic systems, this can result in chemical heterogeneity of the resulting solid and will govern the chemical evolution of the residual melt [*Fox-Powell and Cousins*]. In analogy with terrestrial petrology of the mantle and lithosphere, minimal variations in melt fraction and composition can have substantial impacts on the thermal and mechanical properties of planetary ice shells [*Buffo et al.*, (in review); *McKenzie*, 1989]. Furthermore, models aimed at interpreting the relationship between ice composition and parent fluid composition and/or habitability (e.g. linking surface ice or plume particle composition to interior ocean composition) should address the potential effects of salt hydrate precipitation dynamics as fractional crystallization could alter the results [*Buffo et al.*, *2020; Fox-Powell et al.*, 2020; *Fox-Powell and Cousins*]. Currently, dynamic models of planetary ices and ice-ocean world systems do not account for the presence or effects of salt hydrates [*Buffo et al.*, *2020; Buffo et al.*, (in review); *Vance et al.*, 2020]. British Columbia's saline lakes provide a unique opportunity to investigate the salt hydrate dynamics within diverse natural ice-brine systems, including salt hydrate precipitation in a saturating reservoir and the presence and geochemical dynamics of salt hydrates in diverse ices. Such results can be leveraged as benchmarks for more accurate models hoping to include the geochemical and thermophysical dynamics of ternary ice-brine-hydrate systems.

5. Conclusion

The hypersaline lakes of British Columbia's Cariboo Plateau provide an exceptional terrestrial analog for planetary ice-brine systems. A range of lake chemistries and concentrations, coupled with intense winters, produces a compositionally diverse spectrum of lake ice covers. The novel observations procured during these field expeditions extends our understanding of the biogeochemistry of relatively understudied but broadly applicable terrestrial analog systems, as ice-brine environments are likely ubiquitous throughout the solar system (e.g., Mars, Europa, Enceladus, etc.). Moreover, this analysis constitutes a methodology for quantifying the habitability of planetary ices that can be utilized to inform upcoming astrobiology mission planning and data analysis. Specifically, we constrain the ionic composition and bioburden in low-temperature high-salinity environments and constrain the relationship between observable ice properties, the underlying brine composition, and the thermal evolution of the system. Additionally, we identify novel physicochemical processes (supercooling, salt hydrate formation) that introduce complexities and uncertainties in the dynamics and longevity of these astrobiologically relevant systems. Future work will utilize the collected profiles to validate reactive transport models of planetary ice formation and evolution (e.g. [*Brown et al.*, 2020b; *Buffo et al.*, *2020*]) that seek to simulate the thermal and physicochemical properties of diverse ice-brine systems. Physicochemical heterogeneities likely play an important role in ice shell geophysical processes [*Barr and McKinnon*, 2007; *Johnson et al.*, 2017; *Pappalardo and Barr*, 2004; *Schmidt et al.*, 2011] and proliferous biological communities are sustained by physical and thermochemical gradients in terrestrial ice-ocean/brine environments [*Daly et al.*, 2013; *Loose et al.*, 2011]. As such, devising methods and models which quantify the thermophysical, biological, and geochemical properties and dynamics of planetary ices has implications for constraining the geophysics of icy worlds and assessing the spatiotemporal habitability of their ice-brine systems.

These observations are particularly important because of the presence of magnesium sulfate and sodium carbonate in the ice. With potentially similar ice chemistry existing on a number of high-priority astrobiology targets (Mars, Europa, Enceladus), constraining the characteristics and evolution of these analog ice-brine systems has direct implications for relating spacecraft observations of surface and ice shell properties to interior ocean/brine properties, as this relies heavily on quantifying the efficiency with which impurities are entrained and transported within the ice shell. With any potential ocean-derived biosignatures subject to similar transport processes, impurity entrainment estimates feed forward into detection limit requirements of any future ice-ocean world surface missions. The empirical data set presented here provides a unique benchmark to validate geophysical and biogeochemical models of planetary ices, a crucial tool in forecasting the dynamics, properties, and habitability of ice-ocean worlds. With a number of upcoming ice-ocean world missions promising to return observations with orders of magnitude higher spatial and spectral resolution than their predecessors and more in situ techniques, leveraging terrestrial analog systems such as British Columbia's hypersaline lakes can provide useful guides for the interpretation of data by linking measurements of ice composition to its thermodynamic history, parent fluid properties, and habitability.

Author Contribution Statement

JJB aided in the collection and analysis of all samples and field measurements, carried out PHREEQC simulations, and lead the writing and editing of the manuscript. EKB aided in the collection and analysis of all samples and field measurements and participated in writing and editing the manuscript. AP aided in the collection and analysis of all samples and field measurements and participated in writing and editing the manuscript. BES aided in the analysis of all samples and field measurements, assisted in planning field work methods and participated in writing and editing the manuscript. BK aided in the flow cytometry analysis of the field samples and participated in writing and editing the manuscript. JL aided in the analysis of all samples and field measurements, assisted in planning field work methods and participated in writing and editing the manuscript. JB aided in the flow cytometry analysis of the field samples, assisted in planning field work methods and participated in writing and editing the manuscript. MG aided in the ionic composition analysis of samples, assisted in planning field work methods and participated in editing the manuscript. JBG aided in the fluorescence microscopy analysis of samples, assisted in planning field work methods and participated in editing the manuscript. TP aided in the analysis of field samples. CC aided in the writing of the porosity and permeability sections of the manuscript. PD aided in the collection of sample and field measurements.

Author Disclosure Statement

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