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Bacterial use of choline to tolerate salinity shifts in sea-ice brines

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Abstract

Bacteria within the brine network of sea ice experience temperature-driven fluctuations in salinity on both short and long temporal scales, yet their means of osmoprotection against such fluctuations is poorly understood. One mechanism used to withstand the ion fluxes caused by salinity shifts, well-known in mesophilic bacteria, is the import and export of low molecular weight organic solutes that are compatible with intracellular functions. Working with the marine psychrophilic gammaproteobacterium, *Colwellia psychrerythraea* 34H, and with natural microbial assemblages present in sackhole brines drained from sea ice in Kanajorsuit Bay (2013) and Kobbefjord (2014), Greenland, we measured the utilization of ^{14}C-choline (precursor to glycine betaine, a common compatible solute) at −1°C upon salinity shifts to double and to half the starting salinity. In all cases and across a range of starting salinities, when salinity was increased, ^{14}C-solute (choline or derivatives) was preferentially retained as an intracellular osmolyte; when salinity was decreased, ^{14}C-choline was preferentially respired to $^{14}CO_2$. Additional experiments with cold-adapted bacteria in culture indicated that an abrupt downshift in salinity prompted rapid (subsecond) expulsion of retained ^{14}C-solute, but that uptake of ^{14}C-choline and solute retention resumed when salinity was returned to starting value. Overall, the results indicate that bacteria in sea-ice brines use compatible solutes for osmoprotection, transporting, storing and cycling these molecules as needed to withstand naturally occurring salinity shifts and persist through the seasons. Because choline and many commonly used compatible solutes contain nitrogen, we suggest that when brines freshen and bacteria respire such compatible solutes, the corresponding regeneration of ammonium may enhance specific biogeochemical processes in the ice, possibly algal productivity but particularly nitrification. Measurements of potential nitrification rates in parallel sea-ice samples are consistent with a link between use of the compatible solute strategy and nitrification.

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Introduction

The sea-ice environment is characterized by temperature fluctuations across a wide range of temporal scales, from short-term (< 1 d) diurnal oscillations to long-term (days to months) seasonal shifts (Mikkelsen et al., 2008; Ewert and Deming, 2013, 2014). These temperature fluctuations cause corresponding changes to the salinity of brines within the porous matrix of the ice (brine salinities, if not measured directly, can be calculated from equations set forth in Cox and Weeks, 1983). The extent of these fluctuations depends on geographic location, depth within the ice, and season. For example, at the Mass Balance Site near Barrow, Alaska, in 2011, brine salinity in the upper ice column (0–10 cm) fluctuated between 144 and 227 ppt during Winter (span of 83 ppt) and between near 0 and 179 ppt (span of 179 ppt) during Spring, while at the ice-snow interface, brine salinity fluctuated between 133 and 223 ppt (span of 90 ppt) in Winter and from near 0 to 191 ppt in Spring (span of 191 ppt; Ewert and Deming, 2014). In more temperate (subarctic) climes, brine salinity varies less: measurements over the entirety of the 2005–2006 sea-ice season (December through May) in Kobbefjord, Greenland, indicated that brine salinity in the upper ice (0–10 cm) varied between 10 and 70 ppt (span of 60 ppt; Mikkelsen et al., 2008).

Because the brine phase throughout sea ice harbors microbial life, particularly bacteria (Junge et al., 2001, 2004; Collins et al., 2008), the sea-ice setting lends itself to the study of organisms that can survive both very low temperatures and correspondingly high salinities as well as wide fluctuations in these parameters. The various roles that bacteria play in the biogeochemical cycles of sea ice (Deming, 2002; Bowman, 2015) depend upon effective strategies for surviving these extreme conditions. Studies of freeze tolerance and cold adaptation in sea-ice bacteria are numerous (reviewed by Deming, 2002, 2010; Bowman, 2008), but an understanding of how these organisms survive the salinity shifts that characterize their habitat is limited (Ewert and Deming, 2014). Previous work on microenvironments within sea ice raised the possibility that extracellular polymeric substances (EPS), observed to fill sea-ice pores and coat individual cells inhabiting the brine phase, may provide a physical buffer against extreme salinities (Krembs and Deming, 2008; Krembs et al., 2011). Work with pure cultures has yielded evidence in support of an osmoprotectant role for EPS in sea ice (Marx et al., 2009; Liu et al., 2013). Genomic studies of psychrophilic bacteria, including from sea ice, have highlighted their genetic potential to import and export compatible solutes to tolerate shifts in salinity (Methé et al., 2005; Bowman, 2008; Casanueva et al., 2010). Although the use of compatible solutes is a well-documented strategy in model mesophilic bacteria (Roberts, 2005), evidence of compatible solute usage by psychrophilic or cold-adapted bacteria in culture, or by microbial communities in natural sea-ice brines, is generally lacking.

Compatible solutes are small neutral or zwitterionic molecules that do not interact in a deleterious manner with the internal environment of a cell (hence the term compatible). Instead, they act as osmolytes that help to balance the osmotic effects of both high ion concentrations and shifting osmotic pressures (Brown, 1974, 1976; Roberts, 2005); when retained in high enough concentration they also provide a degree of freeze protection (Casanueva et al., 2010). High ion concentrations are often damaging to the intracellular components of microorganisms, especially to proteins, which are susceptible to denaturing via electrostatic forces (except in some extreme halophiles adapted to high internal ion concentrations) (Gilles, 1997; Bremer and Krämer, 2000). Fluctuating osmotic pressures present a host of potential threats to microbial integrity, including the threat of cell lysis due to a sudden downshift in external salinity and a concurrent rapid influx of water. In response to this circumstance, compatible solutes may be expelled from the cell on extremely short (subsecond) time scales (Ajouz et al., 1998; Bremer and Krämer, 2000); with more time, they can be metabolized into a form that no longer serves as an osmolyte (Kiene, 1998). The use of compatible solutes is, in short, an effective microbial mechanism for withstanding both extreme and fluctuating salinities.

Numerous small organic compounds, particularly sugars and amino acids, can serve as effective compatible solutes; among these, glycine betaine (*N,N,N-trimethylglycine*) is considered a potent compatible solute and is used by a wide spectrum of marine microorganisms (Kiene, 1998; Keller et al., 1999; Armbrust et al., 2004; Collins and Deming, 2013). Halophilic eubacteria, for example, accumulate betaine intracellularly in proportion to the salinity of the culturing medium (Imhoff and Rodriguez-Valera, 1984). Here we build upon work by Kiene (1998), who used ^{14}C-choline uptake and conversion to glycine betaine to show that natural microbial communities in subtropical estuarine waters use the compatible solute strategy to tolerate environmentally relevant shifts in salinity. We focused on the use of ^{14}C-choline by natural microbial communities present in another, more extreme environment also characterized by shifts in salinity — the subzero brine in sea ice. Estimates of choline concentrations in sea-ice brines were not available prior to our study, but choline had been measured in coastal seawater and inferred to be widespread in the marine environment based on its common presence in the membranes of phytoplankton and other eukaryotes (Kiene, 1998). Choline contains nitrogen, making it a valuable organic resource for heterotrophic bacteria; if respired, choline would also represent a possible source of NH_4^+ to the environment (Roberts, 2005). This aspect, coupled with reports of ammonia cycling in sea ice (e.g., Rysgaard and Glud 2004; Thomas et al., 2010), provided additional motivation for focusing on choline in this study.

To complement the work with field samples, we also examined the use of choline by the marine psychrophile, *Colwellia psychrerythraea* 34H, a gammaproteobacterium originally isolated from subzero arctic marine sediments, but considered to be well-adapted to inhabiting sea ice, where the species has since been found (Methé et al., 2005; Boetius et al., 2015). *C. psychrerythraea* 34H possesses genes that code for the specific transport of choline (and other compatible solutes) into the cell, the conversion of choline to glycine betaine (and beyond), and the release of solutes like choline through mechanosensitive ion channels (Collins and Deming, 2013; Ewert and Deming, 2014), yet kinetic studies on the use of choline or any other solute by this psychrophile had not been performed. Another cold-adapted (psychrotolerant) marine gammaproteobacterium, *Psychrobacter* sp. 7E, isolated from high salinity (128 ppt) brine of arctic winter sea ice and capable of growth at subzero temperatures (Ewert and Deming, 2014), was included in selected experiments for comparative purposes; its maximum growth salinity is much higher than that of *C. psychrerythraea* 34H (>125 ppt versus 50 ppt; Huston, 2003; Ewert and Deming, 2014).

Overall, this study examines the hypothesis that microbial communities present in the subzero brine phase of sea ice use the compatible solute strategy to survive the salinity shifts that occur in this extreme habitat. We worked with brines drained from late winter sea ice near Nuuk, Greenland (a study area selected for its logistical advantages), as well as with cold-adapted bacteria in culture. ^{14}C-choline was used in a series of

experiments, all conducted at −1°C (to avoid convoluting temperature effects with salinity) and involving one-way salinity shifts, in order to test for: 1) uptake and preferential retention of labeled solute following osmotic upshift, 2) preferential release or respiration of labeled solute following osmotic downshift, and 3) capacity for rapid efflux of labeled solute following an abrupt osmotic downshift, the greater challenge to a microorganism. To examine recoverability from an osmotic downshift, ^{14}C-choline was used in a two-way (downshift-upshift) experiment using *C. psychrerythraea* 34H in an attempt to validate the effectiveness of compatible solutes as a strategy for surviving the natural salinity fluctuations inherent to sea ice.

Materials and methods

Sample collection and processing

Brine sampling took place on the sea ice of Kanajorsuit Bay (N 64.44632, W 51.57724) and Kobbefjord (N 64.15340, W 51.42275) near Nuuk, Greenland (Figure 1), during the late winter periods of 17 March to 5 April 2013 and 12–21 March 2014, respectively. The sampling periods were selected to coincide with maximum sea-ice thickness and minimum air temperatures to the extent possible, based on data in Mikkelsen et al. (2008) and advance communications from the Greenland Climate Research Center (GCRC) in Nuuk. Multiple "sackholes" were cored in the ice cover (following Eicken et al., 2009) to a depth of ~ 25 cm in sea ice that was 49–58 cm thick (Table 1). In one case, snow cover was removed from the intended sampling site 3 days before the sackholes were cored in an attempt to get colder ice and thus saltier brine. Brine was allowed to drain into the sackholes for 1–3 hours, then collected and pooled into sterile 10-L cubitainers using sterile (ethanol-rinsed) tools and techniques. Sackhole brine salinity was measured using a hand-held refractometer. Brine samples were kept in insulated coolers at *in situ* subzero temperatures (Table 1) until returned to the GCRC, where they were held at approximately −1°C in a temperature-controlled room. Subsamples were taken for immediate experimentation with ^{14}C-choline, with the remaining volume processed for supporting measurements in the cold room within 6 hours of collection.

On brine-sampling days a full "physical" ice core (Eicken et al., 2009) was obtained using a 9-cm diameter ice corer (MARK II Coring system, Kovacs enterprises). The temperature profile was determined immediately on site, following Eicken et al. (2009). The core was then sectioned at 5-cm intervals into sterile plastic bags for subsequent melting and analysis of bulk salinity.

The ammonium concentration in the melted sections was also measured, using the salicylate-hypochlorite method (Bower and Holm-Hansen, 1980). For samples collected in Kanajorsuit Bay in 2013, an additional core was collected on five separate occasions (between 15 March and 24 May) and three 10-cm sections of it (upper, middle and bottom) were used to measure potential nitrification rates. The middle section, which included the depth of the sackhole brines, was collected only for the last three sampling dates (from 5 April to 24 May). These additional sea-ice sections were transported and melted in closed bottles at 4 ± 1°C overnight in darkness before further processing.

Figure 1

Map of the study area and location of sampling sites in 2013 and 2014.

Samples were taken in (A) Kanajorsuit Bay, Greenland (N 64.44632, W 51.57724), between 27 March and 5 April, 2013, and in (B) Kobbefjord, Greenland (N 64.15340, W 51.42275), between 12 and 21 March, 2014.

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Table 1. Characteristics of the sea-ice (sackhole) brine samples used for choline experiments

Location, Year	Sampling day	Ice thickness (cm)	Snow depth (cm)	Air temperature[a] (°C)	Ice temperature[b] (°C)	Brine salinity (ppt)	Bacterial abundance[c] (mean ± S.D. × 10^5 mL^{-1})
Kanajorsuit Bay, 2013	02 April	49	4	−2.0	−1.2 to −3.2	50	1.76 ± 0.25
Kobbefjord, 2014	13 March	49	22	−7.0	−1.3 to −1.5[d]	62.5	2.93 ± 0.11
	18 March	53	30	-6.5	−2.2 to −3.1	54	2.20 ± 0.22
	21 March	58	26	-10	−2.2 to −4.2[d]	51	2.53 ± 0.01
	21 March[e]	58	0[e]	−10	−3.7 to −5.7	73	2.63 ± 0.00

[a]Recorded at time of sackhole drilling
[b]Range of temperatures recorded for the upper sections of the "physical" ice core corresponding with the potential brine drainage zone
[c]Mean of duplicate samples
[d]These ice temperatures appear anomalously warm relative to sackhole brine salinity as the ice cores were collected later in the day (after sackhole drilling) when air temperature was −3.5°C on 18 March and −7.3°C on 21 March.
[e]This site had been cleared of snow 3 days prior (note highest brine salinity in the absence of an insulating snow cover), although wind had created snow drifts (10–35 cm) on a portion of the drilling site.
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On selected days in both years, under-ice seawater was collected through holes in the ice using sterile tubing and receptacles and a portable pump. Measured volumes of the sackhole brines and seawater samples were filtered in the cold (−1°C) room for analysis of suspended particulate matter (SPM), particular organic carbon (POC) and nitrogen (PN), and chlorophyll *a* (Chl *a*) and phaeopigments (Phaeo), according to standard procedures as in Kellogg and Deming (2009). Additional aliquots were processed to determine: bacterial abundance, using epifluorescence microscopy (described below); the particulate fraction of extracellular polysaccharide substances (pEPS) in terms of glucose equivalents (glu-eq), as determined by the phenol-sulfuric assay, as in Ewert et al. (2013); and the abundance of viral-like particles (VLP) present in sample filtrate (< 0.2 μm), using epifluorescence microscopy and the procedures of Wells and Deming (2006). Only samples from 2014 were available for determining VLP abundance. All samples for microscopy were fixed with 0.2 μm-filtered formaldehyde to a final concentration of 2% and stored at 4°C in the dark until further processing, within two months of collection. On one occasion (18 March 2014), a sufficient volume of recently frozen sea ice (between sampling days) was sampled into a sterile plastic bag (as an ice-seawater slush) to enable a ^{14}C-choline salinity shift experiment and bacterial and viral counts.

Cold-adapted bacterial isolates

Colwellia psychrerythraea 34H and *Psychrobacter* sp. 7E were subcultured at −1°C from aliquots of the original isolates stored at −70°C since purification (Huston, 2003; Ewert and Deming, 2014). The growth medium was half-strength Marine Broth 2216 (Difco Laboratories) diluted with sterile, 0.2 μm filtered artificial seawater (ASW: NaCl, 24 g L^{-1}; KCl, 0.7 g L^{-1}; $MgCl_2 \cdot 6H_2O$, 5.3 g L^{-1}; $MgSO_4 \cdot 7H_2O$, 7.0 g L^{-1}; TAPSO buffer, 1.3 g L^{-1}) to retain seawater salinity. Cultures were harvested in late logarithmic growth phase for subsequent experimentation.

Bacterial abundance

Bacterial abundance in both field and laboratory samples was determined using epifluorescence microscopy, as in previous studies (Marx et al., 2009; Ewert and Deming, 2014). For field samples, a dual staining approach was used. To a 5-mL aliquot of fixed sample, 2–3 drops of surfactant (Triton-X) and 0.1% *N,N,N′,N′*-Tetramethylacridine-3,6-diamine (Acridine Orange) were added with mixing. Samples were vacuum-filtered onto 0.2 μm filters and stained for 10 min with the DNA-specific stain 4'-6'-diamidino-2-phenylindole (DAPI; 20 μg mL^{-1}). For suspensions of laboratory cultures, the aliquot was treated identically, except that Acridine Orange was not added. To follow bacterial abundance in field samples and laboratory suspensions incubated with ^{14}C-choline, replicate samples receiving equal concentrations of unlabeled choline were incubated and subsampled in parallel. All samples were examined with a Zeiss Universal epifluorescence microscope, with a minimum of 20 fields or 200 bacteria counted for each sample.

Choline uptake kinetics

To verify that ^{14}C-choline uptake followed Michaelis-Menten kinetics and to determine optimal concentrations of ^{14}C-choline to add in salinity shift experiments, the uptake rate of ^{14}C-choline versus added concentration of ^{14}C-choline was examined for samples of brine and seawater and for suspensions of *C. psychrerythraea* 34H. Ten-mL aliquots of sample were distributed into pre-chilled, sterile (acid-washed, combusted at

450°C > 6 hours), glass 30-mL serum bottles at –1°C (in an ice-water bath), spiked with ^{14}C-choline (Perkin Elmer, 55.2 mCi mmol^{-1}) to yield a range of final concentrations between 5 and 300 nM, and incubated for 1 hour at –1°C. Incubation was terminated by vacuum filtration of samples onto 0.2 µm Supor® filters (Pall Corporation), followed by a rinse with 3–5 mL of 0.2 µm filtered isohaline salt solution. Filters were placed in 5-mL or 20-mL scintillation vials and filled with Ecolume. The filter-captured ^{14}C-choline activity was measured using a Packard liquid scintillation counter (LSC) and kinetic data were analyzed in the manner of Wright and Hobbie (1966), following Kiene (1998) and Kiene et al. (1998).

Choline respiration and retention

The fate of ^{14}C-choline in field samples and in laboratory suspensions of *C. psychrerythraea* 34H was determined by adapting methods described by Kiene (1998). Field samples were subjected to each of three salinity treatments: 1) downshift, where deionized water was added to halve the starting salinity; 2) no shift, where NaCl solution was added to match the starting salinity; and 3) upshift, where NaCl solution was added to double the starting salinity. All diluting solutions were sterile, particle-free (filtered), and chilled prior to use; all dilutions were performed in the cold room at –1°C. Because a range of salinities characterized the sackhole brines (50–73 ppt; Table 1) and seawater samples (30–35 ppt), the upshift and downshift salinities varied accordingly. The resulting series of samples at 0.5x, 1x, and 2x *in situ* salinity, however, had an internally consistent dilution factor of 0.5x for all organisms and other components of the samples. Replicate sample treatments were distributed in pre-chilled, sterile (acid-washed, combusted at 450°C > 6 hours) glass serum bottles, spiked with 100 nM ^{14}C-choline (at < 1% total volume), and incubated at –1°C. Triplicate samples were processed at each of three time points over a period of 0–118 hours.

Laboratory cultures of *C. psychrerythraea* 34H, harvested in late-logarithmic growth phase, were pelleted and washed 3x with pre-chilled (–1°C) ASW via microcentrifuge (15,000 rpm for 30 seconds) and resuspended to a concentration of ~ 1 × 10^6 mL^{-1} (cell densities were verified by epifluorescence microscopy). Resuspensions were subjected to each of three salinity treatments as described above. The starting salinity was 31 ppt, the downshift salinity was 15.5 ppt, and the upshift salinity was 62 ppt. Samples were distributed as described above, spiked with 200 nM ^{14}C-choline (at < 1% total volume) and incubated at –1°C for 1–18.5 hours.

At each incubation time point, respiration (Resp) and retention (Ret) of ^{14}C-choline were quantified. Respiration was measured by acidifying 10 mL of sample with 0.4 mL 4N H_2SO_4, capturing the released CO_2 with a phenylethylamine-soaked wick (0.2 mL) in a sealed vial (after ≥ 1 hour of shaking at ~ 150 rpm at room temperature), and quantifying radioactivity (disintegrations per minute or DPM) present. During 2013 fieldwork, retention was measured by filtering 10 mL of sample through a 0.2 µm Supor filter, rinsing with 3–5 mL of 0.2 µm filtered isohaline salt solution, and quantifying DPM present. In 2014, the additional step of filtering the 10 mL of acidified sample (through a separate 0.2 µm Supor filter) was added to distinguish incorporation of ^{14}C-choline into macromolecules from retention in intracellular pools (Baross et al., 1975). Retention was calculated as the difference between DPM on each of these filters (unacidified sample minus acidified sample). DPM in all samples were quantified by LSC using EcoLume-filled scintillation vials (5 or 20 mL). For all treatments, parallel control incubations included blanks, whereby sample DPM values were corrected for background blank values, and formaldehyde-fixed samples, which showed no respiration or retention. For comparative purposes between treatments and field samples, ratios of respiration to retention (Resp/Ret) were calculated from midpoint and endpoint data.

Short-term efflux of choline

A rapid filtration assay from Ajouz et al. (1998) for evaluating short-term efflux from the mesophile *E. coli* was modified for use with cold-adapted bacterial isolates. Cultures of *C. psychrerythraea* 34H and *Psychrobacter* sp. 7E were resuspended at concentrations of ~ 1 x 10^6 mL^{-1} following 3x pelleting and washing with pre-chilled ASW (–1°C; 15,000 rpm for 30 seconds via microcentrifuge). Duplicate samples were amended with 200 nM ^{14}C-choline and incubated at –1°C for 72 hours. A small aliquot (100 µL) of sample was then withdrawn and placed on a sterile 0.2 µm Supor filter and a constant vacuum (15 psi) was applied. Initial work confirmed that both isolates consistently retained intracellular ^{14}C-choline for at least 30 seconds compared to control manipulations (as described in Ajouz et al., 1998). Immediately after applying vacuum to filters with the adsorbed bacteria, volumes of shock solution (pre-chilled deionized water) ranging from 500 µL to 5 mL were applied to the filters. Under these conditions, the permeation rate of the shock solution was consistently found to be 0.5 mL second^{-1}; therefore, the cells adsorbed to the filter were exposed to the shock solution for between 1 and 10 seconds. Smaller volumes were not used because they did not successfully wet the entire filter. Filters were then placed in scintillation vials filled with scintillation fluid (EcoLume) and ^{14}C-choline activity was quantified via LSC as before.

Recovery of choline uptake mechanism

Following work described in Ajouz et al. (1998), a long-term recoverability assay was modified to accommodate use with cold-adapted bacterial isolates. A culture of *C. psychrerythraea* 34H was resuspended at a concentration of ~ 1 x 10^6 mL^{-1} following 3x pelleting and washing with ASW (–1°C; 15,000 rpm for 30 seconds via microcentrifuge). Sample suspensions, in triplicate, were amended with 200 nM ^{14}C-choline and incubated at –1°C. Periodically (24–72 hours) aliquots were taken to assess the uptake of ^{14}C-choline (1 mL vacuum-filtered onto 0.2 µm Supor filter and rinsed with ASW). After the suspensions reached and maintained a stable level of ^{14}C-choline uptake (after 528 hours), one was downshifted to half of starting salinity (by addition of pre-chilled deionized water), the second was similarly diluted but with no salinity shift (by addition of pre-chilled ASW), and the third was downshifted to half of starting salinity (as for the first) but was immediately returned to starting salinity (via upshift performed by addition of a small pre-chilled volume of concentrated NaCl brine). Each treatment was subsampled in duplicate and filtered as described above (2 mL through 0.2 µm Supor filters) at periodic intervals (24–72 hours) over an additional incubation period of 500 hours at –1°C. All sample filters were quantified via LSC as before.

Potential rates of nitrification

For each sea-ice section, 10 mL of the melted ice was added to each of five exetainers (Labco, Inc.) and spiked with $^{15}NH_4^+$ to final concentration 10 µM. The samples were incubated in the dark in a temperature-controlled room at –1°C. One subsample of each section was terminated after 0, 12, 24, 120 and 240 hours by freezing the sample at –18°C. Produced $^{15}NO_x$ was chemically converted to N_2 gas by methods described in Füssel et al. (2012) and McIlvin and Altabet (2005) and the ^{15}N-labeled N_2 ($^{29}N_2$ and $^{30}N_2$) was subsequently measured using mass spectrometry (Mass Spectrometer: Isotope Ratio Mass Spectrometer Hydra 20-20, SerCon; Autosampler: SerCon; Separation unit: Gas Solid Liquid Sample Prep Unit, ANCA GSL, SerCon) after inserting a helium headspace of 5 mL. Nitrification rates were calculated as the produced amount of NO_x according to incubation time accounting for the *in situ* NH_4^+ concentrations.

Results

Environmental context

The sites drilled to collect sackhole brines for choline experiments had ice that was sufficiently thick (49–58 cm) and cold (down to –5.7°C) to yield brines that ranged in salinity from 50 to 73 ppt (Table 1). Clearing snow from one of the sites 3 days before drilling the sackholes led to colder ice and saltier (73 ppt) brine (Table 1). The physical ice cores yielded generally low ammonium concentrations, ranging from near detection limit to 5.59 µmol $L_{sea\ ice}^{-1}$ (median of 1.38 µmol $L_{sea\ ice}^{-1}$).

Potential nitrification rates in the sea ice were also low, ranging from 3.1 to 35.6 nmol $L_{sea\text{-}ice}^{-1}$ d^{-1} (scaled to ice volume; Figure 2). Throughout the sampling period, the upper and middle ice sections yielded measurable rates (significantly different from zero; simple Student's *t-test*, p-value < 0.05, df > 3), including highest rates at depths corresponding to the sackhole drainage area; no rates were measurable in bottom ice during the latter part of the sampling period (Figure 2).

The concentrations of most particulate variables (SPM, POC, Chl *a*) in the sackhole brines and seawater samples were also generally low, based on study-wide mean values (Table 2) and comparisons with the

Table 2. Particulate variables measured on field samples from Kanajorsuit Bay and Kobbefjord for environmental context

Variable or ratio	Brine (mean ± S.D.)	*n*	Seawater (mean ± S.D.)	*n*
SPM (mg mL^{-1})	0.221 ± 0.056	11	0.202 ± 0.072	12
Chl *a* (µg L^{-1})	1.23 ± 0.58	12	0.95 ± 1.92	12
POC (µg C mL^{-1})	0.116 ± 0.080	12	0.022 ± 0.015	12
pEPS (µg glu-eq mL^{-1})	0.106 ± 0.056	12	0.084 ± 0.075	12
Bacteria (× 10^5 mL^{-1})	2.07 ± 0.64	12	3.70 ± 1.87	12
VLP (× 10^7 mL^{-1})[a]	2.31 ± 0.97	4	3.37 ± 2.69	3
VLP:bacteria[a]	81 ± 2.0	4	59 ± 30	3
Chl *a*:Phaeo	1.98 ± 0.62	12	2.45 ± 3.19	12
C:N (mol:mol)	6.0 ± 1.3	10	4.2 ± 1.7	10

[a]VLP data were only available for 2014 samples.

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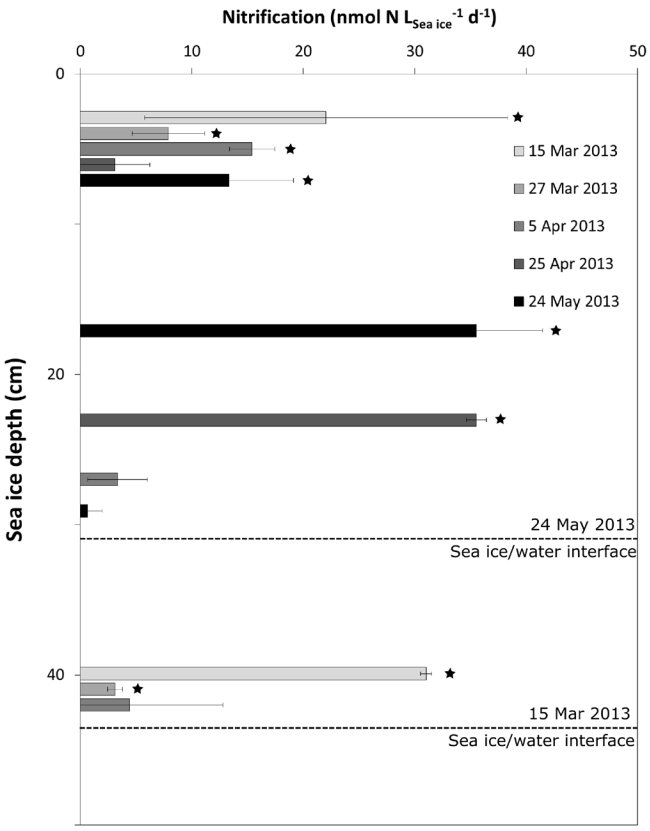

Figure 2

Potential rates of nitrification in sea ice sections.

The seasonal succession in potential rates of nitrification measured in Kanajorsuit Bay 2013, which includes samples from the upper and bottom 10 cm of the ice, while sections from the middle of the ice column were included from the middle of the season. Error bars represent S.D. (n = 5). Stars indicate rates significantly different from zero. Measurements clustered around sea ice depths of 5 cm and 40 cm are staggered for clarity; all measurements in those clusters were taken from 5 cm and 40 cm, respectively.

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literature (see Discussion). Mean concentrations of POC and Chl *a* were higher in the brines than in underlying seawater, but all values were low: ranges of 0.00–0.24 µg C mL^{-1} for POC and 0.04–2.38 µg L^{-1} for Chl *a*. The mean pEPS content of the brine was only slightly higher than that of seawater (Table 2), with ranges of 0.04–0.23 and 0.003–0.22 µg glu-eq mL^{-1}, respectively. Ratios of C:N and Chl *a*:Phaeo were always low (Table 2). The ranges of bacterial abundances in the sackhole brines and seawater samples overlapped, with the higher values observed for seawater: 0.82–2.63 x 10^5 mL^{-1} brine and 2.85–8.78 x 10^5 mL^{-1} seawater. VLP abundances ranged from 1.77 to 2.10 x 10^7 mL^{-1} brine and from 1.18 to 2.77 x 10^7 mL^{-1} seawater, yielding a mean VLP:bacteria ratio of 81 in brine and 59 in seawater (Table 2). The new sea ice sample contained 2.71 x 10^5 bacteria and 1.34 x 10^7 VLP mL^{-1} meltwater, for a VLP:bacteria ratio of 49.

Experiments with natural microbial communities

Substrate saturation experiments using field samples indicated that a suitable concentration of ^{14}C-choline to add in the salinity shift experiments was 100 nM (Figure 3A). A Wright-Hobbie plot of linearized data from sackhole brine (Figure 3B) gave the value of 97 nM for $K_t + S_n$, which provides an upper bound for the natural substrate concentration in the sample (S_n). A double reciprocal Lineweaver-Burk plot (Berg et al., 2002) of the same data (Figure 3C) gave values of 42 nM for K_t (inverse of the x-intercept) and 9.0 nM hour^{-1} for V_{max} (inverse of the y-intercept), where K_t is the cellular transport constant in Wright-Hobbie terminology (Wright and Hobbie, 1966; equivalent to the half-saturation constant K_m in Michaelis-Menten enzyme terminology) and V_{max} is the maximum uptake velocity achieved under the conditions provided (–1°C and 64 ppt). A lower bound of 56 nM for S_n was calculated using the Lineweaver-Burk estimate of K_t ($[K_t + S_n] - K_m$). As both methods of boundary estimations are indirect with numerous caveats, these values provide only an approximate range (56–97 nM) for natural choline concentrations in the brines of this study. Similar analyses of seawater kinetic data yielded estimates for an upper bound for S_n of 110 nM and a V_{max} of 2.9 nM hour^{-1} (data not shown).

Time-course experiments with natural sea-ice brine samples, incubated at –1°C, revealed the microbial fate of ^{14}C-choline following a salinity shift (Figure 4). Following a decrease in salinity, little choline was transported or retained initially; over time considerable choline was taken up, most of which was respired (Figure 4A). When salinity was left unchanged from *in situ*, the microbial community transported and retained choline, eventually respiring a small portion of it (Figure 4B). Following an upshift in salinity, however, choline transport was immediate, with virtually all of it retained intracellularly (none respired) for the duration of the experiment (Figure 4C). Using endpoint Resp/Ret ratios to compare between treatments

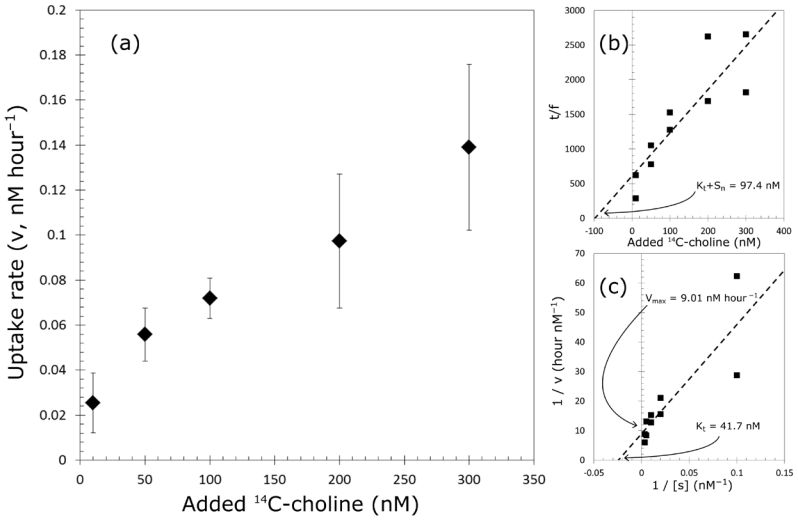

Figure 3

Uptake kinetics of ^{14}C-choline by natural microbial communities in sea-ice brine.

^{14}C-choline uptake data from an experiment with sea-ice brine collected from Kobbefjord, Greenland, indicating Michaelis-Menten saturation kinetics (a) and linearity when transformed for a Wright-Hobbie plot (b), as in Kiene et al. (1998). The x-intercept in (b) gives a value of 97.4 nM for K_t + S_n, as an upper bound for natural substrate concentration (S_n). A double reciprocal Lineweaver-Burk plot of the data (c) gives a value of 41.7 nM for K_t (inverse of x-intercept) and 9.01 nM hour^{-1} for V_{max} (inverse of y-intercept). See text for more details.

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and samples, from both locations and years, revealed that these patterns were universal in sea-ice brines across sampling dates and varying *in situ* conditions (Figure 5, Table 1). The highest ratios, indicating preferential respiration of the choline, always characterized the microbial response to a downshift in salinity; extremely low ratios, indicating negligible respiration and preferential retention of the choline, always characterized the response to a doubling of the salinity. These general trends were apparent regardless of midpoint or endpoint calculations of the Resp/Ret ratio (Figure 6).

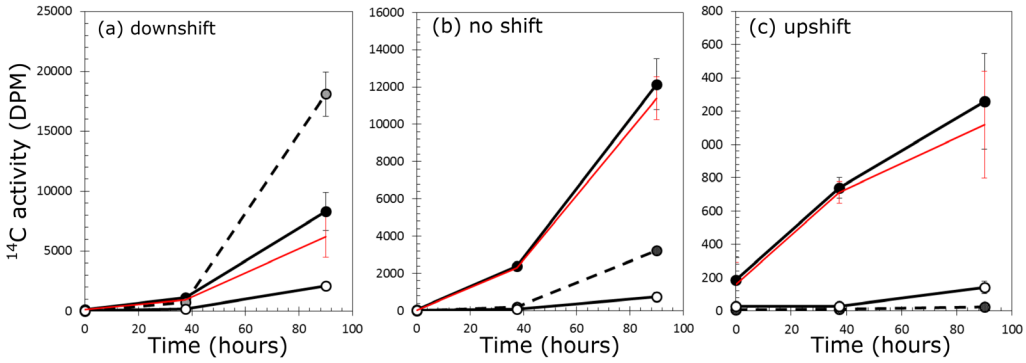

Figure 4

Use of ^{14}C-choline by natural microbial communities in sea-ice brine following salinity shift treatments.

Examples of time-course data on the use of ^{14}C-choline by natural microbial communities in sea-ice brine following a downshift (a), no shift (b), or upshift (c) in salinity, all at –1°C. Grey circles (dashed lines) represent respiration, measured as $^{14}CO_2$ released upon acidification of the sample. Filled circles (solid black line) represent total amount retained intracellularly, measured as filterable material collected pre-acidification. Open circles (solid black line) represent amount incorporated into macromolecules, measured as filterable material collected post-acidification. The red line represents osmolyte retention, calculated by subtracting amount incorporated into macromolecules from total amount retained intracellularly. Error bars indicate S.D. of the mean (n = 3); where no bar is visible, it falls within the dimensions of the symbol for the mean. Note different scales between y-axes.

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Microbial use of ^{14}C-choline was also observed in seawater samples and the new sea ice sample, although at much lower levels (< 500 total DPM endpoint values; data not shown). Preferential behavior as described above was only distinguishable in the case of a salinity upshift, when the microbial communities retained most of the limited amount of transported choline as intracellular solute. No bacterial growth was observed during the subzero incubations of any of the diluted field samples.

Experiments with cold-adapted bacterial isolates

Time-course experiments at –1°C with laboratory suspensions of *C. psychrerythraea* 34H, following salinity shifts and addition of 200 nM ^{14}C-choline (based on substrate saturation; data not shown), resulted in patterns similar to those observed for natural microbial communities in the subzero sea-ice brines (Figure 7). Following a salinity downshift, most of the transported choline was respired (Figure 7A); following a doubling of the salinity, virtually all of the transported choline (or its derivatives) was retained intracellularly (Figure 7C). Comparison of endpoint Resp/Ret ratios for *C. psychrerythraea* 34H again showed highest Resp/Ret ratios under downshift conditions and very low Resp/Ret ratios under upshift conditions (Figure 8). No bacterial growth was observed in these experiments.

Experiments designed to assess the fate of intracellular choline (or its derivatives) following a sudden and extreme downshift in salinity (downshock, as described in Ajouz et al. 1998) indicated a near-complete cellular efflux of the ^{14}C signal within 1 second of administering the downshock (Figure 9). This result was obtained for both *C. psychrerythraea* 34H and *Psychrobacter* sp. 7E (Figure 9).

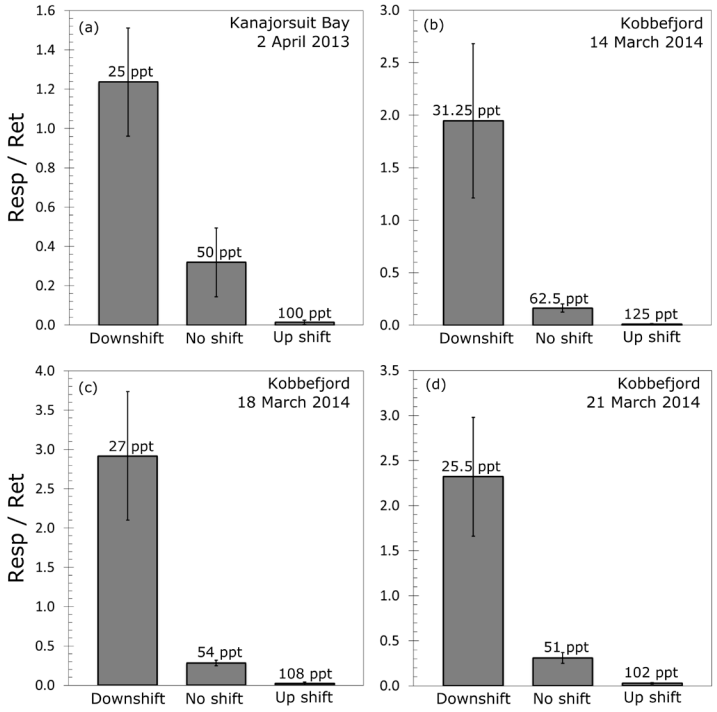

Figure 5

Respiration/retention ratios in natural microbial communities in sea-ice brines following a salinity shift.

Comparison of endpoint respiration/retention (Resp/Ret) values for natural communities present in sea-ice brines from Kanajorsuit Bay in April 2013 (a) and Kobbefjord in March 2014 (b–d) following shifts (down, no, up) in salinity at –1°C. Incubation salinities (ppt) are given above each bar; the no-shift salinity is the *in situ* brine salinity. A Resp/Ret ratio of 1 implies equal respiration and retention of choline; Resp/Ret > 1 indicates more choline respired than retained; and Resp/Ret < 1 indicates more choline retained than respired. Error bars indicate S.D. of the mean (n = 3 except for (a) where n = 2); where no bar is visible, it falls within the dimensions of the symbol for the mean.

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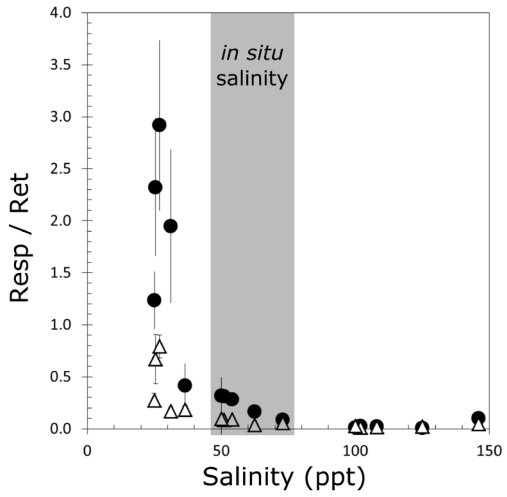

Figure 6

Respiration/retention ratios in natural microbial communities in sea-ice brines by incubation salinity and time.

Comparison of all respiration/retention (Resp/Ret) values obtained from salinity-shift time-course experiments using sackhole brines from Kanajorsuit (April 2013) and Kobbefjord (March 2014). Open triangles indicate Resp/Ret values at mid-points in the experiments (34–48 h); solid circles indicate Resp/Ret values at the endpoint (90–96 h). The shaded box encompasses the no-shift samples and thus the range of *in situ* salinities encountered in this study. Data to the left of the shaded box indicate salinity down-shift treatments; data to the right indicate salinity up-shift treatments. Error bars indicate S.D. of the mean (n = 3); where no bar is visible, it falls within the dimensions of the symbol for the mean.

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Experiments designed to examine the recoverability of the hypothesized compatible solute mechanism, by following a salinity downshift with an upshift (again adapting methods from Ajouz et al. 1998), showed that ^{14}C retained intracellularly by *C. psychrerythraea* 34H decreased rapidly after cell suspensions were exposed to a salinity downshift, but rose quickly to near-control levels (in suspensions never subjected to a salinity shift) when external salinity was returned to starting level (Figure 10). Bacterial growth occurred slowly throughout the experiment, with no differences in growth rate or morphology observed between treatments and controls.

Discussion

Environmental context

The temperature and thickness of the sea ice we sampled, with snow removal in one case, yielded brines with an ideal range of salinities for performing salinity shifts in both the upwards and downwards directions to test for bacterial osmotolerance. The salinity upshifts in our choline experiments allowed for the exposure of organisms to salinities up to 146 ppt, characteristic of Arctic winter sea-ice brines (Ewert and Deming, 2014), while the downshifts (to 25 ppt) exposed them to the freshening that occurs during the melting

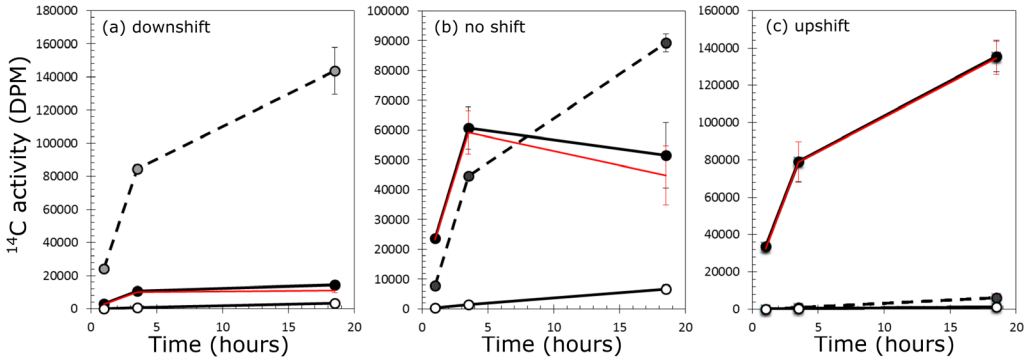

Figure 7

Use of ^{14}C-choline by *Colwellia psychrerythraea* 34H following a salinity shift.

Time-course data on the use of ^{14}C-choline by *Colwellia psychrerythraea* 34H following a downshift (a), no shift (b), or upshift (c) in salinity at –1°C. Grey circles (dashed lines) represent respiration, measured as $^{14}CO_2$ released upon acidification of the sample. Filled circles (solid black line) represent total amount retained intracellularly, measured as filterable material collected pre-acidification. Open circles (solid black line) represent amount incorporated into macromolecules, measured as filterable material collected post-acidification. The red line represents osmolyte retention, calculated by subtracting amount incorporated into macromolecules from total amount retained intracellularly. Error bars indicate S.D. of the mean (n = 4, except for time zero, where n = 2); where no bar is visible, it falls within the dimensions of the symbol for the mean.

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season, thus paralleling the range of salinities (and salinity shifts) that occur within Arctic sea ice. The brines were also derived from sea ice in which algal primary production had not yet begun, as indicated by very low concentrations of Chl *a* (and low Chl *a*/Phaeo ratios), POC, and SPM, and consistent with other studies that included Arctic winter sea ice (Mikkelsen et al., 2008; Collins and Deming, 2011a; Ewert et al., 2013; Niemi and Michel, 2015). The ice we sampled was instead the domain of bacteria and viruses, with both VLP:bacteria and C:N ratios being consistent with metabolically active bacterial populations (e.g., Wells and Deming, 2006; Deming, 2010), as subsequently observed in our incubation experiments.

The pEPS content of the brines used in our choline experiments fell at the lower end of the range reported for upper winter sea-ice brines in a methodologically comparable study (0.01–0.51 µg glu-eq mL^{-1}; Ewert et al., 2013). These values represent only a fraction of the pEPS concentrations measured during an ice-algal bloom (e.g., Krembs et al., 2002; Riedel et al., 2008). High amounts of pEPS in sea ice had prompted the suggestion that extracellular polymers serve as a physical buffer to osmotic shifts (Krembs and Deming, 2008). The observation of only low pEPS concentrations in the brines of this study implies that a physical buffer of pEPS was not the primary osmotolerance strategy.

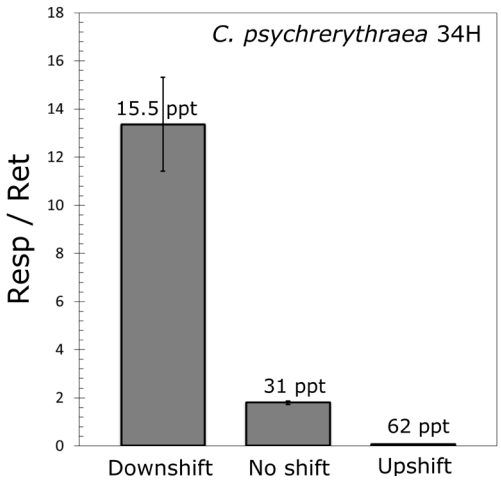

Figure 8

Respiration/retention ratios in *Colwellia psychrerythraea* 34H following a salinity shift.

Comparison of endpoint respiration/retention (Resp/Ret) values for laboratory suspensions of *Colwellia psychrerythraea* 34H following shifts (down, no, up) in salinity at –1°C. Incubation salinities (ppt) are given above each bar; the no-shift salinity is the salinity of the starting suspension. A Resp/Ret ratio of 1 implies equal respiration and retention of choline; Resp/Ret > 1 indicates more choline respired than retained; and Resp/Ret < 1 indicates more choline retained than respired. Error bars indicate S.D. of the mean (n = 4); where no bar is visible, it falls within the dimensions of the symbol for the mean.

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Choline as a compatible solute precursor

All results from our ^{14}C-choline experiments point to use of the compatible solute strategy by microbial communities in sea-ice brines to survive osmotic challenges. Resp/Ret ratios obtained from the salinity shift experiments show a clear and consistent trend of preferential respiration of ^{14}C-choline (high Resp/Ret) when salinity was decreased and preferential retention of ^{14}C-solutes (low Resp/Ret) when salinity was increased. This trend was observed regardless of year or location of field sampling or whether considering microbial communities in subzero brines or a psychrophilic bacterium in pure culture at –1°C (Figures 4, 5, 7). This predictable pattern of Resp/Ret ratios according to salinity shifts provides strong evidence of compatible solute usage in sea-ice brines, broadening the microbial use of this strategy from estuarine settings (Kiene, 1998) to the extensive oceanic regions that harbor sea ice.

Details of the compatible solute strategy have been explored with laboratory isolates; for example, osmoregulating *E. coli* releases osmolytes very rapidly upon experiencing severe hypoosmotic shock (Ajouz et al., 1998). When tested for their responses to an abrupt downshock, both *Colwellia psychrerythraea* 34H and *Psychrobacter* sp. 7E, despite different salinity requirements for growth, behaved in the same manner,

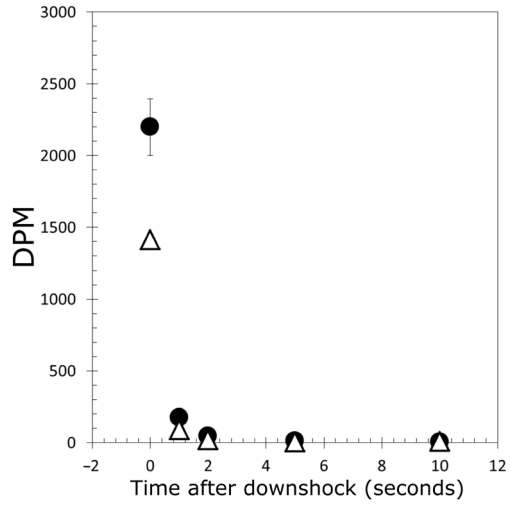

Figure 9

Efflux of ^{14}C-choline from *Colwellia psychrerythraea* 34H and *Psychrobacter* sp. 7E following extreme salinity downshift.

Rapid efflux of ^{14}C-choline from cells of *C. psychrerythraea* 34H and *Psychrobacter sp.* 7E immediately following salinity downshock at –1°C. Efflux was measured as loss of DPM (^{14}C-choline or derivatives) present on 0.2 µm filter. Error bars indicate S.D. of the mean (n = 2, except for time zero, where n = 4); where no bar is visible, it falls within the dimensions of the symbol for the mean.

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rapidly expelling ^{14}C-solute on a subsecond timescale (Figure 9). In sea ice, such downshocks can be expected to occur when bacteria long entrained in the brine phase of the ice are suddenly exposed to freshwater, as in snow-derived melt ponds on the surface of sea ice or within rapidly melting sea ice in summer.

For the compatible solute strategy to be effective in sea ice, the mechanisms involved must be recoverable following a salinity shift, including an abrupt one. The most competitive natural microbial communities would be dominated by organisms that can survive multiple salinity shifts in the absence of gene induction. Experiments with *C. psychrerythraea* 34H show that the compatible solute strategy is fully recoverable (Figure 10). Despite quick, mechanosensitive expulsion of choline upon an abrupt decrease in salinity, the bacteria immediately resumed choline uptake when returned to starting salinity and retained intracellular ^{14}C-solutes at a level similar to their counterparts not subjected to an abrupt downshock. *C. psychrerythraea* 34H thus retains osmolytes at levels proportional to external salinities, adjusting internal concentrations in response to changing external salinities. To the extent that *C. psychrerythraea* 34H represents other cold-adapted heterotrophic bacteria in sea ice, natural brine communities can be expected to exhibit similar recoverability and maintenance of internal osmolyte concentrations. This expectation is strengthened by the evidence that natural communities utilize choline in a consistent manner across a wide range of salinities, with the highest Resp/Ret ratios observed at the lowest salinities, and the lowest Resp/Ret ratios at the highest salinities (Figure 6).

Taken together, these results can be used to describe a seasonal scenario of compatible solute usage in natural sea-ice brines. In early winter as the brines become colder and saltier, resident microbes scavenge the available choline (estimated at 50–100 nM based on choline uptake kinetics) and retain most of its derivatives as osmolytes to balance the osmotic pressures caused by increasing external salinity. A small fraction of the

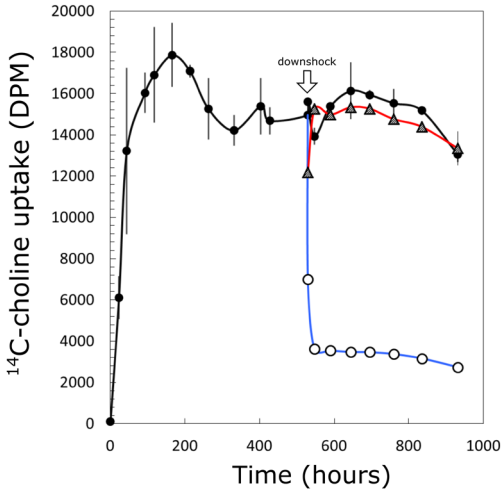

Figure 10

Recoverability of ^{14}C-choline uptake as an osmotolerance mechanism in *Colwellia psychrerythraea* 34H.

The effects of sudden osmotic downshock (open circles, blue line) and of sudden osmotic downshock followed by immediate return to previous osmotic conditions (triangles, red line), in comparison to a control where no downshock was administered (filled circles, black line), on the uptake of ^{14}C-choline by *C. psychrerythraea* 34H at –1°C. DPM values have been scaled to volume, accounting for dilution factors involved in experimental manipulations. Error bars indicate S.D. of the mean (n = 3 before the downshift, n = 2 afterwards); where no bar is visible, it falls within the dimensions of the symbol for the mean.

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choline is incorporated into macromolecules for cellular maintenance (open circles in Figures 3, 6). Results from experiments with the saltiest brines collected (in Kobbefjord) show that natural microbial communities continue to exhibit this behavior at salinities as high as 146 ppt (equivalent to an ice temperature of –9°C; Cox and Weeks, 1983), albeit at reduced rate. Experiments at high salinity and very low temperatures are needed to validate choline-based activity under *in situ* sea-ice conditions during Arctic winter.

As winter progresses to spring, microbes in sea-ice brine experience clear and often wide diurnal shifts in external salinity (Ewert and Deming, 2014), with potential microscale shifts on more abrupt timescales. Depending on the osmotic pressures generated by the downshifts, cells may either quickly expel osmolytes or steadily respire them (producing labeled CO_2 as in our experiments). Rapid or steep downshifts place a cell in danger of lysis, unless the associated osmotic pressures can be negated quickly. Rapid expulsion of osmolytes is accomplished by mechanosensitive channels, membrane pores directly responsive to the tension on a cell membrane. Cells may have one or several classes of mechanosensitive channels: mini (MscM), small (MscS) and large (MscL) (Naismith and Booth, 2012). A number of cold-adapted bacterial species possess multiple copies of one or more of these mechanosensitive channels, including *Colwellia psychrerythraea* 34H (MscS), *Psychrobacter arcticus* (MscS and MscL), and *Psychroflexus torquis* (MscS and MscL) (Ewert Sarmiento, 2013). The expression of this survival mechanism by the cold-adapted gammaproteobacteria we examined (Figure 9) may help to explain the dominance of this group of bacteria in natural sea-ice communities (Deming and Collins, 2016).

Over gradual seasonal decreases in brine salinity (from winter to summer), bacteria can be expected to respire that portion of their osmolytes no longer required for osmotic balance, as always observed in our downshift experiments. Osmolyte respiration would yield energy for metabolism and growth (consistent with the genetic potential of *C. psychrerythraea* 34H and its ability to grow on choline; Collins and Deming, 2013) in the absence of any external input of resources for heterotrophs (or autotrophs). Thus could individual bacteria (and archaea) in sea-ice brines withstand the seasonal increase in salinity with the approach of winter and decrease in salinity with the onset of warmer seasons, as well as any transient or abrupt shifts in salinity.

Environmental selection for choline-based osmotolerance

The primary focus of this study was the behavior of microbial communities in sea-ice brines, but the inclusion of seawater and new sea-ice samples in salinity shift experiments provided instructive comparisons. The use of ^{14}C-choline by the microbial communities in these samples was observed, but at greatly reduced levels compared to sea-ice brines, despite comparable bacterial abundances and the same amount of added ^{14}C-choline. These results suggest that the phylogenetic selection known to occur between seawater and sea ice (Collins et al., 2010; Bowman et al., 2012; Boetius et al., 2015) may be based in part on the ability of ice-entrained organisms to use the compatible solute strategy in the brines they come to inhabit.

The concept of organism selection on the basis of choline use as an osmotolerance strategy is supported by data from a metagenomics study conducted in parallel to ours (Maccario, 2015). Numerous osmotic stress genes involved in the use of choline and its immediate derivative glycine betaine were more prevalent (normalized by total abundance of reads) in sackhole brines than seawater; e.g., glycine betaine transporter OpuD, betaine aldehyde dehydrogenase EC 1.2.1.8, high-affinity choline uptake protein BetT, and HTH-type transcriptional regulator BetI.

Choline-based osmotolerance strategies may be favored in the confines of the ice-brine network if choline is more abundant than other compatible solutes or precursors. Although we did not measure other compatible solutes or precursors, sufficient choline was present in the brines to support choline-based osmotolerance and ^{14}C-choline kinetics indicated enhanced uptake by the brine community. In addition to seawater at freeze-up, a potential source of choline (and various compatible solutes) within the ice-brine network is virally mediated cell lysis (Collins et al., 2011b). The relatively high VLP:bacteria ratios of this study and abundance of viral genes detected in the brines (Maccario, 2015) support this possibility.

Choline as a metabolite

In addition to functioning as a compatible solute precursor, choline is respired (and to a limited degree, incorporated into macromolecules) by natural sea-ice brine communities and laboratory isolates (Figures 3, 6). $^{14}CO_2$ was generated in our experiments, but the fate of the nitrogen in the molecule is unknown. The potential for generating significant ammonium via respiration of nitrogen-containing solutes, previously stored in excess for osmotolerance, is of particular interest given current understanding of sources of ammonium in sea ice. For example, in sea ice on the Mackenzie shelf of the Canadian Arctic, heterotrophic regeneration was reported as accounting for 67% of ammonium concentrations, as measured in incubation experiments using melted ice (Riedel et al., 2007). Our results suggest that a large portion of this ammonium regeneration may have been due instead to compatible solute respiration triggered by the downshift in salinity when the ice was melted and allowed to incubate. In natural sea ice, ammonium regenerated from compatible solutes could be available for cellular release into the brine network, providing an additional nitrogen source for algal productivity. As the sea ice we examined was not supporting an algal bloom and had only low concentrations of ammonium (< 3 μM), we can neither confirm nor reject this hypothesis, but similar research during the productive season could be informative.

An alternative fate of ammonium derived from compatible solutes would be uptake and oxidation by nitrifying bacteria inhabiting the brine network. Evidence that nitrification occurs in sea ice exists for both Arctic and Antarctic sea ice (Priscu et al., 1990; Bowman, 2015; Fripiat et al., 2014, 2015), but rates have

rarely been quantified (Baer et al., 2015). The potential nitrification rates that we measured (3–36 nmol N $L^{-1}d^{-1}$) are generally higher than those reported for Chukchi Sea ice (0.6–5.4 nmol N $L^{-1}d^{-1}$; Baer et al., 2015) and fall within the range reported for pelagic sites in the northern hemisphere (0–120 nmol N L^{-1} d^{-1}, as compiled by Capone et al., 2008), but these sea-ice rates are likely underestimates of *in situ* rates because they were measured in melted incubated samples. Accounting for the relative brine volume in the unmelted ice, our rates could be as much as 25 times higher *in situ*. The likely dilution of labeled ammonium by the production of unlabeled ammonium in the melted samples (as nitrogen-containing osmolytes were respired during the salinity downshift associated with melting) means that the *in situ* rates may have been even higher. These corrections place the measured nitrification rates at the upper end of the range for the pelagic realm, pointing to nitrification as a more important pathway in the sea-ice nitrogen cycle than previously assumed, as also recently suggested by Fripiat et al. (2014) and Baer et al. (2015).

Parallel metagenomics work at our study site (Maccario, 2015) provides supportive evidence of the potential for both bacterial and archaeal nitrification in the sackhole brines and seawater that we sampled. An abundance of nitrifying taxa (relative to all taxonomically annotated sequences) was detected: Thaumarchaeota (0.5–8.4%), dominated by Nitrosopumilales; Nitrosomonadales (0.029–0.48%), dominated by *Nitrosospira* and *Nitrosomonas*; Nitrospinales (0.012–2.12%), dominated by *Nitrospina*; and Nitrosospirales (0.004–0.015%), dominated by *Nitrospira* (L. Maccario, personal communication). Genes for enzymes involved in nitrification (e.g., ammonia monooxygenases) were also present in our samples (0.0001–0.013% of all KEGG annotated genes), as were genes for the broader function of ammonia assimilation, including ammonia transporters (0.4–0.6%; L. Maccario, personal communication).

In the low productivity sea ice we sampled, the proposed coupling between the compatible solute osmotolerance strategy of sea-ice microorganisms (and the potential production of ammonium during salinity downshifts) would not be with ice algae but with the nitrifying component of the ice community. This interpretation is consistent with the highest potential nitrification rates being measured in the upper sea ice (Figure 2) at depths corresponding to the sackhole drainage area that yielded brine samples for the salinity-shift experiments. Tight coupling between nitrification and ammonium generation from compatible solutes could lead to potential loss of nitrogen from sea ice, through nitrification-based N_2O production (e.g., Randall et al., 2012) and, in more biologically productive ice, through denitrification-based N_2O and N_2 production in oxygen-depleted microniches (Rysgaard and Glud, 2004). Regardless of the specific nature of the nitrogen cycling involved, our results show how an ability to use compatible solutes for osmotolerance can help to explain community selection between seawater and sea-ice brine and how the resulting microbial diversity within the brine network influences the biogeochemical cycles of the sea ice, particularly nitrogen-based dynamics.

Conclusions

In this study, both natural microbial communities in sea-ice brines and the cold-adapted marine bacteria, *Colwellia psychrerythraea* 34H and *Psychrobacter* sp. 7E, employed the compatible solute strategy for osmotolerance. This strategy appears sufficient to allow natural sea-ice communities to survive long-term (seasonal), short-term (diurnal or transient) and abrupt shifts in salinity (with ice melting). With nitrogen-based compatible solutes, the potential exists for a seasonal synergy with algal productivity or a closer coupling between this general microbial response to changing salinities and the biogeochemical activities of specific subgroups in the brine network, particularly the nitrifying community.

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Contributions

- Contributed to conception and design: EF, SDC, HS, REC, JWD
- Contributed to acquisition of data: EF, SDC, HS, REC, JWD
- Contributed to analysis and interpretation of data: EF, SDC, HS, REC, JWD
- Drafted and/or revised the article: EF, JWD
- Approved the submitted version for publication: EF, SDC, HS, REC, JWD

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Competing interests

The authors have declared that no competing interests exist.

Data accessibility statement

The following datasets were generated:

- Environmental parameters: Submitted to the Polar Data Catalogue.