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Structure and Stability of Microbial Assemblages in Seasonal Lake Ice: Miquelon Lake, Alberta, Canada

by

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Abstract.

Prokaryotic life has been identified in every terrestrial icy/frozen environment studied to date [for review see: (27, 29, 94, 98)]. There is evidence of microbial activity and replication within ice as well (63, 83), but there has been little research done on the seasonally frozen portion of the cryosphere. The present study focuses on the inter- and intra-seasonal microbial assemblage composition within the lake ice of Miquelon Lake, a shallow saline lake in Alberta, Canada. The prokaryotic diversity was investigated by screening diversity with denaturing gradient gel electrophoresis (DGGE) and subsequently constructing bacterial and eukaryotic clone libraries of the 16S and 18S rRNA genes, respectively. The nearest neighbors (according to BLAST) indicate the presence of a complete microbial food web within the lake ice throughout the duration of the winter season. Furthermore, the presence of a chlorophyll *a* peak at a conserved ice depth throughout the duration of the season gives indication that at lease a portion of the photosynthetic ice consortia might be active or reproducing within the ice.

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List of Symbols and Abbreviations.

A_w	water activity
BLAST	Basic Local Alignment Search Tool
bp	base pair(s)
CAP	cold-acclimation proteins(s)
CTC	5-cyano-2, 3-di-4-tolyl-tetrazolium chloride
DGGE	denaturing gradient gel electrophoresis
DMS	dimethylsulphide
DMSP	dimethylsulfoniopropionate
dNTP	deoxyribonucleotide triphosphate
DOM	dissolved organic matter
EDTA	ethylene diamine tetraacetic acid
EPS	extra-cellular polymeric substances
FYI	first-year sea ice
IMB	ice mass balance
ML	maximum likelihood
MYI	multi-year sea ice
NCBI	National Centre for Biotechnology Information
PAR	photosynthetically active radiation
PCR	polymerase chain reaction
ppt	parts per thousand
psu	practical salinity units
S	salinity
S_b	brine salinity
SSU	small subunit
T	temperature
T-RFLP	terminal restriction fragment length polymorphism
TLI	[^{3}H] leucine incorporation
TTI	[^{3}H] thymidine incorporation
UV	ultraviolet
Va	air volume

Vb	brine volume
ρ	density

Chapter One: General Introduction

Section One: *The Biology of Ice Over Water Systems*

Frozen and permanently cold (<5ºC) environments dominate the extraterrestrial solar system; in fact, ice is the most dominant phase of water on not just planets, but their moons, asteroids, and comets(92). Even the Earth is dominated by predominantly cold (<5°C) or sub-zero temperature regimes. Permafrost exists below 20% of the Earth's land mass(6), sea ice covers approximately 10% of the world's oceans(119)and roughly 90% of the worlds oceans are permanently cold (<5°C) (104). In total, over 85% of the global biosphere exists in a permanently cold (<5°C) or frozen state (76, 108).

Today, prokaryotic life has been identified in every icy/frozen environment studied to date [for reviews see: (28, 30, 83, 93, 98, 103, 126)]. Further highlighting the significance of the total biomass present (and possibly active) in permanent terrestrial ice, Lanoil et al. (2009) reported that prokaryotic life in subglacial waters might equal the prokaryotic abundance estimated to be present in terrestrial freshwater systems (71). Kottmeier and Sullivan (1990) also reported that sea ice brine channel bacterial load was 10 times higher than that found in the underlying sea waters (68), implying some degree of preferential growth within or attachment to the ice. Evidence of prokaryotic activity has been found occurring at -15°C in permafrost brine channels (43), between -2 and -20°C in the brine channels of sea ice (65), and, indirectly, at -40°C in deep glacial ice (99). All of this implies that organisms bound in ice might be capable of maintaining a degree of activity that makes metabolism and even reproduction

plausible. Interestingly, the existence and prolonged activity of these permanently ice-bound prokaryotes might have major implications for studies of extraterrestrial life on Europa and the ice caps of Mars (56, 57). It should be stressed, however, that these aspects of this research are complex and presently remain in their infancy. Much more research in this area is needed to ascertain the degree of activity, metabolism, and proliferation that is possible within ice.

1.1 *The Importance of Sea Ice over the Polar Oceans.*

Sea ice makes up the majority of the portion of the global cryosphere that exists as ice freezing above a water source; seasonal and perennial lake ice make up the remaining portion. This aspect of the cryosphere is of particular climactic importance because of the role that ice-over-water systems play in global temperature regulation and increasing global albedo. And recently global sea ice has seen the largest and most rapid decline of global sea ice cover in recorded history(113), which makes the study of these systems all the more important. Sea ice plays an essential role in decreasing global air-water heat exchange (128), increasing global albedo (94, 95), and buffering the negative impacts that changes in climate patterns will have on the global oceans (113). Polar sea ice is considered one of the earth's largest habitats, covering 14 million square miles in the Arctic and an additional 16 million square miles in the Antarctic during the winter maximum(22). Over 80% of Arctic sea ice was identified as being multi year ice (MYI), i.e. ice that has survived at least one full year, as recently as 20 years ago (125). Yet today, MYI is the smallest fraction of ice still remaining in the Arctic: in March 2011, MYI made up 45% of the total Arctic ice pack and the

oldest ice (>5 years old) made up <10% of the total Arctic MYI (21, 78). The amount of sea ice in the polar oceans is drastically decreasing due to increasing global temperatures: current estimates are that by 2050, more than 95% of the polar oceans will be ice-free throughout the summer (113). Dramatic sea ice loss has already been thoroughly documented across the polar oceans; these losses include reductions in summer ice-cover extent(20, 23, 111), decreasing ice thickness(52, 70), and lengthening of the summer melt season(77).

The negative impacts of this rapid loss of MYI will dramatically impact not just the ecosystems of the polar oceans, but also global ecosystems (31, 45). It will cause a shift in the chemistry and likely the biology of the world's upper oceans and may lead to a complete dependence on oceanic first year ice (FYI), which is seasonal ice only remaining frozen throughout the winter, for thermal regulation and albedo in the polar oceans (113). It will also increase the total solar heat input to the upper ocean, further enhancing summer melting while simultaneously increasing the proportion of sunlight reaching the upper ocean (95). This will, in turn, warm the upper ocean and lead to enhanced loss of ice thickness, higher primary productivities of microbes and algae in the ocean waters (95) and weakened natural oceanic CO_2 sink (13, 39). Additionally, there will likely be significant shifts in the community composition and possibly function of the presently well adapted sea-ice microbiota (3, 32, 42, 119) to planktonic organisms that can reside within the brine channels of sea ice for a season and then re-inhabit the underlying waters, as they likely do in regions dominated by FYI or seasonal lake ice cover. All in all, this ambient temperature driven ice loss

will dramatically change the scope of the global cryosphere; therefore, identifying the small-scale microbial responses to this dramatic sea ice loss might be an indication of what is to come globally as larger and larger portions of the cryosphere melt away (39).

Sea ice is the most widespread ice-over-water frozen ecosystem on Earth. Apart from the expected extreme of sub-zero temperatures, organisms inhabiting sea ice are faced with a number of additional potentially limiting factors. These extremes include seasonal freeze/thaw cycles(123), varying salinities (e.g. brine channel salinities vary from ~34 to >150 PSU (124)), and a lack of sustainable amounts of bioavailable liquid water within the ice. These physical and chemical constraints on life are commonly found in all types of ice over water systems, but there are several additional extremes that should be considered when researching seasonally frozen environments. First, organisms surviving within lake ice are constrained by the seasonality of the ice ecosystem, meaning that the ecosystem melts and reforms over the course of a year, so organisms must be adapted to both the sympagic (within ice) and planktonic (within water) environments. Second, organisms surviving within seasonal ice might undergo multiple freeze-thaw events over the course of one lifecycle, which may result in seasonal shifts in assemblage composition due to the selective pressures of freeze-thaw events. Finally, seasonally frozen systems interact and exchange with the underlying waters, so ice-assemblages might interact with the organisms persisting throughout the winter in the lake water. Lake ice systems differ from longer lasting sea ice systems for several reasons; first of all, they are typically covered

with thinner and warmer ice. Secondarily, even small ice-covered lakes are typically maintained at higher temperatures than the surface waters of the ocean, which are typically -1.8 ºC (124). So it is possible that there is a higher degree of interaction and exchange between the warmer underlying waters and the overlying ice in seasonal lake ice (<5 months old) systems than has been previously identified in FY (7 months old) and MY (>3 years old) sea ice (10). However, even though these between the perennially icy systems and seasonally icy systems might exist, the lack of current research on the topic makes comparing the ecologies of the two systems impossible.

The remainder of this work will use sea ice research as a jumping off point for diving into the unknown qualities of seasonally frozen ecosystems, like Miquelon Lake. Wherever it is appropriate I will attempt to highlight central differences between the two sympagic systems in order to begin to make a first assessment about seasonally frozen systems.

1.2 *The Importance of Lake Ice over confined Lake Waters.*

Lake ice is a seasonal microbial habitat that has not been studied extensively in the current literature. The lake ice ecosystem is made up of lake water ecosystem and associated assemblages, which means that the ecosystem formation, structuring, and evolution all occurs in the short life of the ice-cover. However, the winter-over ecology of lake systems have not been addressed in the current literature and the microbial ecology of seasonal lake ice has been largely overlooked. Even so, these lakes are similar to previously studied systems like sea ice.

Saline lakes are scattered across every continent on earth, with total volumes roughly equaling the volume of terrestrial freshwater lakes(72). From an industrial, economic, and ecological standpoint they are a critical aspect of the Canadian Great Plains where hundreds of these ephemeral lakes make up the only standing water available in landlocked Canada. Prairies lakes, the majority ranging from brackish to saline, play a role in Saskatchewan's mineral industry (11) and act as storage reservoirs of excessive spring runoff, thereby limiting spring flooding and maintaining late summer agriculture when water levels are sufficiently high(122). Several of the larger lakes are naturally the primary habitats or stopover points for migratory birds, including Canadian geese and ducks (117)that can take advantage of the game(82)and stickleback fish surviving in the waters(122). Others support numerous algal species(54), which have been extensively documented (9, 35, 55), as well as complete prokaryotic food webs (48, 116, 118).

1.3 B*ioavailable Liquid Water within Ice.*

Bioavailable liquid water is necessary for life (41). Without liquid water there would be no solvent for enzymes, no capability for substrate/solute diffusion (92), diminished ability to transfer nutrients or small biomolecules between cells (110), and a decreased ability to replicate (40). For example, DNA directly requires (on average) 20 molecules of water for every nucleotide, just to maintain its integrity and proper formation (40). Water is one of the only liquids on earth that is less dense as a solid than as a liquid (81), which is why ice is able to float. Additionally, water naturally exists in all three phases (solid, liquid, and vapor) on

Earth (40) and, surprisingly, liquid water has been isolated (in a supercooled, but liquid state) within the crystalline matrix of solid ice (40, 41, 84).

Eutectophiles are defined as organisms surviving in water at the interface between liquid and solid phases (28). The unavoidable extremes for eutectophiles are the severely cold temperatures and associated lack of liquid water. In fact, microbial life has been found to be supremely capable of adapting to survival in cold temperatures (for review see: (67) (106)), but there must be sufficient water activity (A_w) associated with the liquid water within the ice in order for microbial life to endure the freezing process and survive (53). By definition water activity or available water is the measure of the energy status of the water in the system, which is calculated as the ratio of the water vapour pressure of the solution (p) to that of the pure water (p_o) at the same temperature [Equation 1]. Vapour pressure is used for this calculation because it is a measure of the free-water molecules that are able to evaporate; all other molecules in the brine are bound in the ice matrix or to cations and anions in the solution and therefore they are not considered to be biologically 'available' or 'free'.

Equation 1: Calculation of the proportion of available 'free' water molecules in solution (53).

$$A_w = p/p_o$$

The existence of active water within an ice matrix is due to several factors known to depress the freezing point of water; these factors include: 1) increased concentrations of cations and anions (e.g. brine) (50), 2) the inclusion of particles (e.g. dust and sediments) within the ice matrix (132), 3) the inclusion of gasses within the ice matrix (26), and 4) the movement (via convection, gravity drainage, and brine flushing) of liquid water through the ice lattice structure (62). There can be enough liquid water within ice to support prolonged microbial existence(16, 17, 38), a degree of metabolic activity (37, 99), and even reproduction within the ice (86).

Section 2: *Biodiversity, Activity, and Ecological Importance of Sea Ice Microorganisms*

Sea ice microbial consortia are primarily restricted to sources of available liquid water, bioavailable nutrients, and ambient temperature within the ice, all of which vary slightly with ice depth. These physical and chemical variations with depth may result in a vertical distribution of these physical and chemical parameters that likely influence the distribution of microbial consortia throughout the sea ice core. In fact, the vertical profile of organisms within the ice or the physical location of organisms which can be separated by depth in the ice: 1) surface: wet snow, surface melt ponds, snow-ice, and the underlying warmer snow-slush; 2) interior ice: can be discussed in terms of a) brine channel surfaces, brine inclusions (non-connected to the major channels), brine solution (non-attached or planktonic survival) and b) the vertical profile of bulk melted sea ice segments moving down through the ice core; 3) under-ice: ice-water interface and surface waters that are continually exchanging with brine via expulsion and convection, respectively. Using these divisions it will be possible to walk through the active algal and bacterial consortia of the sea ice, the diversity of the consortia, the degree of interaction they may have with one another, and their ecological importance to the sea ice system as a whole.

Current estimates of the vertical and horizontal diversity of sea ice have reported that the distribution of organisms is 'patchy' in both directions (121), which might be due to the strong chemical gradients associated with sea ice cover (124) or might be due to the high degree of variability in the microbial

composition of the ocean waters themselves, which are then frozen together randomly during the freezing process resulting in a patchy distribution. The lack of a definitive vertical profile is not as surprising when considering the manner in which ice formation occurs in rough ocean waters. Rough oceans freeze first into 1-5mm frazil ice crystals, which then clump together into chunks of pancake ice (29), which can collide and cause ridges and change the salinity profile and community profile of the ice column (73). Additionally, PAR irradiation will allow for increased rates of photosynthesis in certain patches of ice, while other snow-cover regions or areas of varying ice structure may inhibit the same degree of light penetration. Furthermore, the resolution of current investigation into the sea ice microbiota is not nearly refined enough to answer questions of this nature, one way of possibly answering this type of question might be to section cores based on ice-type, porosity, and crystal composition in order to glean new information about the possible role microorganisms play in the horizontal banding patterns visible in sea ice (19).

2.1 *Sea Ice Snow Cover: A Biological Perspective.*

The snow-ice layer is physically different than the underlying ice that might lead to different bacterial and algal assemblages surviving in these uppermost layers of the sea or lake ice because of these differences, which would in turn result in infiltration assemblages from the snow-cover percolating down into the underlying ice system. Physically the snow-ice layer is formed by falling snow compressing and hardening into snow-ice, which means that the incorporation of assemblages into the ice matrix occurs very differently in this

layer than it does in the remainder of the underlying ice. As the snow-ice forms dust particles and fresh snow are deposited on top of the sea ice and are frozen as they land. Because the now layer is forming from freezing freshwater and Aeolian debris, the upper snow and snow-ice layers are also chemically different than the underlying sea ice, for instance the snow ice tends to be less saline, but holds significantly more total and dissolved nitrogen than the underlying sea ice, which may enhance algal growth in the uppermost ice layers, particularly when percolation into the ice is possible (47). Additionally, it has been noted that surface snow might insulate an upper slush layer of a higher temperature (existing below the snow-ice, but above the top of the sea ice) (109, 129), which might lead to a more diverse and active portion of microbial consortia in the uppermost layers of sea ice. Indeed, Kottmeier and Sullivan (68) reported that the slush layer was 77 times more active than the underlying waters, which was likely due primarily to increased PAR, though lower bulk salinity, due to freshwater drainage, may be an additional factor. Furthermore, snow-ice and the freshwater slush layer of the upper ice can lead to the creation of melt pools/lakes on the ice surface that may ultimately drain into the brine channels. This process will further exacerbate the brine flushing with a mixture of freshwater (decreasing overall brine salinity) and increasing the proportion of new allochthonous (likely photosynthesizing microalgae and bacteria carried in with the snow/wind). Interestingly, the degree of influence these new transient aquatic organisms have on the resident ice-assemblages remains to be seen.

In contrast to sea ice ecosystems, lakes can be surrounded by forests, which can greatly decrease the wind-driven accumulation of snow on lake ice. Miquelon Lake for instance has been found to typically have <0.2 meters of snow on it at any period during the winter season (8) and this and other lakes are typically not found to have areas of surface water or melt ponds. Combined this means that internal ice assemblages are likely more similar to the underlying waters, the only constant source of microorganisms, than sources of Aeolian deposition. For this and other reasons detailed here, it is probable that lake ice has less of a vertical assemblage profile than does sea ice.

2.2 *Sea Ice: A Biological Perspective.*

Generally the sea ice biota have been established as diverse and active members of the ecosystem (42, 65, 124). The following section will expound on the location of microorganisms and necessary nutrients within the sea ice that allow for continued survival in this icy biome. The sea ice brine channels form naturally due to the exclusion of dissolved cations, anions, and microorganisms from the crystalline ice matrix. Keep in mind that, for the purpose of this thesis, resident sea ice organisms have been defined as those organisms that survive attached to the inner brine channel walls and are therefore not disrupted by the movement of brine via convection or expulsion.

2.3.1 *Organisms Abundance and Activity: Brine Solution versus Internal Ice.*

In one study published in 1997 (86), researchers used a syringe to extract brine solution from the ice core and subsequently melted some of the intact ice core portions to compare nutrient concentrations in the brine solution, ice, and

underlying waters. Interestingly, the study showed that nitrate and silicate concentrations increased in the brine solution throughout the season, but the concentrations of the same nutrients in the underlying waters were also increasing throughout the season (86), which implies that nutrient exchange, as well as upwelling events (34), may play a big role in Baltic Sea ice. Meanwhile, the bacterial load of the internal brine solution was also shown to be increasing, but those data are also inconclusive because the bacterial load of the underlying waters were increasing at similar rates throughout the season (86). Which, unfortunately, made it impossible to ascertain from the organisms in the ice brine were actively growing or whether their numbers were being replenished by the influx of underlying waters. The authors did use [^{3}H] thymidine incorporation (TTI) and [^{3}H] leucine incorporation (TLI) rates to show that the organisms in the brine solution were more active than the organisms in the underlying waters from Feb-Mar; however, by late March the activity rates (based on TTI rates) of the organisms in the underlying waters increased 15 fold (86). This exceptionally high increase in under-water growth rates was assumed to be caused by increased irradiance (associated with longer days and thinner ice-cover) of the upper ocean during the spring ice melt, but no chlorophyll *a* data was obtained to confirm this assumption (86). All in all, this study hinted at the high activity rates of organisms surviving planktonically in the brine solution. However, one problem with this study was that they extracted the brine solution using a syringe, which means it is possible that attached organisms may have been disrupted and effectively sucked out of the brine channels with the brine solution. In future, a

better way to differentiate organisms in the brine solution from those attached from the ice might be to 'flush' the system with sterile water and compare the melted bulk ice to the flushed out brine solution in order to decipher which organisms are the most active and which are more similar to the underlying waters.

If organisms surviving planktonically in the brine solution are merely psychrotolerant organisms from the underlying waters and that they are being passively drawn into the ice rather than actively establishing a population within the brine channels, then these organisms might be part of the transient consortia. However, if on the other hand, organisms in the brine make up the majority of the ice population (e.g. density in brine solution >> density in bulk ice-melt), then it might be that organisms capable of vertical movement with gas vacuoles or other means (60, 61), could be actively inhabiting a particular ice depth in order to maximize nutrient concentrations or brine volume to meet specific needs. Conversely, this method of flushing brine solution from the channels would also determine whether organisms are clearly attached to the brine channel walls, rather than assuming that ice-bound organisms are surviving in biofilms based on higher extra-polymeric substances (EPS) concentrations within the ice than in the underlying waters.

However, the process of describing the microbial distribution of sea ice bacteria based on a vertical profile through the ice column has been well established in the literature. In fact, sea ice-cover has been described as an

'upside-down benthic' ecosystem, because of the inherent vertical distribution of organisms that was assumed to be present throughout a sea ice core (88).

2.2.1 *The Vertical Profile of Sea Ice: A Biological Perspective.*

Vertical chemical and thermal profiles are initially created as the ice forms and the entire process leads to the establishment of a vertical organismal profile as well. However, it remains to be seen whether the vertical microbial distribution is directly influenced by the vertically changing parameters such as: available space (brine volume), bioavailable nutrients and liquid water, Photosynthetically Active Radiation (PAR), salinity gradients, and the movement of external nutrients and organisms through the system. Systems with higher rates of exchange with the external environment should have a less stable vertical profile, but when stability throughout the season is established, regardless of high rates of exchange, it could be a sign that the microbial consortia is able to manipulate the environment to ensure a given position in the ice matrix.

2.2.2 *Brine Volume Size and the Vertical Profile of Sea Ice.*

The distribution and size of brine channels changes throughout the ice core, which creates a vertical profile of brine channels that might impact the distribution of organisms within the ice. Sea ice brine volumes can make up 5-20% of the total ice volume (124), but more recently estimates of <10% brine volume have been reported (96, 115). Additionally, experimental studies have reported that capillary brine channels of insufficient size were not occupied, but that organisms were most highly concentrated within capillaries only slightly larger than the organisms present (69). Interestingly, given the restrictions placed

on organisms occupying brine channels, there is still a degree of predator-prey interactions occurring within the ice. For instance, rotifers can occupy channels that are 57% of their own diameter, allowing them to traverse the brine channel network and occupy a unique niche in the sympagic food web (69). Typically the larger, less flexible, predators are unable to enter or survive in the ice, but in that case they might attached to the bottom slushy layers and tendrils, thereby taking advantage of attachment and a constant stream of nutrient rich brine exclusion from the internal network (74). Additionally these grazers and lower ice algae might be supplying the upper layers of ice with some nutrients in the form of wastes and byproducts that are carried into the brine channels via natural temperature driven convection (74).

2.2.3 *Vertical Profile of Salinity in the Ice.*

Brine channel size and geometry are directly dependent on: the amount of water exchange that is occurring throughout the bottom portion of the ice, as well as on the salinity and temperature of the brine at that ice depth (87). By definition, the brine volume will be smallest in regions with the lowest internal ice temperatures and associated higher internal brine salinities. Additionally, the temperature gradient throughout the ice impacts the concentration of the brine salinity (46), when the ice is coldest the brine is more concentrated in the upper portion of the ice, but this leads to gravity driven brine movement down the ice core to the lower portions. Other processes, such as brine expulsion and flushing also impact the distribution of brine throughout the ice (58).

The reason for this salinity fluctuation is physical and chemical, but it has major biological implications, which suggests that the composition of the bacterial consortia in the brine solution will vary with ice depth (89). Organisms surviving in the underlying saline waters of the ocean or lakes must deal with moderate salinities of anywhere from the 6-15 ppt typical of the diluted central Baltic sea waters (63), which freeze at 0°C, to the more typical sea water conditions, where salinity is around 34 ppt and water freezes at -1.8°C (87). However, cryoconcentration and the freezing process act to potentially make the internal brine channel salinity as much as an order of magnitude higher than the underlying waters. For instance, for standard seawater (salinity 34.3 ppt), the internal brine salinity ranges from 37.6 ppt at -2°C to 235.6 ppt at -30°C (25). These fluctuations mean that organisms trapped within sea ice must be capable of surviving in a wide range of salinities. Furthermore, based on the shapes of the bulk salinity curves (FYI typically has C shaped curve: with increasingly high salinities being typical of ice at the top and bottom portions of the ice-cover (resulting in a C-shaped bulk salinity graph) (33)), the organisms thriving in the middle portions of the core are faced with the lowest brine salinities, while organisms at the top and bottom sections must be capable of surviving in higher salinities.

Section 3: *Microbial Activity and Interactions within Environments*

Biodiversity studies are used to assess the species richness of a given system, but biodiversity alone cannot be used as an estimate of ecosystem functionally. In fact, given all of the recent advances in microbial sequencing procedures it cannot even be said that the identification of microbial DNA or markers in an ecosystem equates to prolonged microbial survival or activity. So making a clear distinction between activity and presence is a critical part of understanding the role these organisms play in frozen ecosystems.

3.1 *Determining the Viability of Microbes Encased in Ice.*

While prokaryotes have been identified in almost every frozen biome on Earth, existence does not necessarily equate microbial activity or viability. In fact, researchers have identified bacterial DNA in permafrost that has been dated to over 1 million years old and even though the DNA was partially degraded, it was still PCR amplifiable (1, 18, 90). This being said it is clear that the identification of bacterial DNA in these extreme ecosystems does not necessarily mean the organisms are still viable and active members of the community. Therefore, a distinction must be made between those organisms identified by PCR and those organisms that have been shown to be biologically active or at least viable via culture based methods.

Psychrophilic and even psychrotolerant organisms are significantly underrepresented in culture based experiments (130). However, there are several other ways to go about indicating that identified microorganisms are still actively metabolizing and/or actively growing in the environment. The

most commonly used *in situ* activity measurements are based on the incorporation of radioactively labelled molecules such as: [^{3}H]-thymidine incorporation into nucleic acids (demonstrating replication), or [^{3}H]-leucine incorporation into proteins, organic ^{14}C incorporation into glycolipids, and ^{14}C incorporation into biomass via $^{14}CO_2$ respiration (all demonstrating metabolism) (99). Another method for demonstrating metabolism is direct visualization of active respiration using incubation with 5-cyano-2, 3-di-4-tolyl-tetrazolium chloride (CTC) followed by filtration and epifluorescence microscopy (107). These metabolite-based methods have been employed to distinguish between identification of bacterial DNA and *in situ* bacterial metabolic activity in several icy or permanently cold environments including snow (14), permafrost (43, 44), glacial ice (2, 4, 17, 66, 100), sea ice (65, 85), and lake ice (101, 105).

It is important to note the following difference between metabolic activity and viability: metabolic activity suggests the presence of an intact cellular membrane, which indirectly indicates viability, but does not confirm it. Furthermore, continued activity and metabolism implies the ability to survive for prolonged periods within frozen/icy systems (30). Recent evidence suggests that ice-over-water organisms are metabolically suppressed during ice formation, but after incorporation into the brine, biomass production increases significantly: 1) over 30% of the total sea ice biomass can be active (49); 2) after freeze-in, the bacterial activity in the ice cover of a high Arctic lake was significantly greater than that identified in the underlying lake waters(36); and 3) primary production

in the sea waters below ice was approximately 2.05 (mg C m^{-3} d^{-1}), while the ice activities averaged 8.9 times higher, pore water was 10 fold higher, the slush layer was 77 times higher, and over 11.9 x10^3 times higher in the surface ponds than the underlying sea waters (68). Higher than expected viability and activity within the sea ice was observed in sea ice from both the Arctic and Antarctic; culturability in sea ice was higher among sea ice samples than nearby seawater samples (12). At any rate, it is clear that organisms are able to metabolize within the ice, which leads to the obvious next question of how are microorganisms able to survive the freezing process, become encased within the ice without dying, and then maintain some level of function even when the surrounding liquid water is freezing solid. The answer is a complicated one and deals with cumulative adaptions of microorganisms, which combine to increase survival and activity within the ice.

In short, the resident sea ice organisms have been defined as being those organisms that survive attached to the inner brine channel walls and are therefore not disrupted by the movement of brine via convection or expulsion. In effect, to make up a portion of the resident consortia bacteria must be so well adapted to surviving in the environment (i.e. sea ice) that sudden environmental disturbances (i.e. brine drainage, brine expulsion, flushing, ice crystal growth, etc.) do not destroy or alter the community composition, in other words the community must be both resistant and resilient to these physical processes to be considered a resident consortia (5, 112). Conversely, the transient consortia are those organisms that are affiliated with the brine solution cycling through the ice and

therefore these transient organisms may not be specialized sea ice microbiota. In fact, they might be transient melt pool, snow melt, rain water, or sea water organisms, entering the ice involuntarily and leaving it in much the same manner, without substantially surviving within the icy biome.

3.2 *Importance of Activity and Reproduction within the Ice.*

Prokaryotic and Eukaryotic activity and reproduction within the ice is a very important estimation of the degree of organism survival during the freezing process, as well as the degree of ecosystem functionality and stability. In fact, current estimates of microorganism activity within ice places the lowest threshold of activity well below -20 °C (65), which was historically considered impossible. The wide range of specified adaptations that enable organisms to survive the freezing process have not been exhaustively researched, but it is clear that organisms can evade impediment on new ice crystals during growth, maintain cell wall integrity (127), stay motile within the ice (64), and even maybe direct the formation or expansion of brine channels during freezing (123, 131). These factors really highlight the several ways in which organisms might physically impact their primary habitat. Additionally, active organisms continue cycling nutrients (15, 28, 91, 102), releasing products to the underlying waters, seeding underlying water algal blooms (75, 114). Furthermore, organisms capable of reproducing within the ice present even more interesting ideas to the field of research (87), particularly because such organisms might be capable of evolving within the ice into divergent species. However, the current questions in this field greatly outnumber the answers and much more research is needed to more

completely understand the impact ice-over water ecosystems have on neighboring systems.

3.2.1 *Evidence of Eukaryotic Activity and Reproduction within the Ice.*

Due to the perennial quality of some Arctic ice, it is possible to find that some ice floes can support species with life spans of more than a year. For example, the amphipods *Apherusa glacialis* and *Gammarus wilkitzkii* have a 2 and 6 year lifespan, respectively, in Arctic MYI (97), suggesting that these organisms are capable of surviving within FYI or young MYI ice without undergoing replication, which could imply that they are not true members of the resident ice-consortia. In contrast, Antarctic copepod species have life spans of less than one year (sometimes reaching two years), which fits with the shorter life-span of their seasonally icy environments. However, the life-span of Antarctic copepods can outlast the span of the ice-cover, so they may not be replicating within the ice, which implies that they are residents of the seawater and merely persist in the sea ice throughout the winter. The majority of copepods move into the warmer waters during the winter season and suffer high losses to activity and survival, but some species are capable of surviving within and under the ice-cover (7).

However, even in MYI it is difficult to find reports of sampling actively reproducing amphipods and copepods in the sea ice (reviewed in (3)) and it is even more difficult to find reports of actively reproducing bacterial species, making it problematic to concretely substantiate this new concept of a truly resident ice species. In fact, only one image of a dividing cell was discovered in

this literature review. In a study that took place on the seasonal ice cover of the Baltic Ocean (12 Feb to15 Mar 1996), the sea ice was sectioned into vertical pieces and bacterial cell morphotypes and biomass were qualitatively analyzed with scanning electron microscopy (86). This study published an electron micrograph of samples obtained 23 Feb (new pancake ice-cover), wherein the authors discovered a dividing bacterium of the genus *Caulobacter* spp. (86).

Section 4: *The Importance of the Seasonally Frozen Lakes of the Canadian Great Plains*

There has been a surprising lack of research regarding the microbial consortia of seasonal ice and the diversity shifts associated with transitioning from the underlying water to the overlying ice. Furthermore, considering the drastic changes that the ice-bound consortia are faced with surviving throughout one winter season, including initially freezing into the ice, increasingly high salinities throughout the ice-bound season, and the eventual thaw, release, and resumption of a planktonic life, it is surprising that these transitionally icy systems have not received more coverage in the current explosion of research of permanently sympagic assemblages.

Seasonally icy systems differ from permanent icy biomes primarily because a fraction of the lake water microbial assemblages must be capable of surviving this annual freezing process, while maintaining functionality and viability in their new icy biome. In this fashion, the lake water assemblages with a previously acquired adaptations (27) may become the initial inoculum of the lake ice and survive within the ice-cover throughout the winter months (Dec. to Apr.). Research on this topic may offer insight regarding the broader ecological effects that may accompany shorter winter seasons and the likely trend of an increasing proportion of seasonal sea ice encroaching on the once perennial ice cover of the high arctic and Antarctic (23, 120). This climate driven switch from multiyear ice to first year ice will lead to changes in the microbial constituents of the sea ice, which may be more dramatic in an already seasonally frozen system,

particularly the small, shallow, secluded lakes which will more rapidly respond to or be impacted by slight fluctuations in weather.

4.1 *Studying the Saline Lakes of the Great Plains: Importance and Future Directions.*

The dominant ecosystems of the Canadian Great Plains include the prairies and grasslands, which span a 350,000 km^2 region touching the provinces of Alberta, Saskatchewan, and Manitoba. The ability of these lakes to store water is directly linked to the amount of freshwater input from spring snow, snowfield, and glacial runoff. Historically, this freshwater input was reliable (80), but is presently diminishing due to steadily increasing air temperatures (59) and seasonal drought. These changes have led to significant water level losses to several of these lakes over time (80), which, in turn, has drastically altered the overall lake water chemistries. Today, the lakes in the region are completely or nearly completely isolated from other waterways for at least part of the summer, leading to annually decreasing water levels and associated increasing solute concentrations. The region is also sporadically challenged by droughts and hot summers during which average mean temperatures are 25°C and temperature extremes of ~30 °C are not uncommon (79). The lakes are also stressed by the general lack of precipitation, which accounts for the high degree of ephemeral lakes is the region (54). Furthermore, if this region follows the projected trend for increasing ambient temperatures we might see air temperatures increasing by 1.8 to 4 °C(59), which will further exacerbate an already noticeable increase in the proportion of ephemeral lakes and ultimately the permanent loss of other lakes

in the region. Moreover, because lake levels are dependent on spring and freshwater input, variations in snowfall during the previous winter, coupled with variations in summer temperatures, might result highly variable chemistries from year to year or possibly the complete loss of certain smaller lakes that were once present throughout the summer.

These inter-seasonal changes in water chemistries occur due to evapoconcentration of the dissolved solids in the lake water. Additionally, these lakes have differing initial salinities, ranging from fresh to hypersaline(11, 24), with the majority of the lakes falling into the range of 20 ppt to >50 ppt(54), which means that slight alterations in overall lake volume over the course of one summer might lead to a cumulative change in the lake salinity, ultimately reshaping the ecosystem that these salty lakes help support. However, slight changes throughout the course of a summer is only one aspect of the seasonal alterations that can occur in a landscape faced with such varying annual mean temperatures in summer and winter as 25°C and –4.1°C, respectively(79, 80). This seasonally changing air temperature with the onset of winter causes annual freeze/thaw cycles. Winter brings with it several changes to the landscape of the Canadian Great Plains, many of which impact the saline lakes and the organisms living within the lakes themselves. With mean temperatures approaching -4.1 °C and minimum temperatures below –61.1 °C were recorded(79), it is likely that the majority of the liquid water in the plains will eventually freeze. Additionally, the process of cryoconcentration takes place throughout the winter; as the ice cover freezes over the lakes freshwater is removed from the lake causing the bulk

lake salinities to increase throughout the winter and then decrease again after the spring melt. Other physical and chemical changes associated with the winter freeze of these lakes include increasing salinity of the internal brine channels relative to the underlying waters, sodium sulphate crystal formation(54), decreased photosynthetically active radiation reaching the lake waters, decreased mixing, and depressed liquid water temperatures, all of which are more noticeably impacted by low ambient air temperatures above small, shallow, and secluded lake systems(54).

Miquelon Lake, located in Miquelon lake Provincial Park roughly 35 km southeast of Edmonton Alberta Canada, was chosen as the specific focus of this study because it is a small (surface area: 8.72 km^2), shallow (mean depth: 2.7m), secluded (residence time of water: >100 years) (122), brackish (6-9ppt) lake of the Great Plains. In fact, though it is one of hundreds of brackish to saline lakes in the Canadian Great Plains, it was chosen specifically for this study because the salinities of these waters so closely approximated those typical of the Baltic Sea (6-15 ppt) (63), a sea ice location thought to be analogous in physical ice structure to Miquelon due to their similar salinities. Additionally, phenomena such as over ice flooding, upwelling of underlying waters onto the ice, the existence of ice ridges and rafting, and the formation of snow-ice are all considered to be distinct sea ice features, which have been observed on Miquelon lake as well (8). Miquelon has been used as a physical ice analogue for sea ice systems by Christian Haas and collaborators for several years (8, 51), and their salinity measurements and radar images both point to the high degree of physical

and structural similarity between the physical structure of the brackish sea and lake ice. However, the degree to which Miquelon lake ice might be a biological analogue to this and other sea ice systems remains undetermined at this time.

The hypotheses of the current research are as follows: 1) ice assemblages are highly similar across depths and throughout the 5-month season; 2) ice assemblages will originate primarily from the underlying waters; 3) seasonally frozen lakes will maintain an actively functioning ecosystem and microbial food web throughout the winter. These questions have been addressed in regard to sea ice, but have not, to my knowledge, been addressed in regard to seasonal lake ice. The remainder of this paper will be aimed at addressing these hypotheses in regard to the assemblages identified in the ice and waters of Miquelon Lake during the winter season 2009/10.

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Composition, diversity, and stability of microbial assemblages in seasonal lake ice, Miquelon Lake, central Alberta

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Abstract.

This study focuses on the inter- and intra-seasonal microbial assemblage within the lake ice of Miquelon Lake, a shallow saline lake in Alberta, Canada. Bacteria and Eukarya dominate the microbial assemblage, which has less than 1% Archaea. The bacterial assemblages were moderately diverse and did not vary with either ice depth or time. The closest relatives of the bacterial sequences from the ice include Actinobacteria, Bacteroidetes, Proteobacteria, Verrucomicrobia, and Cyanobacteria. The eukaryotic assemblages were less conserved and had very low diversity. The nearest neighbors of eukaryotic gene sequences were dominated by green algae; however, a copepod and cercozoan were also identified, indicating the presence of complete microbial loop. The persistence of a chlorophyll a peak at 30-35 cm below the ice surface, despite ice migration and brine flushing, indicates possible biological activity within the ice. This is the first study of the composition, diversity, and stability of seasonal lake ice.

Introduction.

Saline lakes are scattered across every continent on earth, with total volumes roughly equaling the volume of terrestrial freshwater lakes (Last, 2002) Specifically, the hundreds of scattered brackish to saline lakes of the Canadian Great Plains are of particular economical, agricultural, and ecological importance to the inhabitants of the region. These plains lakes are used in the mineral industry (particularly in regard to potassium sulfate and sodium sulfate extractions) (Bowman & Sachs, 2008) and act as storage reservoirs of excessive spring runoff, thereby limiting spring flooding and maintaining late summer agriculture when water levels are sufficiently high (Swanson & Zurawell, 2006) . Additionally, several of the larger lakes are the primary habitats or stopover points for migratory animals, Canadian geese, and ducks (Sorenson *et al.*, 1998) , which can take advantage of the game and stickleback fish surviving in the waters (Mitchell & Prepas, 1990; Swanson & Zurawell, 2006) . The lakes also support numerous algal species that have been extensively documented (Haynes & Hammer, 1978; Hammer, 1978; Bierhuizen & Prepas, 1985; Evans & Prepas, 1996) , as well as complete microbial food webs (Sørensen & Teske, 2006; Sorokin *et al.*, 2006; Grasby & Londry, 2007) . The physical and chemical aspects of the lakes located in the Canadian plains have been studied since 1972 (Mitchell & Prepas, 1990) . These ephemeral bodies of water range from fresh to brackish, the majority having salinities ranging from 22 to 180 ppt (Hammer, 1978) , making them classified as ranging from brackish (20-50 ppt) to hypersaline (>50ppt) (Beadle, 1959) . However, little is known about how the

lake water organisms are influenced by the winter weather dynamics, annual freeze-thaw cycles of the upper waters, or the progression from being an open-water to becoming an ice-covered lake.

Little is known about the winter ecology of the Great Plains lakes of Canada, especially the dynamics of the organisms persisting in the ice-cover and the underlying waters. There are some studies of perennially frozen lakes and the microbial assemblage distribution of lake ice cover in Antarctica (Priscu *et al.*, 1998; Dieser *et al.*, 2010; Foreman *et al.*, 2011) , but similar studies of seasonally ice-covered lakes are rare. Accounts of ongoing winter dynamics of phytoplankton and zooplankton during the winter of some northern lakes have been published (Bowers *et al.*, 1990; Vanderploeg *et al.*, 1992) , but we are aware of no such studies regarding the stability of the bacterial assemblages of the ice-cover or the underlying waters. However, there have been numerous accounts of bacterial and algal abundance (Garrison, 1991; Gosink *et al.*, 1993; Gradinger & Ikävalko, 1998; Priscu *et al.*, 1998; Deming, 2002; Díez *et al.*, 2012) , activity (Grossmann, 1994; Mock & Gradinger, 1999; Junge *et al.*, 2004; Mock & Thomas, 2005; Pusceddu *et al.*, 2009) , and reproduction (Mock *et al.*, 1997) within the brine channels of sea ice have been reported since the first discovery of sympagic or 'ice-associated' organisms, little is known regarding the activity and abundance microbes in the seasonal ice-cover of lakes.

There have been few assessments, to date, of bacterial abundance, activity, and diversity in the lake waters of perennially frozen lakes in the Arctic (Bahr *et al.*, 1996) and Antarctic (Simmons *et al.*, 1993) , while even less is known about

the prokaryotic assemblages surviving within the lake ice itself (Priscu *et al.*, 1998; Priscu *et al.*, 1999; Gordon *et al.*, 2000) . And even less is known regarding the *in situ* activity of these lake ice organisms (Junge *et al.*, 2004) . There is extensive literature regarding sea ice assemblages and the likely degree of activity/production occurring within the sea ice as well as the degree to which sea ice harbours a fully functioning microbial loop [for review see (Garrison, 1991; Brierley & Thomas, 2002; Arndt & Swadling, 2006)]. Therefore, as sea ice has been directly compared to sea ice in terms of diversity (Junge *et al.*, 2004) , it seems fitting to use the following assessment of the sea ice microbial loop as a hypothetical appraisal of the likely prokaryotic assemblages that might be present and active in lake ice.

High rates of primary production was the first indication of biogeochemical cycling within the sea ice, in fact, in some samples of Antarctic sea ice the annual primary production has been estimated to be as high as 63 to 70 Tg C yr^{1-} (Lizorre, 2001) . Additionally, it has been noted that there is an indication that heterotrophic bacteria (Junge *et al.*, 2004) , heterotrophic flagellates, and heterotrophic euglenophytes have also been identified in sea ice floes, which could demonstrate the presence of complete cycling occurring within ice (Kuosa *et al.*, 1992) . Positive correlations between bacterial number, biomass, production, and Chl *a* and primary production has been used to suggest that bacterial growth may be coupled to microalgae growth (Kottmeier & Sullivan, 1990) , which indicates that bacterial growth during ice formation may be stimulated by ice-algae blooms, which would be subsequently maintained at a

given depth of increased irradiance. Ice microalgae might provide bacteria with dissolved organic matter (DOM) as an energy source (Kottmeier & Sullivan, 1987) , while bacteria might provide algae with inorganic nutrients for prolonged sympagic survival (McGrath Grossi *et al.*, 1984) . Furthermore, diverse populations of microheterotrophs (e.g. protozoans, dinoflagellates, ciliates, and amoebae) complete the microbial loop (Garrison *et al.*, 1984; Kottmeier & Sullivan, 1988) . However, Archaea, previously identified and confirmed as being persistently active in seawater during winter (Murray *et al.*, 1999) , have not been identified consistently within sea ice and when they have been identified they represented very low percentages of the total ice-population (Junge *et al.*, 2004) . This degree of evidence of a complete microbial loop within sea ice speaks to the fact that there might be a similar fully functioning ecosystem existing within lake ice, even though the there is indication that lake ice might have less of a diverse prokaryotic assemblage due to the lack of frazil ice scavenging during lake ice formation (Garrison *et al.*, 1983; Junge *et al.*, 2004) .

The present study represents the first characterization of microbial diversity in the ice-covered lakes of central Alberta and the first attempt to identify spatial and temporal shifts in the microbial assemblage composition. We explored the inter- and intra-seasonal shifts in microbial assemblage composition both within the lake ice and underlying lake water of Miquelon Lake, Alberta CA. The central hypotheses of this study are as follows: 1) the physical environment in the ice is the primary driver of community structure within the ice; thus, the seasonal lake ice has communities similar in composition to other frozen ice-over-

water systems (e.g. sea ice or perennial lake ice); 2) the microbial community in the ice may is further structured by the chemical environment within the ice matrix, indicating that the vertical chemical gradient within the ice restricts populations to specific depths in the ice and/or points in the season; 3) seasonally frozen lakes of the Canadian North maintain an actively functioning ecosystem and microbial food web throughout the Canadian winter.

Methods.

Study site. Miquelon Lake, located in Miquelon Lake Provincial Park Edmonton, Alberta at 53.25°N, 112.90°W (Swanson & Zurawell, 2006) was chosen as the example of small (surface area: 8.72 km^2), shallow (mean depth: 2.7m), secluded (residence time of water: >100 years), and brackish (6-9 ppt) lakes of the Great Plains (Swanson & Zurawell, 2006) . Miquelon Lake is fed primarily by precipitation, spring flow, and agricultural runoff (Swanson & Zurawell, 2006) , but even so the water levels continue to decrease and the lake has not overflowed into the North Saskatchewan River for over 60 years (Swanson & Zurawell, 2006) . Miquelon Lake waters are dominated by prokaryotic life. Photosynthetic algae and cyanobacteria are also present; in fact, based on the chlorophyll-*a* levels measured throughout the open-water season, Miquelon Lake is classified as a mesotrophic system (Swanson & Zurawell, 2006) . Furthermore, Miquelon Lake may make a good physical analog for some sea ice systems. Temperature and salinity regimes indicate that this lake is a mixed system until the appearance of overlying ice (Jan-April), which acts to insulate the underlying waters resulting in a slight thermohalocline in the underlying waters.

Sample collection and processing. Ice cores and underlying lake water samples were collected every two weeks throughout the 4-month 2009/10 winter season. Two ice cores with a 9 cm diameter were collected with a Kovacs Mark II corer (Kovacs Enterprises Inc.; Lebanon, NH): one for biological sampling and one for bulk salinity measurements. Ice thickness measurements were taken at the time of sampling by measuring the precise length of the removed ice core (after removal).

The core for biological sampling was kept in the dark and frozen at -20°C until returning to the lab where it was aseptically sectioned into 4-7 cm sections using a flame sterilized 15 cm long drywall saw. The sections were melted in the dark at 4°C (12-16 hours per section) and then immediately processed. Following removal of subsamples for cell enumeration and chlorophyll *a* concentrations (see below), samples were filtered through 0.22 μm pore size 47 mm polysulfone filters (Pall Corporation; East Hills, NY). Filters were stored frozen at -80°C in sterile sealed Seal-a-Meal® bags (Sunbeam® Products Inc.; Neosho, MO).

Surface water samples were collected in 1 L sterile acid washed Nalgene® bottles (VWR) after removing the ice core. Samples were kept in the dark at 4°C and processed within 24 hours of sampling following the same procedure as the melted ice core segments.

In-situ Measurements. In-situ measurements of snow and ice temperatures were acquired using automated measurements by two Ice Mass Balance Buoys (IMB) (MetOcean/CRREL and SAMS IBMs) (Figure 2-1). This instrument is designed to measure thermodynamic changes in the mass balance of ice cover using pressure and temperature sensors in the ice. Temperature sensors are typically accurate to 0.1ºC (Richter-Menge *et al.,* 2006) . For the purpose of this paper this instrument was responsible for acquiring the air temperature and internal ice temperature data.

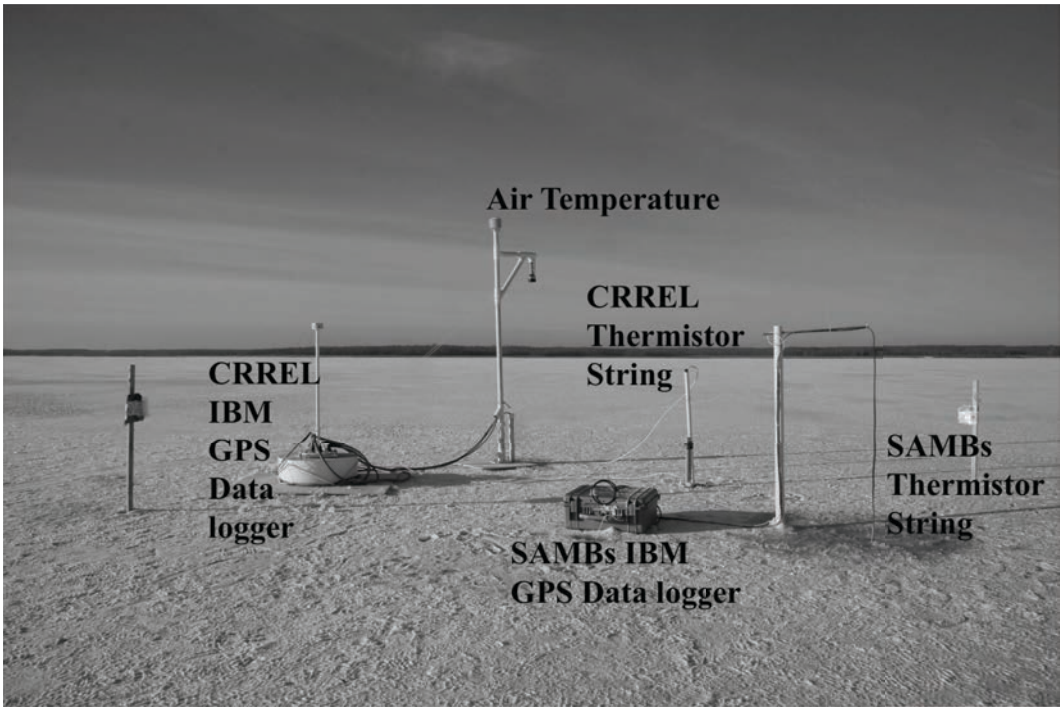


Figure 2-1. Shows the set up of the IMB buoys on the lake in December 2009. The MetOcean/CRREL (Cold Regions Research and Engineering Laboratory; Dartmouth, Nova Scotia) IMB consists of the logger buoy, surface and subsurface (not shown) sonic rangers, and the thermistor string. The SAMS (Scottish Association for Marine Sciences©; Oban, Argyll) IMB in the foreground consists of the logger/modem enclosure and the digital thermistor circuit.

Ice Salinity Measurements. The core for bulk salinity measurements was sectioned onsite into 3-4 cm pieces immediately after removal and placed into sterile plastic tubs to melt. Measurements of water temperature (*in situ*), lake water salinity (*in situ*), and bulk ice core melt salinity were acquired using a MultiLine® IDS WTW Cond 330i conductivity meter (Wissenschaftlich-

Technische Werkstätten (WTW) Inc./Xylem Inc.; Weilheim, Germany), which was calibrated prior to use according to manufacturers specifications.

Brine Salinity Calculations. The brine salinity of the ice (S_b) is calculated as follows in Equations 4 and 5.

Equation 4: Brine Salinity Approximation for T ≥ -23°C (Petrich & Eicken, 2010) **.**

$$S_b = \left(1 - \frac{54.11}{T}\right)^{-1} \times 1000$$

Equation 5: Brine Density approximation (Petrich & Eicken, 2010) **.**

$$\rho_b = 1000 + 0.8S_b$$

Brine Volume Calculations. Please note the following symbol definitions (List of Symbols): Vb is the brine volume within the ice, Va is the air volume within the ice, ρ is the ice density (kg m^{-3}), S is bulk salinity of the ice (ppt), and T is temperature (°C). The subscripts i, b, and si denote pure ice, brine, and sea ice, respectively. The coefficients for a_i, b_i, c_i, and d_i for both F_1 and F_2 are listed in Table 2-1. For the purpose of these calculations we assumed that V_a = 1 ppt (0.001) because it has been documented that the volume of air in sea, brackish, or freshwater ice is typically 1 ppt, so this assumption was made for the following calculations (Cox & Weeks, 1982) .

Equation 1: Calculation of Brine Volume; as calculated in (Leppäranta & Myrberg, 2009) .

*The coefficients for a_i, b_i, c_i, and d_i for both F_1 and F_2 are listed in Table 2-1.

$$\frac{V_b}{V} = \left(1 - \frac{V_a}{V}\right)\frac{\rho_i S_{si}}{F_1(T) - \rho_i S_{si} F_2(T)},$$

where

$$F_i(T) = a_i + b_i T + c_i T^2 + d_i T^3$$

Equation 2: The density of pure ice (ρ) as calculated in (Petrich & Eicken, 2010) **.**

$$\rho_i = \frac{(917 - 0.1403\,T)}{1000}$$

Table 2-1. Coefficients for functions $F_i(T)$ for different temperature intervals. Adapted from (Cox & Weeks, 1982; Leppäranta & Myrberg, 2009; Petrich & Eicken, 2010) .

T (°C)	a_1	b_1	c_1	d_1
$0 \geq T > -2$	-0.041221	-18.407	0.58402	0.21454
$-2 \geq T \geq -22.9$	-4.732	-22.45	-0.6397	-0.01074
$-22.9 > T \geq -30$	9899	1309	55.27	0.7160
T (°C)	**a_2**	**b_2**	**c_2**	**d_2**
$0 \geq T > -2$	0.090312	-0.016111	1.2291×10^{-4}	1.3603×10^{-4}
$-2 \geq T \geq -22.9$	0.08903	-0.01763	-5.330×10^{-4}	-8.801×10^{-6}
$-22.9 > T \geq -30$	8.547	1.089	0.04518	5.819×10^{-4}

Cell enumeration. Formalin-fixed (3.7% v/v) subsamples (total volume: 25 mL) of the ice cores and lake water were filtered on polycarbonate black membrane filters (pore size: 0.22 μm; diameter: 25 mm; Whatman; VWR) and stained with 4',6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich) for 15 minutes in the dark, and bacterial abundances were determined by fluorescence microscopy as previously described (Porter & Feig, 1980). The analyses were limited to nonfilamentous and non-autoflorescencing bacterial morphotypes (<5 mm cell length).

Chlorophyll *a* measurements. Chlorophyll *a* (Chl-*a*) concentrations were assessed for the ice depth profile and the underlying water. GF/F glass fiber filters (Whatman, VWR) for chlorophyll analysis were precombusted in an Isotemp Programmable Muffle Furnace (Fisher) at 450-500°C for at least 12 hours. 60 mL sample aliquots were filtered though a 25 mm precombusted Whatman GF/F glass fiber filter in the dark. Filters were stored frozen at –20°C until processing. Duplicate samples were taken randomly and used as quality controls throughout the extraction and measurement process: the quality controls totaled 10% of the total number of samples.

Chl-*a* was extracted by overnight incubation in 95% ethanol in the dark using standard extraction methods and calculations (Bergmann & Peters, 1980). Samples were measured directly after completion of the extraction process on a RF-1501 spectrofluorophotometer (Shimadzu Scientific Instruments; Columbia, MD U.S.A.). The minimum detection limit of this protocol was 1 μg/L for 200 ml sample volume. Optimum sensitivity for Chl-*a* extract measurements is

obtained at an excitation wavelength of 436 nm and an emission wavelength of 680 nm (Bergmann M. and R.H. Peters, 1980) . Chl-*a* from *Anacystis nidulans* algae (Sigma) was used for the production of a daily standard curve.

Nucleic acid extraction. The remaining water from the ice core sections (100 to 300 mL depending on the segment size and volume) and lake water samples (900 mL) was filtered through sterile polyethersulfone membrane filters (pore size: 0.2 μm; diameter: 47 mm; PALL Corporation, Ann Arbor, MI), which were immediately frozen at -80°C until DNA extraction. DNA was extracted from the frozen filters using FastDNA® extraction kit according to the manufacturer's protocol (MP Biomedicals, Solon, OH). DNA was eluted in 200 μL of warm DNAse free commercial water (Life Technologies, Grand Island, NY). The solution was buffered to 1x TE concentration (10 mM Tris, pH 8.0, 1 mM NaEDTA) and stored at -20°C.

PCR: Partial bacterial 16S rRNA genes were amplified with previously described primers 341F and 518R, with a 40 mer GC clamp on the 341F primer (Table 2-1; (Myers *et al.*, 1985)) with running and PCR conditions as previously describe (Kulp *et al.*, 2006) . PCR specific primers were used for eukaryotic amplification of 18S rRNA gene and for the nested amplification of archaeal 16S rRNA gene as well (Table 2-2, (Bano *et al.*, 2004; Labrenz *et al.*, 2010) , and references therein).

Table 2-2. PCR Primers used in this study.

Primer set	Target	Sequence (5'-3')	Reference
341F*	Bact 16S	CCTACGGGAGGCAGCAG	Muyzer et al. (1993)
518R	rRNA	ATTACCGCGGCTGCTGG	Muyzer et al. (1993)
Euk1A	Euk 18S	CTGGTTGATCCTGCCAG	Diez et al. (2001)
Euk516R*	rRNA	ACCAGACTTGCCCTCC	Diez et al. (2001)
A21F	external Arc 16S	TTCCGGTTGATCCYGCCGGA	DeLong (1992)
1492R	universal rRNA	GGTTACCTTGTTACGACTT	Labrenz, M. et al. (2010)
344F*	internal Arc 16S-V3	ACGGGGCGCAGCAGGCGCGA	Labrenz, M. et al. (2010)
519R	internal rRNA	GGT DTT ACC GCG GCK GCT G	Sørensen KB, Teske A. (2006)
*GC clamp (40 bp) added for DGGE-PCR (Myers et al. 1985). 5′-CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCC-3′			

In an attempt to minimize PCR bias all PCRs were preformed in triplicate and then pooled (Polz & Cavanaugh, 1998) prior to running on DGGE or sequencing.

Denaturing gradient gel electrophoresis (DGGE). Initial screening of bacterial and eukaryotic PCR products was performed via DGGE using a D-CODE system (BioRad, Hercules, CA) as previously described (Kulp *et al.,* 2006) . 400ng of DNA were run for every sample on the DGGE, normalization of DNA concentrations was performed using standard agarose gel electrophoresis.

DGGE banding patterns were analyzed with the program GelCompar II (version 4.0; Applied Maths, Austin, TX) using a 2% band position tolerance to determine band locations. The corresponding cladogram was generated using an unweighted pair group method based on Dice correlation coefficients, which are based on the presence/absence of a band regardless of absolute band intensity,

previously described in detail (Kulp *et al.*, 2006) . Bands were visualized under UV light after staining the gel for 15-30 minutes in SYBR Green stain (Molecular Probes, Eugene, OR), according to the manufacturer's instructions.

qPCR Analysis. DNA from lake ice and water samples was homogenized, resulting in one bulk sample for ice (all ice core depths and sampling dates) and one bulk sample representing the underlying lake water (all sampling dates). The relative abundance of Bacteria, Eukarya, and Archaea 16S rRNA genes in the lake ice and lake water samples was determined using primers described in Table 2-2.

qPCR was performed in triplicate 10 µl reactions containing 5 µl Rotor-Gene SYBR green PCR kit (Qiagen, Inc), 1 µM concentration of primers, 2µl template and 1µl Qiagen RNase-Free water. Reactions were performed in a Rotor-Gene Q (Qiagen, CA) qPCR machine. Cycles were 40 cycles of 95 °C for 10 s and 60 °C for 15 s. Gene copy number was calculated in relation to a standard curve included in all runs using *Escherichia coli* genomic DNA.

Clone Library Construction. Three PCR reactions were pooled and 1 clone library per environment (lake ice and lake water) was constructed for Bacteria and Eukarya using the TOPO® TA Cloning® Kit (Invitrogen) according to the manufacturer's instructions. Libraries of clones were selected from the Bacteria, lake ice (n = 123), Bacteria, lake water (n = 191), Eukarya, lake ice (n = 123), and Eukarya, lake water (n = 39) samples.

Restriction fragment length polymorphism (RFLP). Preliminary grouping of clones was performed by RFLP analysis using restriction enzymes Hha1 and MSP1, following previously described techniques (Skidmore *et al.*, 2005) .

Clone insert orientation was determined by unidirectional PCR using the previously discussed conditions and parameters but with only M13F primer in the master mix. The representative members of each RFLP grouping were unidirectionally sequenced with M13F or M13R, depending on orientation. One clone for every ten members in a given OTU (based on RFLP analysis) was chosen for sequencing. All clones chosen for sequencing were rerun on a DGGE prior to sequencing to confirm band position in reference to the original samples as previously described (Kulp *et al.*, 2006) .

Sequencing was carried out with a BigDye Terminator Cycle Sequencing Kit (Life Technologies, Carlsbad, CA, USA). Concentrations used were as follows: 2 X BigDye terminator sequencing buffer, 1 X Ready Reaction premix, 100 - 300 ng of template and 3 - 5 pM primers. The PCR cycling used was an initial denaturation of 96 °C for 1 min, followed by 30 looped cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min. The resulting products were analyzed by the Department of Biological Sciences Molecular Biology Facility on a 3730 DNA Analyzer (Life Sciences), chromatograms visualized with FinchTV (PerkinElmer,Waltham, MA, USA).

Phylogenetic Tree. Sequences were trimmed and processed using standard methods (Lanoil *et al.*, 2001) . All sequences were subjected to CHIMERA_ CHECK (Cole *et al.*, 2004) to ensure the absence of chimeric sequences prior to beginning phylogenetic analysis. To obtain our initial phylogenetic relationships for the sequenced clones, nearest neighbours were determined by BLASTn analysis against the GenBank database (www.ncbi.nlm.nih.gov/BLAST/).

Sequences from this study and their close relatives (obtained from the GenBank database) were aligned in Genious 5.5.8 (Biomatters Ltd.; New Zealand) using 25 alignment iterations and the FastAligner function to align sequences. All alignments were manually refined by eye and shared gaps were eliminated. Maximum likelihood-based phylogenetic analysis was conducted with the PYLym module in Genious (Drummond *et al.*, 2011) using only those sequences with nearly complete coverage (sequence length ranging from 300-600 bp) sequences. Subsequently, the robustness of tree topologies was tested by making a new tree with a truncated version of all the sequences, in which all gaps in all of the sequences were removed, resulting in a truncated sequence (~250 bp). The phylogenetic relationships in both trees were identical and the phylogenetic relationships were therefore considered robust. The robustness of the branching order was confirmed by bootstrap analysis (100 re-samplings).

Statistical analyses. Good's coverage estimate (Good, 1953) was calculated manually for the clone libraries.

Nucleotide sequence accession numbers. Sequences will be submitted to the Genbank database at the time of submission for publication.

Results.

Throughout the 2009-2010 winter season (Nov-Apr), air temperatures at Miquelon Lake ranged from a low of –40°C (13 Dec 09) to a high of +10 °C (29 Mar 10), with an average winter air temperature of –10.6 °C. During that time period, Miquelon lake ice grew from 0 to 0.4 m in total thickness (Figure 2-2), had internal ice temperatures ranging from approximately –1 to –4°C (Figure 2-3). Seasonal average ice temperatures and underlying water temperatures were very stable, depicting a small range of subzero temperatures faced by organisms in the system (Figure 2-4). Miquelon lake water salinity averaged throughout the 2009/10 winter season. Brine salinity, which is directly determined by ice temperature, varied with depth, ranging from a high of 60 ppt to a low of ~10 ppt (Figure 2-3). The brine volumes make up an average of 10% of the total ice volume throughout this season, with the lowest brine volume (V_b) occurring at the same depth as the lowest brine salinities throughout the season (Figure 2-3).

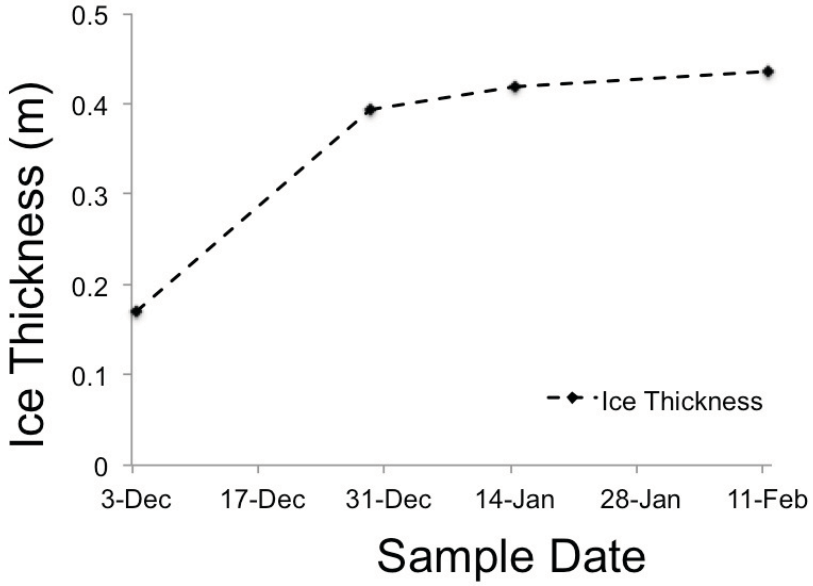


Figure 2-2. Ice growth throughout the winter season 2009/10.

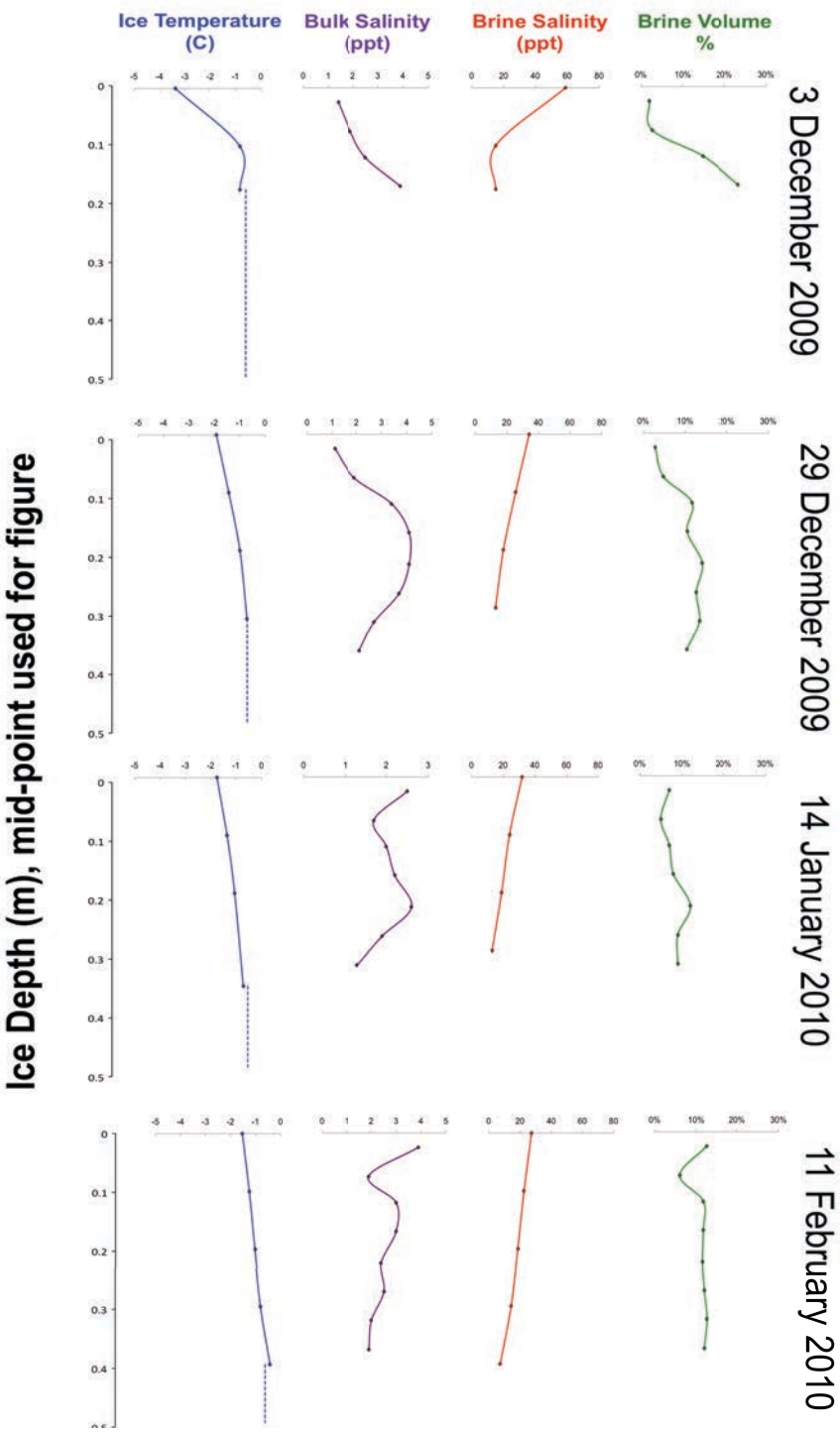


Figure 2-3. Vertical lake ice profile of (a) temperature (internal ice temperature: solid line; beneath ice water temperature: dashed line), (b) bulk salinity (ppt), (c) brine salinity, and (d) % brine volume. Four dates (corresponding to sampling dates) were graphed: 3 December 2009, 29 December 2009, 14 January 2010, and 11 February 2010 (moving down from top to bottom).

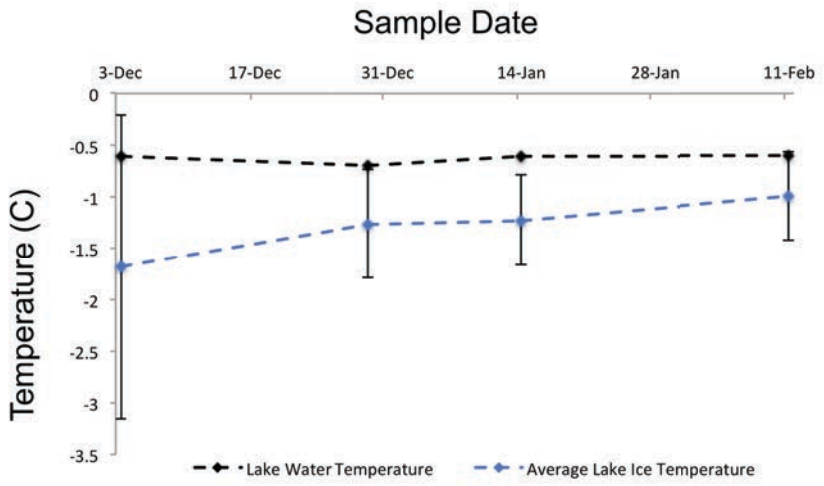


Figure 2-4. Progression of lake water temperature and ice temperature throughout the season, illustrating the short duration of ice-cover over Miquelon and the relatively small range of temperatures facing the organisms in both the water and ice-cover (total range: -0.5 to -1.5).

Microscopic enumeration showed 2.4 x 10^6 cells mL^{-1} in the ice and 5.61 x 10^6 cells mL^{-1} in the lake water (Figure 2-5), ca. 15% of which are auto-fluorescing (and therefore likely photosynthetic) cells. qPCR results showed small subunit rRNA gene copy number relative abundance of 50% Bacteria, 50% Eukarya, and <1% for Archaea (data not shown).

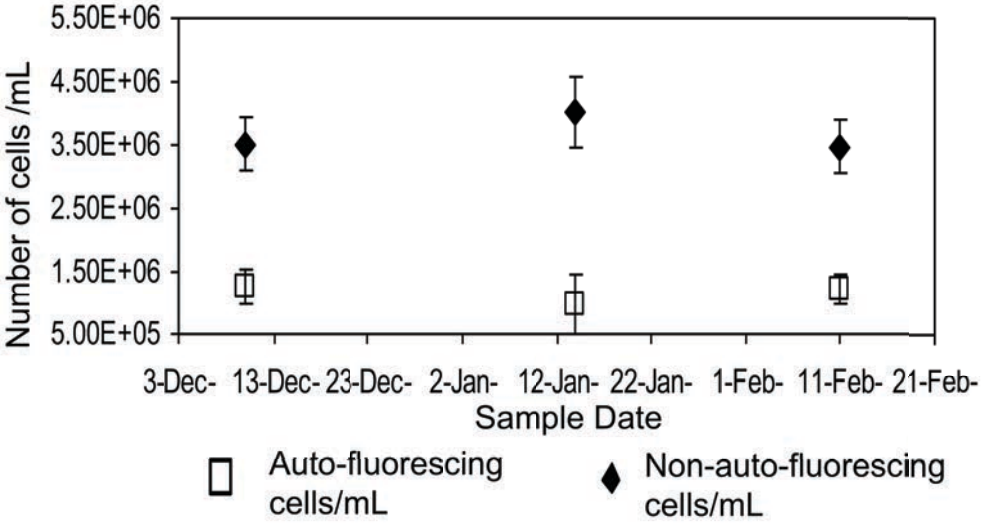


Figure 2-5. Integrated cell abundance of Miquelon Lake ice for three time points, using direct DAPI cell count data. Cell types differentiated by autofluorescence.

There was a sustained Chl-*a* peak at a depth of ~0.26 to 0.3 m throughout the season (Figure 2-6). The peak, which was 2-2.5 times higher at this depth than at any other depth in the core, was sustained for the months where ice was not prohibitively thin.

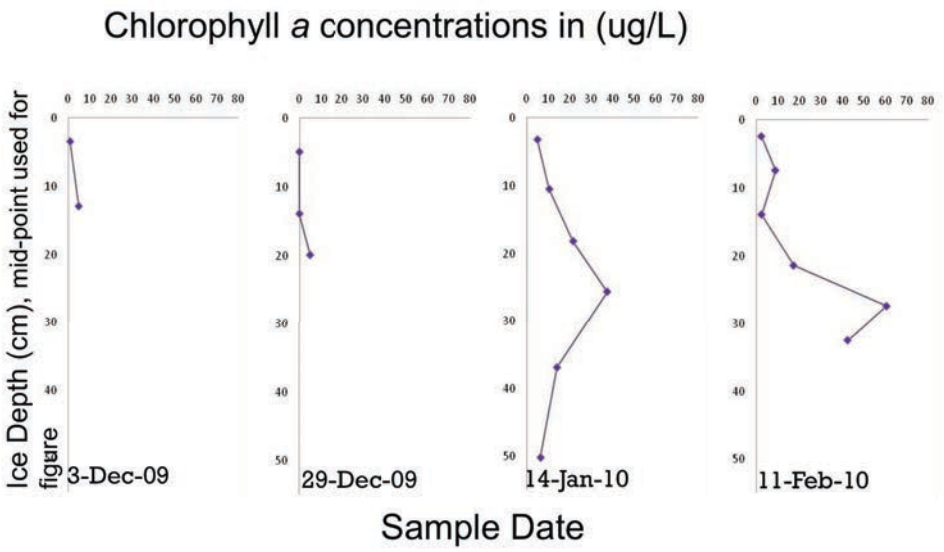


Figure 2-6. Time series of a vertical depth profile of chlorophyll *a* concentrations (ug/L) in lake ice cores.

Cluster analysis of DGGE banding patterns of both Bacteria and Eukarya ribosomal RNA genes for all of the ice samples, regardless of spatial or temporal variables, indicates the presence of a conserved microbial assemblage within the ice (Figure 2-7a). Bulk lake ice samples and water samples were ~66% similar based on the DGGE cladogram analysis (Figure 2-8a), regardless of sampling date.

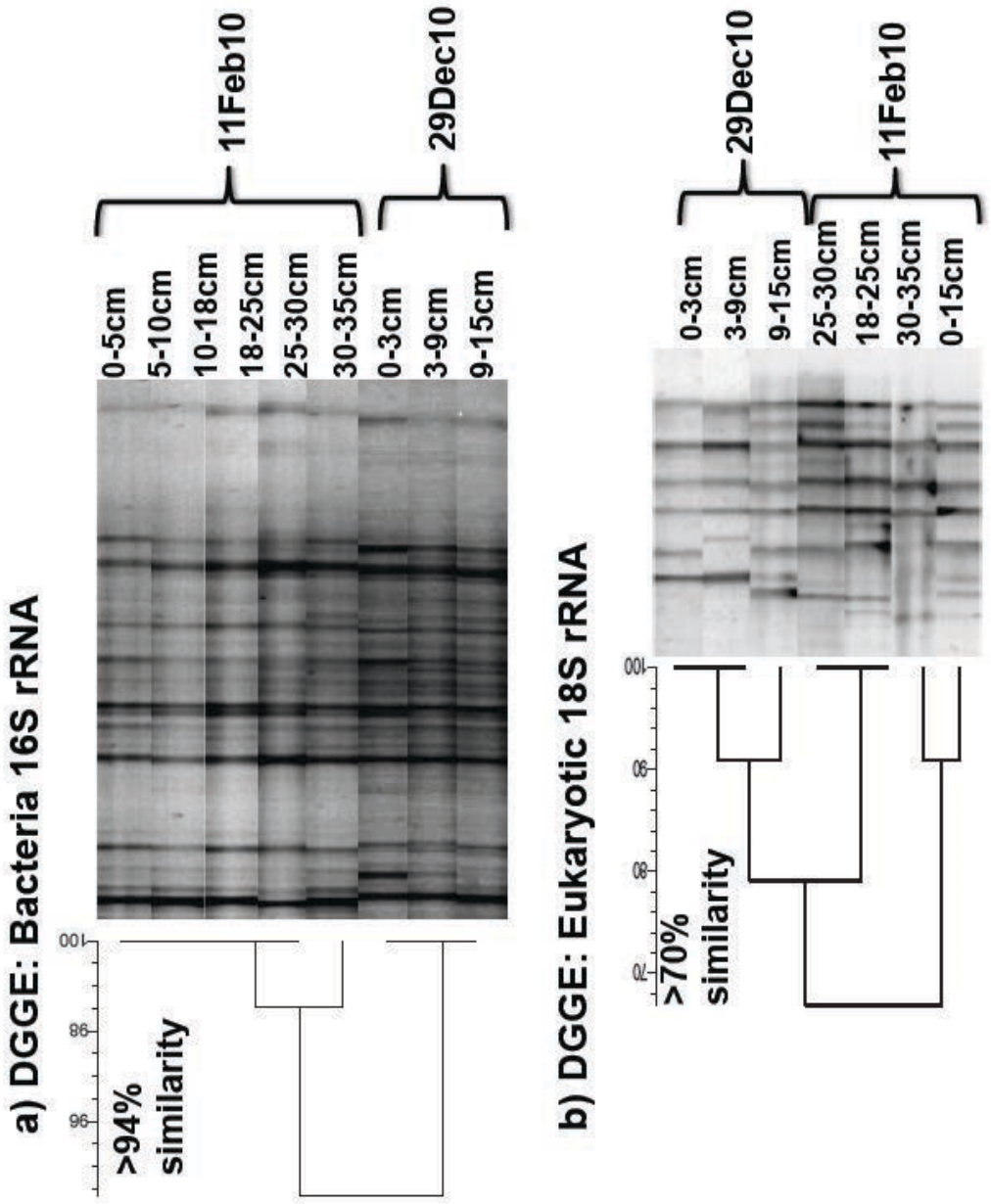


Figure 2-7. Spatial depth profile of Miquelon Lake ice cores for two sampling time points. a) Bacterial 16S rDNA assemblage composition; b) Eukaryotic 18S rDNA assemblage composition.

The Eukarya consortium appears to be less divers due to lower band richness (based on the DGGE analysis) than the bacterial consortium is (Figure 2-8b). The Eukarya assemblages were still very conserved (>70% similar) throughout ice depth and the season. The Eukarya consortia had more shared DGGE bands with the underlying water than the Bacteria consortia.

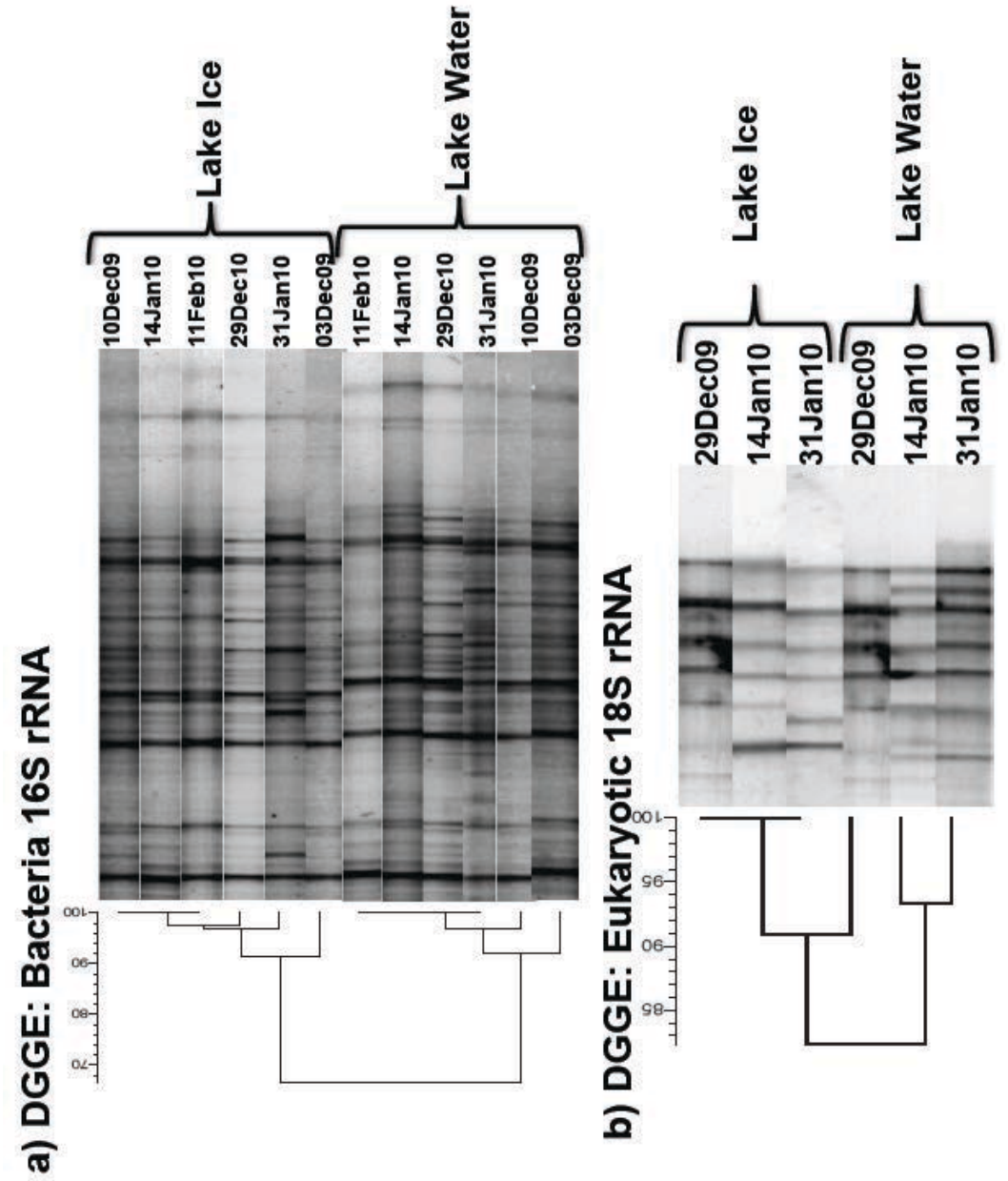


Figure 2-8. Temporal survey of assemblages in Miquelon Lake ice cores and lake water. a) Bacterial 16S rRNA gene amplicons of ice and water assemblages; b) Eukaryotic 18S rRNA gene amplicons of ice and water assemblages.

In order to identify the origin of the dominant bands and elucidate the differences between the ice and water consortium, we constructed a clone library of the bulk samples (mixed all ice depths and all sample dates from throughout the season, due to the high similarity observed with the DGGE). The Bacteria clone library had a total of 314 clones (with 213 clones remaining after removal of the mitochondrial plastid sequences). Based on RFLP results, these clones were grouped into 19 unique OTUs. Eleven of these OTUs were found in both ice and the underlying waters, with the remainder only present in one of the clone libraries (Figure 9a). The dominant band in the bacterial DGGE (Figure 2-7a) (initially making up 71% of the bacterial ice clone library and 41% of the bacterial water clone library) was identified as a mitochondrial rRNA sequences were closely related to *Nannochloropsis oceanica* and *Chlorella minutissima* mitochondrial plastids based on Blast nearest neighbor results (Figure 10 a and b), and were therefore excluded from further clone library analysis. Miquelon Lake ice and water show a surprising rank abundance curve (Figure 2-9a), with half of the OTUs being represented by roughly equivalent numbers of clones and the remainder of the OTUs comprising a short tail of singletons. A more standard rank-abundance curve, where a few OTUs dominate the clone library and the remaining OTUs are a long tail of singletons, is the pattern seen in the Eukarya rank abundance curve [Eukaryal Lake Ice (n = 123), and Eukaryal Lake Water (n = 39)]. (Figure 2-10b).

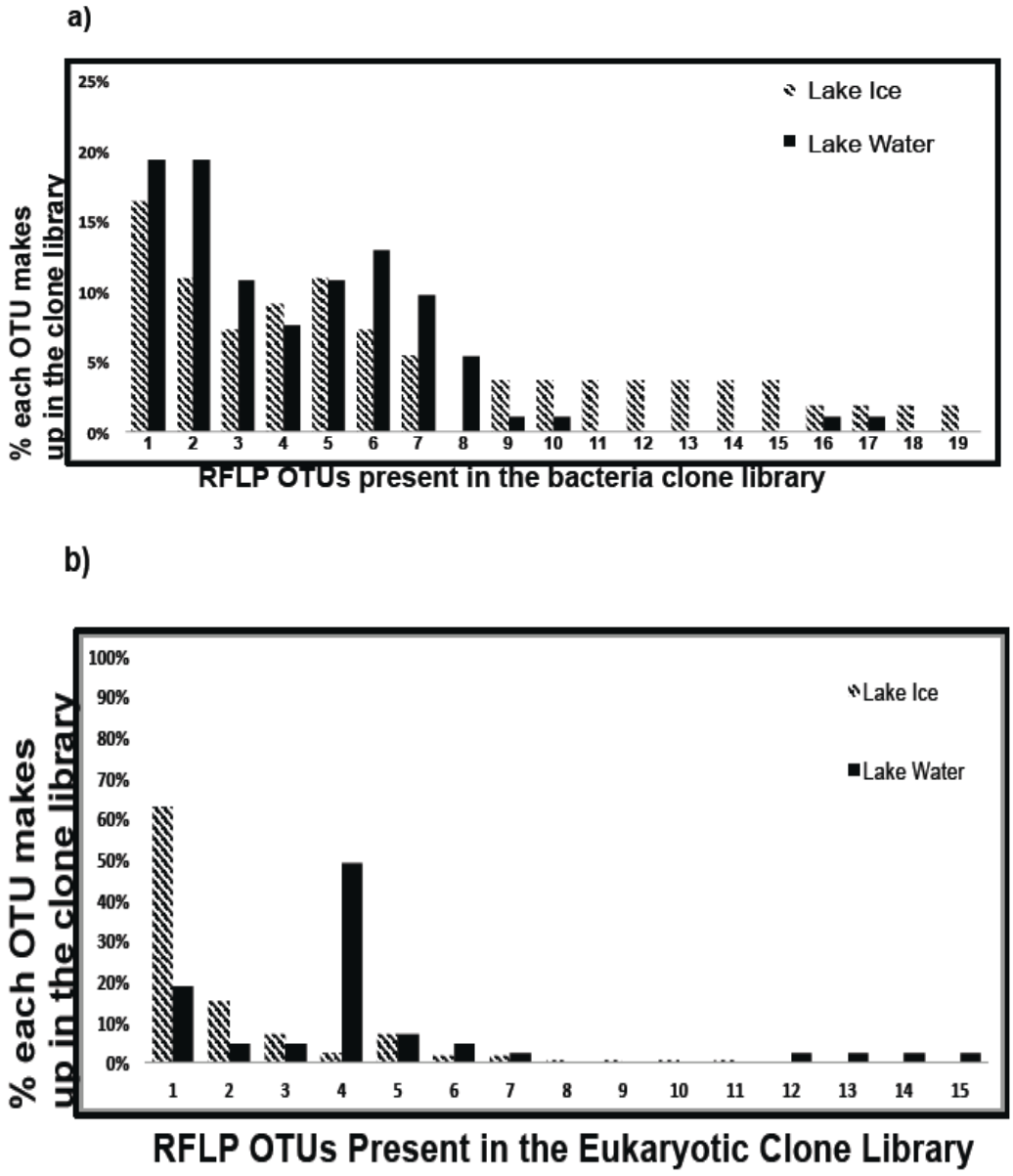


Figure 2-9. Rank abundance curve presented in percentages of the total clone library (after removal of mitochondrial DNA) for bacterial (a) and eukaryal (b) clone libraries. Clone libraries consist of the following total number of clones; Bacteria: lake ice (n = 123), lake water (n = 191); Eukarya: lake ice (n = 123), lake water (n = 39).

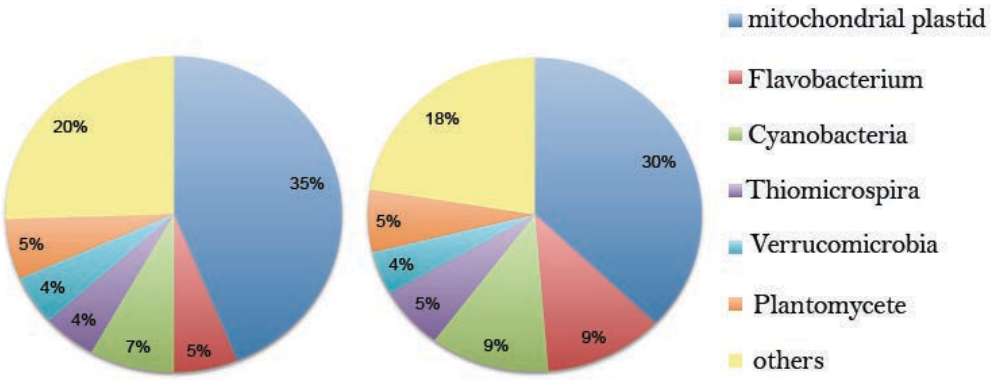


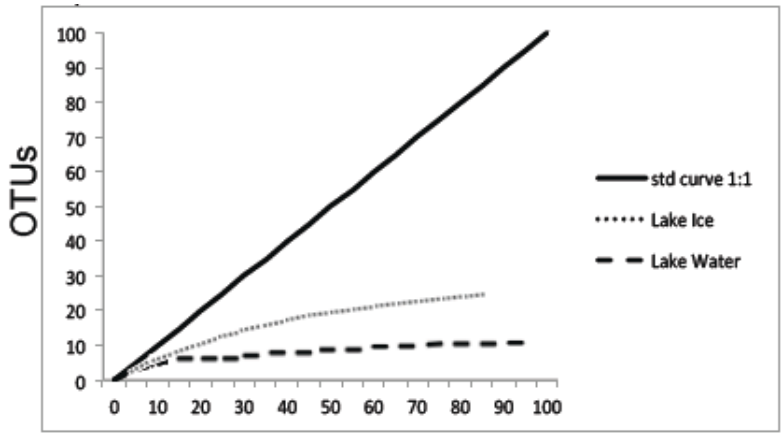
Figure 2-10. Proportion of most dominant bacterial phyla in the (a) lake ice and (b) lake water clone libraries. Phyla are based on nearest neighbor BLAST Genbank identities. The 'other' represent the portion of the clone library that was not sequenced, the reason being that each of the included OTUs represented <10% of the overall clone library.

Based on rarefaction curves, both the ice and water bacterial diversity was sampled to completion (Figure 2-12a). Separately, these libraries accounted for 50% and 72% of the overall likely diversity, based on Good's coverage estimate for the ice cover and water, respectively; however, a combined estimate for bacterial coverage is 92% (Table 2-2). The lower coverage of the ice cover was primarily due to the higher proportion of Mitochondrial plastid sequences (71% and 41% of the total ice and water libraries, respectively), but due to the degree of overlap in the clone libraries combined estimates are more representative of total diversity coverage. The ice Eukarya diversity was sampled to completion, but the coverage of the water clone library was lower (Figure 2-12b) and the Good's estimate agreed with lower coverage for the Eukaryal water clone library, but the combined estimate for ice and water had 97.8% coverage (Table 2-2).

Clone Library name	# of Clones (without mitochondrial plastids)	# of OTUs (without mitochondrial plastids)	Good's Coverage
Bacterial Ice	55	18	50%
Bacterial Water	98	12	72%
Eukaryal Ice	123	11	95%
Eukaryal Water	39	10	37%

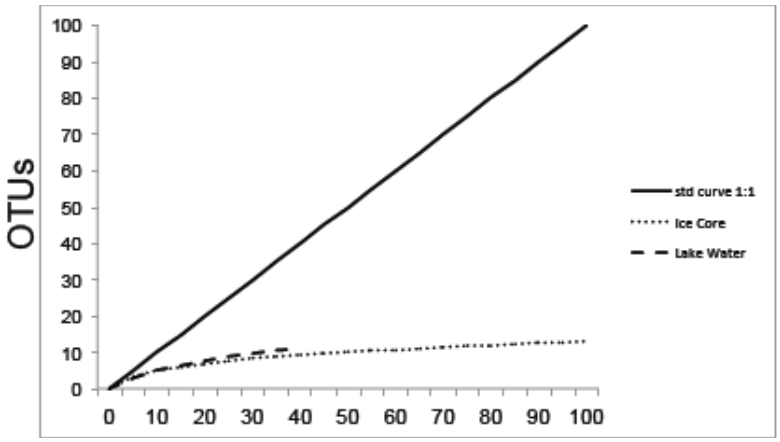
Table 2-2. Good's Coverage estimate for clone libraries, as calculated manually based on (Good, 1953).

a)



Number of occurrences of each OTU in the Bacterial clone library.

b)

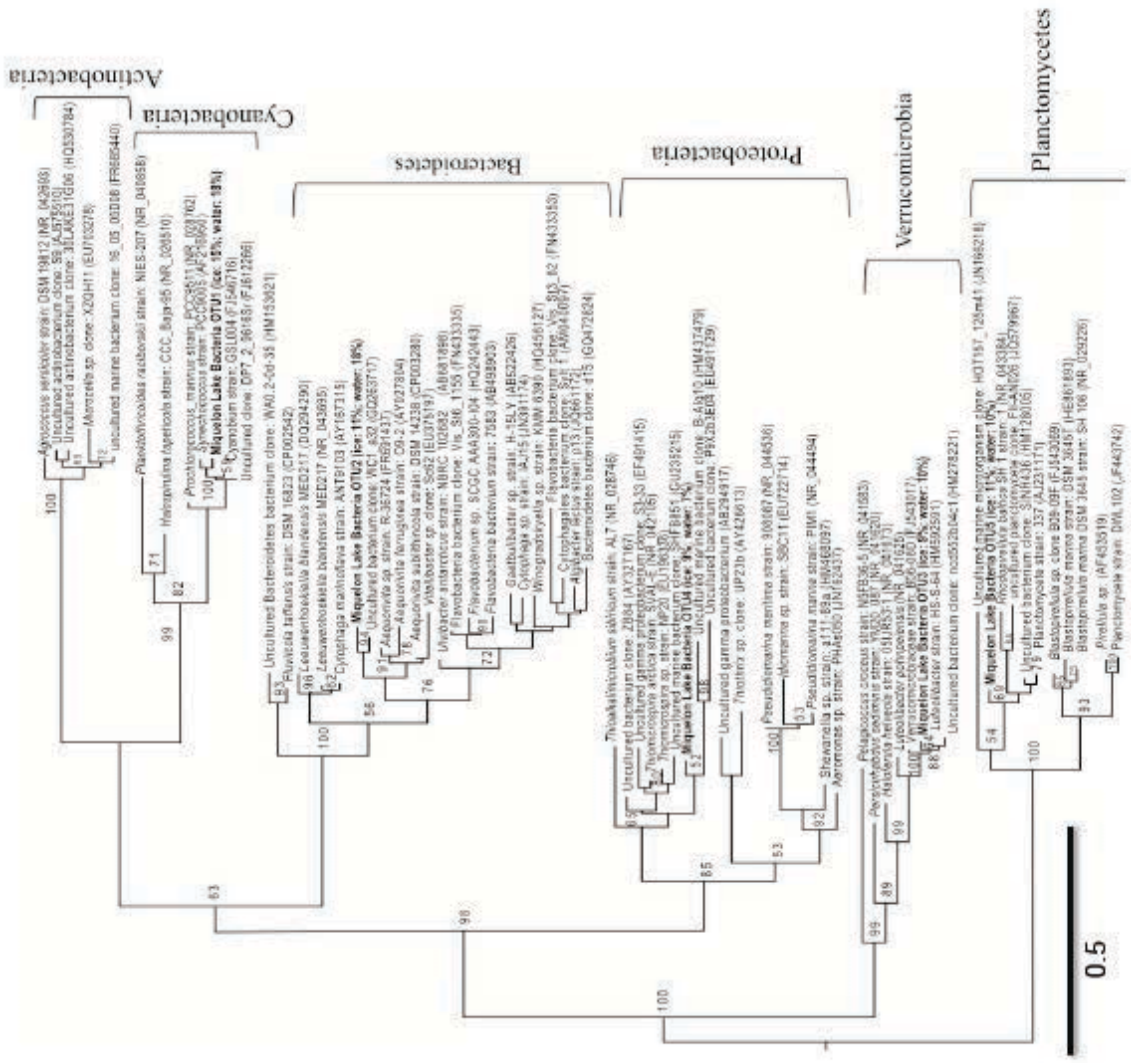


Number of occurrences of each OTU in the Eukaryal clone library.

Figure 2-12. Rarefaction curves for (a) bacterial clone libraries (ice and water) and (b) eukaryotic clone libraries (ice and water).

The closest relatives of the OTUs obtained in the bacterial clone library include the phyla Actinobacteria, Bacteroidetes, Proteobacteria, Verrucomicrobia, and Cyanobacteria (according to BLAST analysis against the GenBank database) (Figure 2-13). The eukaryotic clone library was dominated almost entirely by OTUs with nearest neighbors from green algae (Figure 2-14 and 2-15), but a copepod and cercozoan were also identified in both the ice and underlying waters

(Figure 2-14), demonstrating the presence of a complete microbial food web within the ice-cover of this seasonally frozen lake.



tree) identified in the bacterial clone libraries of the lake ice and water [ice: % of clone library; water: % of clone library]. Reference sequences chosen using nearest neighbors and other various arctic or marine reference sequences (based on BLAST Genbank analysis).

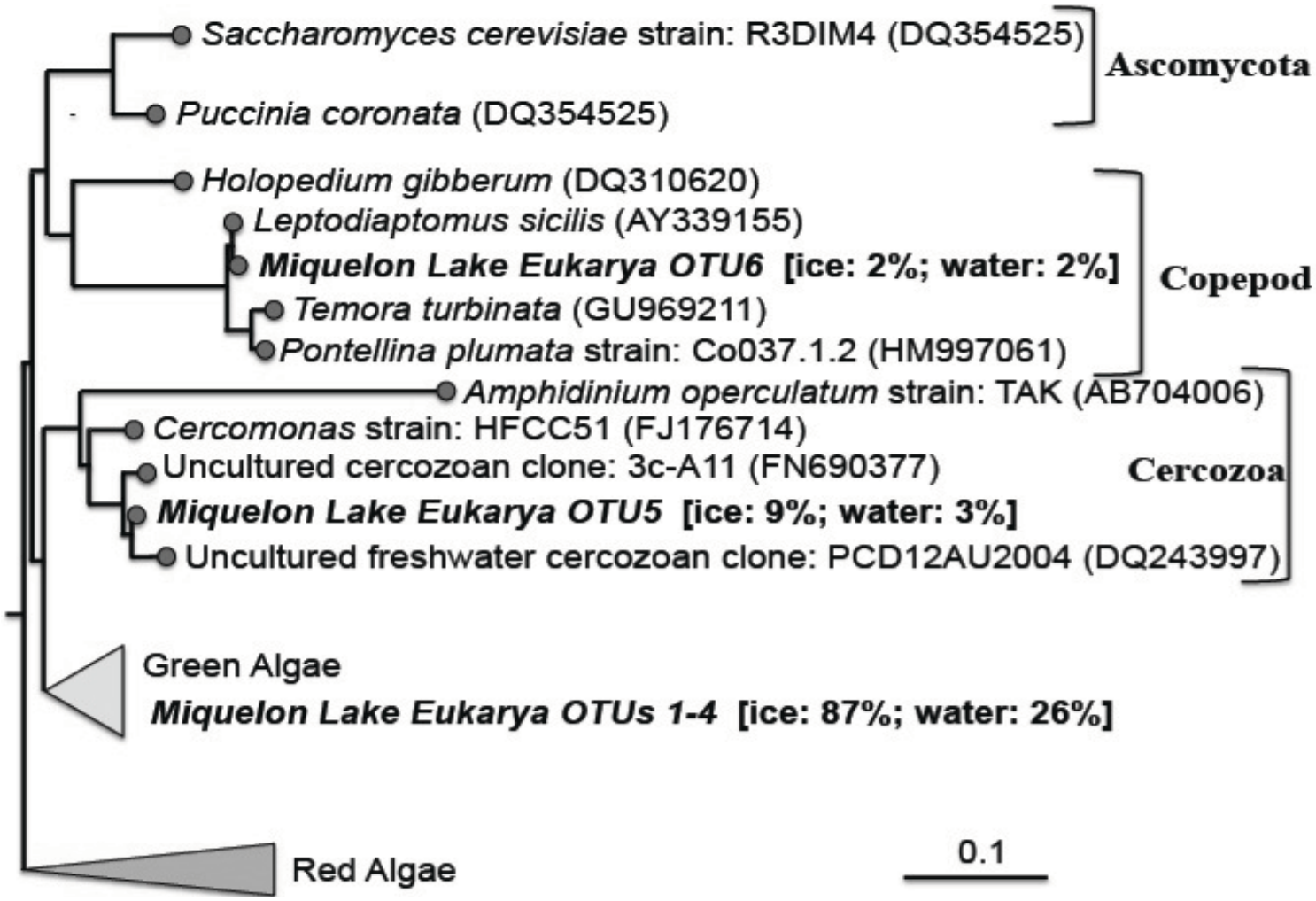

Saccharomyces cerevisiae strain: R3DIM4 (DQ354525)
Puccinia coronata (DQ354525)
Ascomycota
Holopedium gibberum (DQ310620)
Leptodiaptomus sicilis (AY339155)
***Miquelon Lake Eukarya OTU6* [ice: 2%; water: 2%]**
Temora turbinata (GU969211)
Pontellina plumata strain: Co037.1.2 (HM997061)
Copepod
Amphidinium operculatum strain: TAK (AB704006)
Cercomonas strain: HFCC51 (FJ176714)
Uncultured cercozoan clone: 3c-A11 (FN690377)
***Miquelon Lake Eukarya OTU5* [ice: 9%; water: 3%]**
Uncultured freshwater cercozoan clone: PCD12AU2004 (DQ243997)
Cercozoa
Green Algae
***Miquelon Lake Eukarya OTUs 1-4* [ice: 87%; water: 26%]**
Red Algae
0.1

Figure 13. Eukaryote phylogenetic tree of cercazoa and copepod sequences (bold in tree) identified in the lake ice and water [ice: % of clone library; water: % of clone library]. Reference sequences chosen using nearest neighbors and other various arctic or marine reference sequences (based on BLAST Genbank analysis).

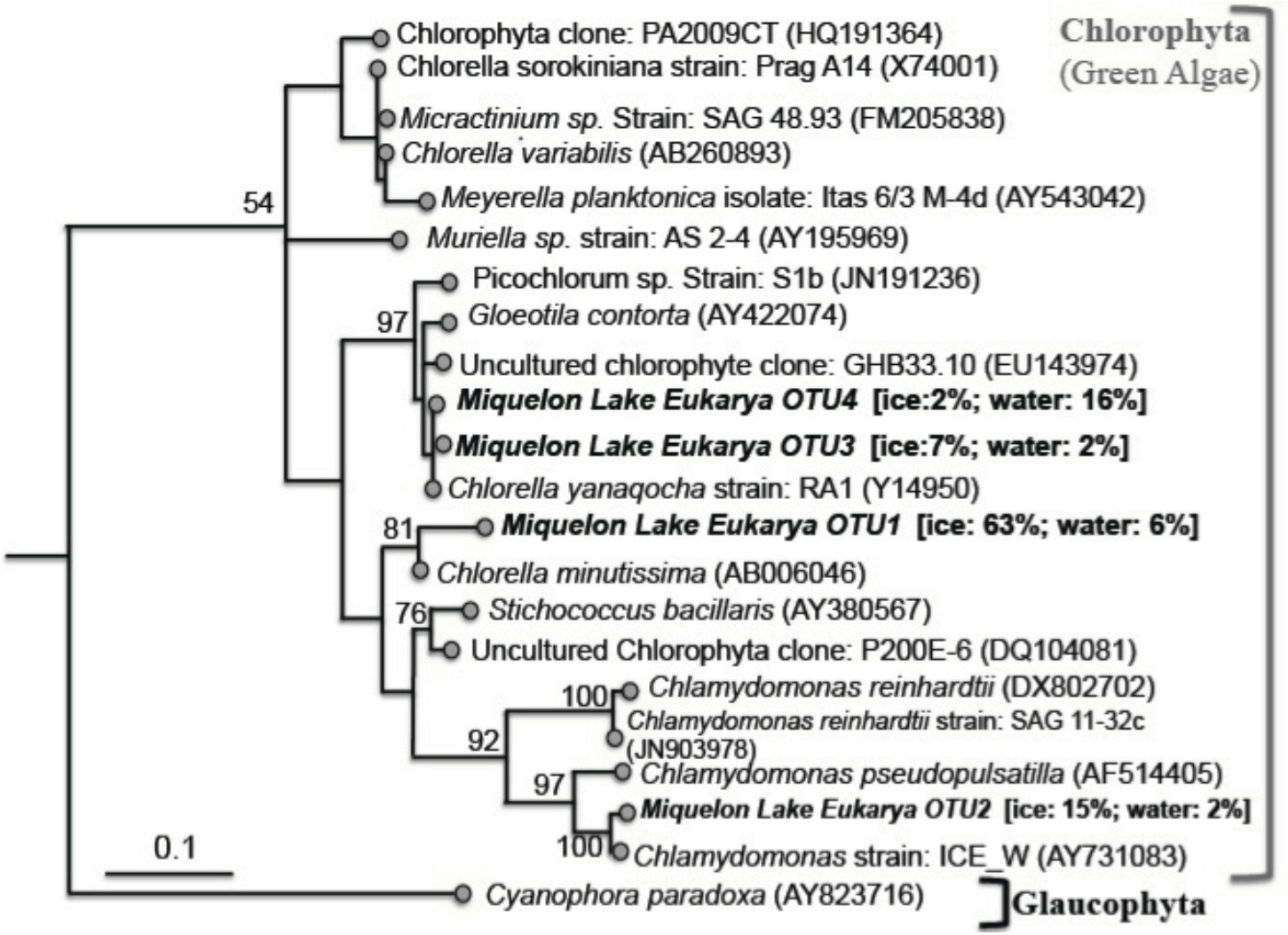

Chlorophyta clone: PA2009CT (HQ191364)
Chlorella sorokiniana strain: Prag A14 (X74001)
Micractinium sp. Strain: SAG 48.93 (FM205838)
Chlorella variabilis (AB260893)
Meyerella planktonica isolate: Itas 6/3 M-4d (AY543042)
Muriella sp. strain: AS 2-4 (AY195969)
Picochlorum sp. Strain: S1b (JN191236)
Gloeotila contorta (AY422074)
Uncultured chlorophyte clone: GHB33.10 (EU143974)
Miquelon Lake Eukarya OTU4 [ice:2%; water: 16%]
Miquelon Lake Eukarya OTU3 [ice:7%; water: 2%]
Chlorella yanaqocha strain: RA1 (Y14950)
Miquelon Lake Eukarya OTU1 [ice: 63%; water: 6%]
Chlorella minutissima (AB006046)
Stichococcus bacillaris (AY380567)
Uncultured Chlorophyta clone: P200E-6 (DQ104081)
Chlamydomonas reinhardtii (DX802702)
Chlamydomonas reinhardtii strain: SAG 11-32c (JN903978)
Chlamydomonas pseudopulsatilla (AF514405)
Miquelon Lake Eukarya OTU2 [ice: 15%; water: 2%]
Chlamydomonas strain: ICE_W (AY731083)
Cyanophora paradoxa (AY823716)
54
97
81
76
100
92
97
100
0.1
Chlorophyta
(Green Algae)
Glaucophyta

Figure 14. Eukaryote phylogenetic tree of green algal sequences (bold in tree) identified in the lake ice and water[ice: % of clone library; water: % of clone library]. Reference sequences chosen using nearest neighbors andother various arctic or marine reference sequences (basedon BLAST Genbank analysis).

Discussion.

Our findings support four main conclusions: 1) the ice assemblage composition is essentially invariant with depth in the ice; 2) the ice assemblage composition is essentially invariant throughout the season; 3) the ice and lake assemblages might be active throughout the winter; and 4) there is complete microbial loop within the ice-cover of this seasonally frozen briny lake in central Alberta.

The invariance in ice assemblage composition with depth might be an indication that the observed variations in temperature, brine volume, and brine salinity (Figure 2-3) did not have a significant impact on the composition of the microbial ice assemblages. Considering that these physical and chemical changes in this briny lake system are relatively mild compared to sea ice, where ice temperatures can reach -40ºC and brine salinities approach salt saturation, this explanation may well be true. Alternatively, there may be a high degree of microbial mixing throughout the brine channels. In fact, the majority (83%) of the bacterial clones were representatives of OTUs that were present in both the lake ice and waters. There were mutually exclusive OTUs present, but only one of them made up more than 5% of the total clone library and was therefore sequenced. The remaining 8 exclusive OTUs are most likely an artifact of low sampling size of the libraries (i.e. the OTU is present, but unsampled) coupled with the higher than expected proportion of eukaryotic mitochondrial plastids in the bacterial library. The discrepancy between the DGGE and clone library diversity estimation results (see Figures 2-7, 2-9) is likely an artifact of lower

overall sampling effort of the clone libraries, particularly the eukaryotic libraries and the fact that the DGGE was found to underestimate diversity because multiple species migrated to the same band location. Such a high degree of overlap between the consortia of the ice and underlying waters might indicate the presence of a mixing event forcing lake water up through the ice column, or it may indicate that transient lake water members of the ice bound consortia were more easily detectable and that a more high thru-put sampling effort is required in the future to analyze fine scale changes in the community structure with depth or time.

Mixing events that force underlying lake water up into the brine channels is a documented phenomenon in sea ice, but it primarily occurs in the bottom 20 cm of the ice, where the brine channels are larger and the warm underlying waters have the most exchange. In a closed system, more exchange with the underlying waters might be expected since the mass of the ice-cover growing down vertically into the underlying waters might be great enough to build up some pressure in the system. This pressure could be sufficient enough to force brine and lake water vertically up out of the ice; such flooding events have been observed at Miquelon Lake (Beckers, J. pers. comm.). This theory is weakly supported by the bulk salinity data which show that in the early season (3Dec) the salinity peak is just forming, by (10Dec) the peak is at 18cm, it this migrates to 22cm (11Jan), and back up to 12cm (14Feb); this migration of the bulk salinity peak throughout the season indicates vertical brine movement, which may have been expelled onto the ice surface during periods of fast ice growth. The level of similarity between the

water and ice communities for both Bacteria and Eukarya further indicates that some degree of mixing between the lake water and ice brine because this level of similarity is unprecedented in the literature (Bowman *et al.*, 2012) , even in seasonally frozen Antarctic lake ice (Foreman *et al.*, 2011) .

The temporal stability of the lake ice and water microbial assemblages demonstrate that after the initial freezing event, the amount of time remaining encased in the ice-cover is not a controlling factor in regard to bacterial community composition. The presence of a seasonally stable Chl-*a* peak indicates some biological activity and growth because this Chl-*a* peak remains at the same ice depth regardless of brine movement and flushing and ice growth and loss, indicating that they must grow in order to maintain the same position.

It was somewhat surprising that Miquelon Lake clones were not similar to clones and isolates from sea ice or the ice covered lakes of Antarctica. Gene sequences from in Miquelon Lake ice were predominantly related to sequences originally found in other cold-water lakes, springs, and saline environments. However, in addition to these expected sequences, two clones identified in the lake ice and waters were over 97% similar to human skin biota and wastewater treatment waters, indicating some level of possible contamination into this secluded system. The similarity to cold, but unfrozen, lake, spring, and saline environments but not to icy environments suggests a lack of specific adaptation to the seasonal icy conditions, unlike in perennially frozen systems.

Finally, this study indicates the presence of a complete microbial loop within the lake ice of this small briny lake. This is the first indication of continued

microbial loop functioning during the winter and it implies that these organisms may be actively cycling organic nutrients throughout the duration of the winter. Thus, it seems clear that the briny lakes of northern Canada are of great ecological importance to the Canadian North, even in winter, where the activity and interactions of mesophilic lakes like Miquelon are frequently overlooked.

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3.0 Chapter Three: *Comparing Microbial Assemblages in Lake Ice-Cover to Those in Sea Ice-Cover*

Sea ice is a characteristic feature of polar waters, and 90% of these waters are covered by first year or seasonal sea ice (FYI) (2), the remainder of the ice being multiyear ice (MYI). Bacteria were first observed in Antarctic sea ice in 1966 (9), and today over 200 microbial species have been identified living on, in, or in association with sea ice (5). This expansive list covers organisms from several trophic levels that create an active food web within the sea ice cover (16, 17). However, comparing the microbial consortia in sea ice to that identified in brackish and freshwater lakes is complicated, because of the wide array of small-scale differences between the two ecosystems, which, in actuality, might substantially constrict a particular consortium from activity or proliferation within the ice or might directly drive overall assemblage composition and adaptation. Furthermore making direct comparisons between the sea ice biota identified in previous studies and the consortia identified in the ice-cover of Miquelon lake in the current study is difficult due to the differences in sequencing efforts used and because the lack of clear distinction between resident ice consortia and transient consortia. However, there are several parameters that can be compared in order to highlight similarities and major differences between the two ecosystems.

First of all the mechanism of ice formation of the rough ocean versus the formation of ice on calm lake waters is a key difference between the two systems that leads to several microstructure variations in the ice crystal lattice. Secondarily, the thickness of the total ice-cover, the length of time the water

remains ice-covered (seasonal versus perennial), and the degree of total mixing from the underlying waters through the ice column all influence the microbial community structure, as well as influencing the proportion of resident versus transient organisms that might be identified. Finally, the existence of a fully functioning and possibly active microbial assemblage in the ice cover of the ocean and seasonally frozen lakes demonstrates the importance of winter ecology, and highlights the ecological significance of the organisms that are currently being lost as ice-cover over the polar oceans continues to rapidly disappear (4, 20).

As previously described in section 1.4 of this work, the manner in which ice over water is initially frozen greatly impacts the overall diversity and distribution of the microbial consortia of the ice cover. These micro-scale variations, perhaps due to the physical ice formation/crystal structure, overall ice thickness and brine volume/permeability, slight variations in brine chemistries, and even the length of the residence time of organisms will eventually impact and shape the composition of the ice-bound microbial assemblages. In fact, the process of sea ice formation inoculates the ice with the first bacteria and eukaryotes via frazil ice scavenging (6) and by the recruitment of bacteria that are directly associated with microalgae or debris that become frozen into the ice (3, 8). In fact it is probable that this phenomena of frazil ice 'scavenging' is the key difference between sea and lake ice formation that has the greatest impact on the overall microbial composition and abundance in the ice (6). Furthermore, organisms in MYI have a much longer residence times in the ice [sea ice: >5 years; Miquelon lake ice: <5 months], and on average, MYI is over three times

thicker than the cores removed on the coldest days at Miquelon lake [sea ice: ~1.5 m (22) ; Miquelon lake ice: ~0.4 m (Figure 2-1)]. Together these differences result in sea ice bound organisms having a substantially longer period of sympagic existence within the sea ice than organisms encased in small, mixed, seasonally frozen systems like Miquelon Lake have, which might result in a more diverse and variable system.

In fact, the frazil ice scavenging phenomenon, coupled with the elevated brine volume in sea ice compared to that found in Miquelon lake ice [sea ice: 5-20% volume (22) ; Miquelon lake ice: ~10% (Figures 2-2)], and longer residence times, might be the key differences between sea and lake ice that ultimately accounts for the disparity between microbial abundances in systems with the scavenging phenomenon [Arctic sea ice: 1.02 x 10^12 cells/mL (21)] and systems without the phenomenon [Baltic Sea Ice: 2.25 x 10^6 cells/mL (18) ; Miquelon lake ice (non-auto fluorescing cells): 3.75 x 10^6 cells/mL (Figure 2-5)]. However interestingly, MYI cores have a strong vertical distribution of these organisms, and it has been notice that up to 93% of the total biomass tends to be localized in the bottom 20 cm of the ice core (21) . Additionally, the bacterial cell counts in sea ice can be 10 times higher than counts for the underlying waters (21) , which is another trend unshared by the Miquelon lake ice (where the underlying waters had 2.3 times higher total cell counts than the overlying ice). Which might be an indication that the Miquelon lake organisms are less adapted to sympagic survival than are their ocean dwelling counterparts. This finding makes sense in terms of Miquelon being a seasonally frozen system, because

organisms must first be capable of planktonic survival and then must adapt to possibly attaching to internal brine channel walls. Whereas the process of frazil ice scavenging in sea ice formation could passively enrich the ice cover with organisms already more suited toward attachment to ice crystals than their planktonic counterparts, in a calm water system the ice grows vertically down around already planktonic prokaryotes. In fact, Kramer et *al.* (14) demonstrated a correlation between the species and distributions of organisms in sea ice and found that they vary depending on the type of ice they were isolated from, which was greatly attributed to the fact that organisms surviving in FYI must survive planktonic for at least a portion of their lifecycle (14) . However, similarly brackish lakes have not been studied as extensively as sea ice microorganisms have, so making extensive parallels between the two systems is difficult.

The indication that Miquelon lake ice has an active and fully functioning ecosystem within the ice-cover is not an unprecedented finding. In fact, active food webs have been found within perennially ice covered brackish and saline lakes of Antarctica as early as 1998 (19) , but those systems are continually frozen, allowing for a degree of adaptation and even evolution within the ice cover over time. Sea ice also has been found very enriched with prokaryotes and larger organisms, such as diatoms and larger scavengers (7) . The lake ice, by comparison is much less diverse than other icy systems that have been studied to date.

There are several reasons for the high degree of stability and the limited diversity that was identified in this study. One reason is the methods used in

analysing samples are aimed at targeting the most prominent members of the consortia, whereas high thru put sequencing methods might indicate a higher diversity in this system. Another reason for this finding might have to do with the fact that ice-cover over water is, geologically speaking, a very recent event in evolutionary history, which might explain the lack of a highly diverse sea-ice bacteria consortia as well (13) . The close phylogenetic relationships between the organisms identified in the lake ice and water of the current study, agree with this indication that there may have been a limited degree of time to available for the evolution of more highly diverse and phylogenetically distinct organisms in lake ice. Directly following the appearance of ice cover of ocean or lake waters the stress of a newly freezing environment is the major stress directing adaptation of the newly ice-associated organisms. Which could lead to the assumption proposed by Junge et al. (13) that resident bacterial communities will be dominated by few populations that are specifically adapted for better survival of the common sea or brackish lake ice stressors (e.g. variable and subzero temperature, salinity, variable and limited brine volume, and variable degrees of nutrient exchange with the external environment).

Even if the biological comparisons are not clear after this small scale study, it is important to make comparisons between small lakes like Miquelon and the greater expanse of sea ice because being small, a lake like Miquelon will demonstrate exacerbated responses to the slight changes in climate and ambient temperatures that are sure to come (10) . Additionally Miquelon has: a shorter winter season (Miquelon: <5 months; Sea ice <7 months to >5 yrs), higher

average air temperatures (–10.6°C for the winter), higher average ice temperatures (Miquelon: –1 to –4°C; sea ice: –2 to –10°C (22)), lower range of brine salinity 5-30 ppt (sea ice: 35-150 ppt (22)), and substantially thinner ice ~0.18 to 0.42 m cover Miquelon versus the 0.3 to 0.8 m covering the Baltic Ocean (23) and over 1.5 m covering much of the Arctic Ocean (22) . All of which compound together to make this small Miquelon lake respond faster to slight climactic changes than the bigger, ocean stabilized, sea ice system.

Additionally, comparing the biological inhabitants of lake and sea ice during the current global warming trends, might lead to a faster understanding of how the sea ice system will respond to climate change in the coming years. As ambient temperatures continue to increase we might find that the sea ice consortia beings to respond in very similar ways that the Miquelon ice consortia respond to each seasonal melt. And based on the rapid rate of Arctic sea ice loss in recent years, decrease in thickness of 1.6 m over the last 50 years (15) , these kinds of comparisons might give us insight into the changing system before all of the sea ice is lost. In fact, we might be able to glean information about how the sea ice microbial consortia will change as sea ice thins from this small thinly ice covered lake Miquelon, at least while it is still frozen throughout the winter.

3.1 *Future Directions.*

Given the findings of the present study: 1) presence of a complete microbial loop (and the notable lack of Archaea) surviving within the ice cover of Miquelon Lake; 2) indication of persisting activity of (at least) the photosynthetic portion of the lake ice assemblage throughout the winter; and 3) the high degree

of temporal and spatial stability in the microbial composition of the lake ice, there are several ways in which future research on Miquelon Lake ice and waters will be of scientific interest. The presences of a complete microbial loop within the ice cover is very interesting and gives indication of continued ecosystem functioning throughout the winter, which is of global interest if organisms frozen in lake and sea ice globally are taking part in global biogeochemical nutrient cycling throughout the winter. However, research indicating the impact of sea ice organisms and activities to the global carbon cycle is still in its infancy. It has been documented that heterotrophic organisms are persistently active within sea ice, which gives indication of the fact that global biogeochemical cycling (particularly carbon cycling) is occurring within sea ice and by extension might be occurring within lake ice as well (13) ; however further research directed at estimating the impact these organisms have on global nutrient cycling is needed to more clearly resolved the global importance of sea and lake ice ecosystems. The finding that Archaea represent <1% of the population in the lake ice might agree with previous studies of sea ice that indicated very small proportions of identifiable and active Archaea in sea ice sample as well (12) , but neither finding seems conclusive and more high thru put sequencing techniques are likely necessary to understand whether or not Archaea are an integral part of the sea ice biota.

Clarification of the degree of *in situ* activity and reproduction sea and lake ice organisms undergo throughout the winter is also of vital importance to understanding the global importance of these lake ice organisms. Presently, sea

and lake ice research is based primarily on bulk measurements from melted ice samples, which precludes researchers from determining their actual distribution and activity of organisms under in situ conditions (11). Unfortunately, as previously discussed in regard to the difficulty in confirming the status of prokaryotes in the ice (e.g. residents or transients), because melting destroys all evidence of microenvironments within the ice environment. However, *in situ* imaging does give indication of the location of organisms within the ice and the visualized fully intact cellular membranes indicate survival of the freezing process (11), but ideally as equipment advances we will be more capable of making *in situ* measurements and activity assessments to clarify the degree of in situ activity and reproduction within ice cover.

Finally, the high degree of spatial and vertical homogeneity of the lake ice prokaryotes is an interesting difference between previous MYI studies and this seasonally lake ice study. Miquelon lake ice seemed more thoroughly biologically mixed than previously studied MYI systems (1). However, even though the organisms seemed to be homogeneously identifiable through the ice core, the bulk salinity and Chlorophyll *a* both had a vertical profile throughout the ice and in the case of Chl *a* the peak was maintained in the same location throughout the duration of the season, which demonstrates a degree of vertical stability (at least at the level of photosynthetic organisms), but this result was not evidenced by the DGGE nor by the clone library analysis. This discrepancy is indicative of a lower than optimal sampling effort of this system, and perhaps in the future high thru put sequencing techniques might lend themselves to

answering these questions better than DGGE and clone library analyses have done.

In closing, this study is a good gateway study for future advancement in the field of lake and sea ice microbiology. Even if brackish lake ice is not a direct biological analogue to sea ice ecosystems, it is an example of a fully functioning microbial loop persisting throughout the winter and likely impacting biogeochemical cycles. Hopefully, winter assessments of prokaryotic diversity, abundance, and activity will continue on other seasonally ice covered freshwater to saline lakes of the Canadian North in order to better understand the effects of winter on the biogeochemical cycling of these scattered lakes.

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