Low-temperature chemotaxis, halotaxis and chemohalotaxis by the psychrophilic marine bacterium *Colwellia psychrerythraea* 34H

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Summary

A variety of ecologically important processes are driven by bacterial motility and taxis, yet these basic bacterial behaviours remain understudied in cold habitats. Here, we present a series of experiments designed to test the chemotactic ability of the model marine psychrophilic bacterium *Colwellia psychrerythraea* 34H, when grown at optimal temperature and salinity (8°C, 35 ppt) or its original isolation conditions (−1°C, 35 ppt), towards serine and mannose at temperatures from −8°C to 27°C (above its upper growth temperature of 18°C), and at salinities of 15, 35 and 55 ppt (at 8°C and −1°C). Results indicate that *C. psychrerythraea* 34H is capable of chemotaxis at all temperatures tested, with strongest chemotaxis at the temperature at which it was first grown, whether 8°C or −1°C. This model marine psychrophile also showed significant halotaxis towards 15 and 55 ppt solutions, as well as strong substrate-specific chemohalotaxis. We suggest that such patterns of taxis may enable bacteria to colonize sea ice, position themselves optimally within its extremely cold, hypersaline and temporally fluctuating microenvironments, and respond to various chemical signals therein.

Introduction

Motility and taxis are important survival strategies for many bacteria and critical to a variety of microbial processes, including biofilmning (reviewed by Guttenplan and Kearns, 2013), pathogenicity (Josenhans and Suhrbaa, 2002) and genetic competence (Meibom et al., 2005). While much foundational knowledge of motility and taxis has been learned from mesophilic organisms like *Escherichia coli* (presented in detail by Berg, 2004) and *Pseudomonas aeruginosa* (reviewed by Kato et al., 2008), these processes are also considered to be widespread, if intermittent and of variable character, throughout the world’s oceans based on seawater studies at temperatures of 15°C or warmer (Grossart et al., 2001; Mitchell and Kogure, 2006).

Chemotaxis in particular has been hypothesized as an important process that allows bacteria to exploit the organic particles, point sources and short-lived, patchy nutrient gradients that characterize the marine environment (Jackson, 1987; Kjærboe and Jackson, 2001; Barbara and Mitchell, 2003a; 2003b; Stocker et al., 2008). Further studies have characterized the unique physical and ecological considerations for chemotaxis in the ocean (Stocker and Seymour, 2012; Stocker, 2012; Taylor and Stocker, 2012). In laboratory studies of bacterial isolates, chemotaxis in seawater has been examined in the context of colonization, association or nutrient consumption, for example with phytoplankton (e.g., Bowen et al., 1993; Barbara and Mitchell, 2003a; 2003b; Seymour et al., 2009; Smriga et al., 2015). Studies of bacterial taxis in situ have shown that ‘infochemicals’, for example DMSP, play an important role in driving chemotaxis and colonization of corals and other marine environments (Thurber et al., 2009; Raina et al., 2010; Seymour et al., 2010; Garren et al., 2014; Tout et al., 2015a). For certain *Vibrio* populations, temperature (31°C) modulates their associations with corals, implying thermal effects on motility (Tout et al., 2015b).

The majority of the world’s ocean, however, is cold (5°C or below), and gradients encompassing more extreme (sub-zero) temperatures characterize the polar oceans. Gradients exist at the seawater/sea ice interface and the unfrozen brines within the ice, which also feature gradients in organic compounds and salt concentrations (Eicken, 2003; Krembs et al., 2011). Yet, the tactic behaviour of bacteria in these environments is unknown. Only a few studies have examined taxis at cold temperatures. Chemotaxis at 5°C has been shown in *Pseudomonas fluorescens* (Lynch, 1980), *Colwellia maris* (Takada et al., 1993) and *Vibrio anguillarum* (Larsen et al., 2004), while Hazeleger and colleagues...
(1998) demonstrated the ability of the enteric bacterium Campylobacter jejuni to chemotax at 4°C. The effects of even lower temperatures on bacterial chemotaxis or halotaxis, and certainly the sub-zero temperatures of sea ice brines, are poorly understood.

There are many reasons to expect that temperature impacts the extent and efficiency of bacterial sensing and taxis. E. coli is known to use a robust, complex kinase cascade to propagate signals from surface chemoreceptors to the flagellum to direct chemotaxis (Alon et al., 1999; Bren and Eisenbach, 2000). The chemical reactions involved are individually very sensitive to temperature fluctuations, but kinetic parameters and enzyme synthesis in the chemotactic response cascade are temperature-compensated, leading to thermal robustness across the physiological range of E. coli (Oleksiuk et al., 2011). With both marine bacterial isolates and mesophilic E. coli, however, these pathways have been investigated only at relatively moderate temperatures (10–35°C) compared with those experienced by bacteria in cold seawater and within the brines of the sea ice matrix, where temperatures can extend below −20°C (Junge et al., 2004).

At cold temperatures, additional physical and physiological processes may reduce or inhibit the receipt of chemical signals that trigger bacterial chemotaxis, including fatty acid transition leading to lipid membrane rigidity (Lofgren and Fox, 1974), high salinities requiring transport actions to maintain turgor pressure (Firth et al., 2016), and increased viscosity slowing diffusion (Schneider and Doetsch, 1974; Stocker and Seymour, 2012). Additionally, the fractal microstructure of sea ice brine pores leads to high surface area to volume ratios (Krembs et al., 2011), possibly favouring surface attachment over motility.

Studying motility and taxis at cold temperatures is challenging. Methods to study bacterial movement have focused primarily on mesophiles, with the recent inclusion of mesophilic marine bacteria and direct visualization using microscopy (reviewed by Son et al., 2015). These studies often use microfluidic devices to observe individual and population responses to the introduction of gradients in nutrients, gases, repellents, and so forth. (Seymour et al., 2008; Ahmed et al., 2010; Seymour et al., 2010). While these methods can produce rich and robust data sets, they can be expensive and difficult to adapt to cold temperatures which fog glass and cause small volumes of liquids to freeze easily. Previous studies have involved high resolution microscopes factory modified for sub-zero conditions, as well as cold rooms or freezers large enough to allow human operators to manipulate samples and microscopes (Junge et al., 2001, 2003). Although a cold stage on a microscope at room temperature (Bar Dolev et al., 2016) could be adapted for taxis studies, the traditional capillary tube assay, first used by Pfeffer (1884) and refined by Adler (1973), provides an imminently feasible and economic means to study bacterial taxis at cold temperature. This and similar techniques have already been adapted to marine bacteria (Larsen et al., 2004; Tout et al., 2015a).

In the classic assay, the chemotactic response of bacteria presented with a gradient in the attractant (or repellent) of interest is evaluated by enumerating those that swim into a capillary tube, the source of the high end of the diffusion-established gradient, relative to a control tube absent the target compound.

Colwellia psychrerythraea strain 34H (hereinafter Cp34H), was selected for this study as a model marine psychrophile (Méthé et al., 2005). Originally isolated from Arctic marine sediments at −1°C (Huston, 2003), Cp34H has subsequently been found in other cold marine environments and is considered highly ice-adapted (Boetius et al., 2015). Cp34H currently holds the low-temperature record for motility at −10°C, first observed in an analogue sea ice brine (along with robust swimming at −8°C, −5°C and −1°C; Junge et al., 2003), and as confirmed by digital holographic microscopy (Wallace et al., 2015). At −1°C, Cp34H also transports compatible solutes (small molecular weight organic compounds) to tolerate salinity shifts inherent to sea ice brines (Firth et al., 2016). Optimal (most rapid) growth occurs at 8°C and 35 ppt in organically rich media, with the growth range spanning −12°C to 22°C, and 15 to 70 ppt salinity (Huston, 2003; Wells and Deming, 2006). The responses of Cp34H, however, to the complex gradients in temperature, salinity and nutrients presented by the sea ice environment have yet to be characterized, leading to this work.

In preliminary work, we had observed thermotaxis by Cp34H; that is, when grown at its optimal growth temperature of about 8°C (and salinity of 35 ppt) but placed at higher and lower temperatures, Cp34H swam towards its growth temperature (Showalter and Deming, Abstr. 115th Gen. Meet. Am. Soc. Microbiol. 2015). These observations of thermotaxis, coupled with previously published work showing optimal chemotaxis at optimal growth temperature for E. coli and Vibrio anguillarum (Takada et al., 1993; Larsen et al., 2004; Oleksiuk et al., 2011), led us to hypothesize that Cp34H would behave similarly, with strongest chemotaxis occurring at its optimal growth temperature. We considered that at cold temperatures suboptimal for growth, the chemotactic response of Cp34H would be stronger to compounds able to provide a greater return of energy or to signal a more optimal environment. Given the co-occurring nutrient and salinity gradients that characterize the sea ice environment, we also hypothesized that when presented with chemotractants under salinities higher and
lower than seawater Cp34H would continue to exhibit chemotaxis, that is, to exhibit chemohalotaxis.

Here, we present the results of capillary tube experiments designed to characterize the chemotactic and halotactic behaviour of Cp34H toward the amino acid serine (0.1 M to 1 M) and the sugar monomer mannose (1 M) across a range of temperatures (−8°C, −1°C, 8°C, 15°C, 22°C and 27°C) and salinities (15, 35 and 55 ppt). Our goal was to provide foundational information on the potential for bacterial taxis within the gradients that characterize sea ice and its interface with seawater. Several factors suggested the use of serine as an experimental chemoattractant, including the finding that serine is the most dominant amino acid in sea ice, at likely millimolar concentrations in the brine (Yang, 1995). Serine is also commonly used as a chemoattractant in tests of laboratory isolates, in part because the tsr receptor, which senses serine, is among the most highly expressed chemoattractant receptors in E. coli (Feng et al., 1997).

Additionally, the tsr receptor is a component of thermosensory systems in E. coli, such that serine impacts how bacteria sense temperature gradients (Clarke and Koshland, 1979; Maeda and Imae, 1979). A survey of the Cp34H genome using the GenBank database (Clark et al., 2016) revealed that Cp34H contains methyl-accepting chemotaxis proteins similar to tsr. Mannose, was chosen because it is a common monosaccharide found in sea ice (Aslam et al., 2016), often dominates the composition of exopolysaccharides produced by sea ice bacteria (Underwood et al., 2010), and might provide more energy (ATP) than serine if assimilated and catabolized. Mannose has also been used in temperate studies of marine chemotaxis in the context of coral reef bacteria, which demonstrated chemotaxis toward 100 μM mannose while bacteria not associated with reefs did not (Tout et al., 2015a). Ultimately, we examined comparative tactic responses of our model organism when grown at its optimal growth temperature (8°C) versus isolation temperature (−1°C), as the latter better represents polar waters and the sea ice environment.

**Results and discussion**

The results of our experiments, across the set of temperature and salinity conditions under which Colwellia psychrerythraea 34H was grown and tested for chemotaxis, halotaxis, and chemohalotaxis are presented in graphical form (Figs 1–4). To our knowledge, these results are unique in their demonstration of bacterial chemotaxis below 4°C, and they establish a new low-temperature record of −8°C (the lowest temperature we tested) for bacterial chemotaxis. They are also unique in demonstrating significant halotaxis and strong substrate-specific chemohalotaxis by a marine bacterium.

![Chemotactic dose-response of Colwellia psychrerythraea 34H to serine at 8°C](image)

**Fig. 1.** Chemotactic dose-response of Colwellia psychrerythraea 34H to serine at 8°C. Cp34H was grown and tested at 8°C and 35 ppt salinity, rinsed to remove dissolved organic compounds, and presented with 10 mM, 100 mM or 1 M serine as chemoattractant (see Supporting Information for details). The dashed line indicates the normalized control value of 1, with values above the line indicating chemotaxis. Asterisks indicate significant (p < 0.05) chemotaxis, as demonstrated by significantly higher bacterial concentrations in capillary tubes with chemoattractant compared with control tubes (see Supporting Information Table S1 for p values). Error bars indicate standard deviation of the mean (n = 3, except where indicated in Supporting Information Table S1).

Obtaining internally consistent results across these conditions, which included warm, cold and sub-zero temperatures, required time-scaling and anti-freeze adjustments to the capillary tube assay, originally designed for room temperature work. Details of these adjustments are provided in the Supporting Information. Bacterial densities in the sets of treatment and control capillary tubes, with p-values for significance of the difference using a Student’s t-test, are listed in Supporting Information Table S1. Significant (p < 0.05) results of two-way ANOVAs, used to examine interactions between experimental factors, are provided in the course of the discussion below.

**Chemotaxis, when Cp34H was grown at 8°C**

To first determine a suitable concentration of chemoattractant to use in this study, a dose-response experiment was conducted with 10 mM, 100 mM and 1 M serine, commonly used concentrations in the pure-culture chemotaxis literature (e.g., Alder, 1973; Takada et al., 1993). Cp34H had been grown in nutrient-rich media (Difco Marine Broth 2216) at optimal growth temperature and salinity (8°C and 35 ppt), prior to rinsing and testing for chemotaxis at the same temperature and salinity. Negative controls of capillary tubes with no chemoattractant accounted for random motility. The organism’s chemotactic response to 1 M
Bacterial taxis at low temperature

Fig. 2. Chemotactic response of *Colwellia psychrerythraea* 34H at temperatures from −8°C to 27°C. *Cp*34H, when grown at 8°C, demonstrated significant taxis to serine at temperatures from −1°C to 22°C, and to mannose more broadly, at all test temperatures. Dark grey bars represent chemotaxis to 1 M serine; light grey bars, to 1 M mannose; shaded area indicates chemotactic responses above the upper growth temperature for *Cp*34H. Chemotactic response was calculated as the ratio of mean concentration of bacteria in the capillary tube with chemoattractant (treatment) to mean concentration of bacteria in the control tube. The dashed line indicates the normalized control value of 1, with values above the line indicating chemotaxis; asterisks indicate significant (p < 0.05) chemotaxis (see Supporting Information Table S1 for individual p values). Error bars indicate standard deviation of the mean (n = 3, except where indicated in Supporting Information Table S1).

![Chemotactic response of Colwellia psychrerythraea 34H at temperatures from −8°C to 27°C.](image)

Fig. 3. Chemotactic response of *Colwellia psychrerythraea* 34H when grown at −1°C. *Cp*34H grown showed strongest chemotaxis at its growth temperature, whether grown at −1°C (this figure) or at 8°C (Fig. 2). Dark grey bars represent relative chemotaxis to 1 M serine; light grey bars, chemotaxis to 1 M mannose. The dashed line indicates the normalized control value of 1, with values above the line indicating chemotaxis; asterisks indicate significant (p < 0.05) chemotaxis (see Supporting Information Table S1 for individual p values). Error bars indicate standard deviation of the mean (n = 3).

serine was strong and significant (Fig. 1). The response to a lower concentration of 100 mM serine, though significant, was weaker, while the response to 10 mM serine was not significant (Fig. 1). As similar results were obtained with mannose (not shown), a 1 M concentration of chemoattractant was used in all subsequent experiments. Organisms grown in nutrient-rich media may express low-sensitivity chemoreceptors because of heavy methylation of chemoreceptors (Terracciano and Canale-Parola, 1984), but the purpose of this study was to examine temperature and salinity conditions that could support chemotaxis, not to decipher receptor sensitivity in *Cp*34H.

In subsequent experiments, *Cp*34H demonstrated significant chemotaxis at all test temperatures (Fig. 2): −8°C, −1°C, 8°C, 15°C, 22°C and 27°C (all at salinity of 35 ppt; incubated for 8, 4, 2, 1.5, 1.5 and 1.5 h, respectively, see Supporting Information), with strongest chemotaxis observed at the temperature at which the organism had been grown (8°C in Fig. 2). Although the strongest response was to serine (at 8°C, with notable activity at −1°C), the temperature range for serine as chemoattractant was narrower than the range for mannose. No significant chemotaxis to serine was detected at the ends of the temperature test range, −8°C and 27°C; in contrast, chemotaxis to mannose was significant across the entire range (Fig. 2). Mannose also tended to be the stronger chemoattractant at the warmer temperatures (Fig. 2), including above maximal growth temperature (18°C, Huston, 2003), although insignificantly when compared with serine.

This pattern of a broad temperature range for chemotaxis with strongest taxis at optimal growth temperature, likely due to methylation of chemotaxis proteins, is generally consistent with results obtained for other bacteria, including two marine bacteria, *Vibrio anguillarum* (optimal growth and strongest chemotaxis at 25°C; Larsen et al., 2004) and *Colwellia maris* (optimal growth and strongest chemotaxis at 15°C; Takada et al., 1993). Also like *V. anguillarum* and *C. maris*, *Cp*34H remained significantly chemotactic at temperatures below optimal growth temperature (8°C for *Cp*34H); that is, at −1 and −8°C (Supporting Information Table S1). Both of these temperatures still fall within the growth range of *Cp*34H, which is bounded by −12°C and 18°C (Wells and Deming, 2006); we were not able to test for chemotaxis at or below the lower growth temperature.

Observations of chemotaxis at 27°C, 9°C above the upper growth temperature of *Cp*34H, suggest that *Cp*34H expends energy for high physiological activity beyond the bounds of growth. This phenomenon has been observed previously for chemotaxis when extending tests below the lower thermal bound for growth; for example, Hazeleger and colleagues (1998)
demonstrated chemotaxis in Camplyobacter jejuni as much as 30°C below its minimum growth temperature. Temperature-dependent motility responses have also been shown in B. subtilis, which produced proteins connected to flagellar synthesis and chemotaxis when shocked with low temperature (Graumann et al., 1996). For Cp34H, a strict psychrophile, we suggest that the chemotactic response at 27°C may indicate a short-term survival response to warm temperatures, a testable hypothesis in future experiments. Previous studies have demonstrated that proper membrane fluidity is required for chemotaxis (Lofgren and Fox, 1974), likely due to membrane-embedded methyl-accepting chemotaxis proteins. The chemotactic responses of Cp34H demonstrate versatility in expressing the membrane rigidity required to respond chemotactically at 27°C, as well as the fluidity to respond at the sub-zero end of its temperature range. As the chemotaxis response of Cp34H above its upper growth temperature stands in contrast to previous work with Colwellia maris, where no chemotaxis was observed above its upper growth temperature (between 20°C and 24°C; Takada et al., 1993), more research on this aspect of chemotaxis is needed.

Chemotaxis, when Cp34H was grown at −1°C

Cp34H grown at the environmentally relevant temperature of −1°C demonstrated chemotaxis toward serine and mannose at similar magnitudes as when grown at optimal growth temperature (8°C); the peak responses, however, were shifted from 8°C (Fig. 2) to −1°C (Fig. 3). This marine psychrophile thus appears to behave as the mesophile E. coli in showing thermal robustness for chemotaxis across its physiological growth range (Oleksiuk et al., 2011). As Cp34H is capable of growth at even colder temperatures (to −12°C; Wells and Deming, 2006), chemotaxis may be expected at such temperatures, as suggested by the significant response to mannose measured at −8°C (Fig. 2). We observed that the chemotactic response to mannose at −1°C was significantly higher when Cp34H had been grown at −1°C (Fig. 3) than when grown at 8°C (t test, p = 0.002; Fig. 2).

A two-way ANOVA comparing the effect of temperature and chemoattractant on chemotactic response of cells grown at −1°C indicated that experimental temperature was the main effect determining chemotactic response (p = 1.06 × 10⁻⁶). Comparing cells grown at −1°C and cells grown at 8°C indicated significant interaction between temperature and growth temperature at the 90% confidence level (p = 0.0665), implying that specific responses to serine versus mannose were connected to temperature.

Although the molecular-level mechanisms accounting for chemotactic and substrate-specific responses at sub-zero temperature remain to be determined, directed bacterial movements under the extreme temperatures we tested have both physiological and environmental implications, as discussed below.
**Halotaxis and chemohalotaxis**

As temperature gradients in sea ice and environs are accompanied by gradients in salinity and organic solutes, we included experiments to test for halotaxis and chemohalotaxis by *Cp34H*. When cultures grown under optimal conditions (8°C, 35 ppt) were presented with a salinity signal (absent organic chemoattractants) of either higher or lower salinity in the capillary tube, with the salts diffusing to create a gradient, *Cp34H* demonstrated significant halotaxis in each salinity gradient at both test temperatures: 8°C (Fig. 4A) and −1°C (Fig. 4B). The strongest halotactic response was observed at −1°C in the gradient towards fresher salinity, simulating the melting of sea ice into −1°C seawater. Strong halotaxis near the freeze/thaw boundary for Arctic seawater (about −1.9°C) might indicate that bacteria use a change in salinity as a signal to colonize freezing sea ice in fall or to leave melting sea ice in spring (Fig. 5). Future studies investigating bacterial taxis *in situ* at the sea ice/seawater boundary may provide tests of this hypothesis.

When a gradient in organic chemoattractant was presented simultaneously with the salinity gradient, significant chemohalotaxis was observed under all conditions (Fig. 4A). Although patterns of response to the different chemoattractants were observed, a two-way ANOVA comparing effects of treatment temperature and chemoattractant indicated that temperature was the most important factor determining chemotaxis (*p* = 0.0006), whereas chemoattractant did not have a significant effect. The strongest chemohalotactic response was recorded at −1°C in a gradient towards higher salinity (55 ppt) and mannose (1 M) (Fig. 4B). This result, and similar results from the earlier chemotaxis experiments, imply that under optimal growth conditions, *Cp34H* may chemotax more strongly to the nitrogen-containing chemoattractant serine (e.g., at 8°C, the response to serine was greater than to mannose [*p* = 1.90 × 10⁻²⁵]). When confronted with suboptimal or stressful conditions, however, the sugar monomer mannose was the stronger chemoattractant (e.g., at −1°C and 15 ppt salinity [*p* = 0.003], and at −1°C and 55 ppt [*p* = 0.003]). From an energetic perspective, this finding is consistent with the hypothesis that *Cp34H* may seek substrate for maximum yield of ATP to counter suboptimal or stressful conditions (6-C mannose catabolism yields 2 ATP per unit, while 3-C serine catabolism yields 1 ATP per unit).

In the context of the sea ice environment, however,

![Fig. 5. A schematic synthesis of suggested bacterial taxis in the context of the sea ice environment. Based on results from this study, we suggest that cold-adapted marine bacteria may use chemotaxis, halotaxis and chemohalotaxis to position themselves in response to environmental gradients encountered at the sea ice/seawater interface and within the sea ice brine network. Chemohalotaxis by the model marine psychrophile *Colwellia psychrerythraea* 34H toward organic solutes (serine and mannose, in this study) at −1°C and salinities higher than seawater (55 ppt) suggest potential movement up gradients toward brine and solutes rejected from sea ice as it forms (A) or toward brine and solutes retained in the ice as it grows (B). Within the sea ice brine network (C), chemohalotaxis and halotaxis by *Cp34H* at the subzero temperatures we tested (−1°C and −8°C) suggest that bacteria may be able to respond to chemical gradients produced by sea ice algae (C and D, in green), EPS or other organic matter, as well as salinity gradients that change internally in the ice matrix as temperatures change. When sea ice melts (E), results of experiments with *Cp34H* at −1°C suggest potential movement toward low-salinity melt waters and associated organics released from the ice. Arrows point in the direction of suggested bacterial movement. Gradients in yellow within arrows represent gradients of organic chemicals analogous to serine or mannose, while gradients in blue represent salt gradients; the highest concentration of chemoattractant or salt is represented by the darkest shade.](https://example.com/fig5)
other explanations may pertain. Sea ice is an algae-rich habitat during the bloom season, and the concurrent production of extracellular polymeric substances (EPS) by both algae and bacteria, including \( \text{Cp34H} \) (Marx et al., 2009), can lead to high concentrations of exopoly saccharides within sea ice brine pockets and at the sea ice/seawater interface (Krembs et al., 2002; 2011), where such complex sugars serve as extracellular cryoprotectants, inhibiting ice formation near cells, and osmoprotectants, providing a hydrated buffer against salinity extremes (Krembs and Deming, 2008; Deming and Young, 2017). Algal EPS most commonly contain the monomers glucose and mannose, in varying concentrations (Aslam et al., 2016), and though not yet possible to quantify on the relevant micrometer scale, gradients in such monomers due to EPS hydrolysis can be expected based on microscopic evidence (Krembs et al., 2011). The strong chemotactic response of \( \text{Cp34H} \) towards mannose may reflect the benefits of seeking algal EPS, which may serve as a source of energy, as protection against the extreme conditions of temperature and salinity that characterize sea ice (Ewert and Deming, 2014), or as a chemical signal to bacteria. The possibility that mannose may also serve as an intracellular osmoprotectant, once found and taken up by an organism, remains to be explored.

At the sea ice/seawater interface, salinity gradients are present both as the ice grows, rejecting high salinity brine into underlying seawater, and as the ice melts, releasing fresh or lower salinity water into the surface ocean (Fig. 5). Our results suggest that \( \text{Cp34H} \) may have the capacity to respond to salinity gradients in both of these contexts, as halotaxis was demonstrated in response to salinities both higher and lower than seawater salinity, albeit under controlled laboratory conditions. These results are unique to \( \text{Cp34H} \) as compared with demonstrations of osmotaxis and halotaxis in \( \text{E. coli} \), which prefers low concentrations of osmolyte under all tested conditions (Li et al., 1988). The difference in halotactic response of \( \text{Cp34H} \) at \(-1^\circ\text{C}\) compared with \(8^\circ\text{C}\) suggests that temperature may influence the osmosensing ability, perhaps as a result of changes in membrane fluidity. Previous analysis of lipid membrane composition in \( \text{Cp34H} \) has suggested that the organism is capable of homeoviscous acclimation at low temperatures (Methé et al., 2005; Nunn et al., 2015), and \( \text{Cp34H} \) has been shown to increase its number of monounsaturated fatty acids at \(-1^\circ\text{C}\) as compared with \(9^\circ\text{C}\) (Huston, 2003). This change in membrane fluidity and fatty acid composition may modulate sensitivity or function of osmo-sensing proteins which are located in the bacterial membrane (Los and Murata, 2004; Poolman et al., 2004).

**Implications for microbial life within sea ice**

Although the results of this study may help to inform bacterial behaviour at the sea ice/seawater interface, the scope of motility and chemotaxis within the ice is still an outstanding question for further investigation. Previous studies have shown that \( \text{Cp34H} \) is capable of motility in sea ice brine analogues at and below \(-10^\circ\text{C}\) (Junge et al., 2003; Wallace et al., 2015), but bacterial motility has not been visualized directly in natural sea ice. Here, we have demonstrated in laboratory experiments that \( \text{Cp34H} \) is capable not only of motility but also of chemotaxis between \(-8^\circ\text{C}\) and \(27^\circ\text{C}\) and 15 to 55 ppt. In particular, \( \text{Cp34H} \) showed strong chemohalotaxis toward mannose at \(-1^\circ\text{C}\) in a salinity gradient (upper bound set by 55 ppt) and chemotaxis toward mannose even at \(-8^\circ\text{C}\) (in 35 ppt salt with 5% glycerol). These findings indicate that chemotaxis may be feasible within the sea ice brine network (Fig. 5). Indeed, 16S rRNA gene sequence data indicate that motile copiotrophic members of the **Gammaproteobacteria** and **Flavobacteria** commonly dominate sea ice bacterial communities (Bowman et al., 2012; Boe tius et al., 2015). Though untested, bacterial taxis toward mannose may allow colonization of sea ice, contributing to community differentiation from seawater.

Several factors, however, appear to argue against chemotaxis within sea ice brines. More than 95% of the bacteria in sea ice reside in the brine fraction, but most of them are associated with surfaces or EPS gels in the brine (Junge et al., 2001; 2004; Meiners et al., 2008). Proteomic investigations of \( \text{Cp34H} \) have indicated loss of flagellar synthesis proteins after 24 h at \(-10^\circ\text{C}\), possibly leading to surface association (Nunn et al., 2015). Nunn and colleagues (2015) also demonstrated upregulation of chemosensing proteins at \(-10^\circ\text{C}\), which may indicate a ‘lie and wait’ strategy by \( \text{Cp34H} \), quickly returning to a chemotactic state upon sensing a lucrative chemical gradient. Indeed, Lindensmith and colleagues (2016) were able to stimulate taxis in non-motile bacteria in sea ice brines by adding marine broth to the brines (Lindensmith et al., 2016), implying the use of a similar ‘lie and wait’ strategy by bacteria *in situ*.

Future studies to determine environmental conditions that favour surface attachment and biofilming over motility and chemotaxis within sea ice will increase understanding of microbial ecology within sea ice. Recently, Bar Dolev and colleagues (2016) showed the production of a ‘molecular fishing hook’ by *Marinomonas primoryensis*, allowing the organism to attach to the surface of ice crystals, while Carillo and colleagues (2015) and Casillo and colleagues (2017) showed that capsular and extracellular polysaccharides produced by \( \text{Cp34H} \) have ice-binding properties. If organisms such as *M. primoryensis*
and Cp34H are capable of biofilming on ice as a surface, they may also experience enhanced horizontal gene transfer and variable viral infection dynamics, as in the well-known mesophilic Vibrio case (Meibom et al., 2005), which could improve understanding of the evolution and function of marine microbial communities in the cold. Tools to visualize bacteria within sea ice brines directly, such as digital holographic microscopy (Wallace et al., 2015; Lindensmith et al., 2016), and to examine viral action directly (Brum and Sullivan, 2015), may allow the probing of these questions in a true environmental context in future studies. The results of this study lay groundwork by demonstrating that cold-adapted bacteria may be able to position themselves within the sea ice matrix by chemotaxis, halotaxis and chemohalotaxis, seeking optimal energy gains or responding to chemical signals prior to attachment.

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**Supporting Information**

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Fig. S1.** Experimental protocol used to test chemotaxis across a range of temperatures. (A) *Colwellia psychrerythraea* 34H cells were grown under desired conditions and harvested in mid-late log phase (when the largest fraction of cells appeared as motile) by washing twice in organic-free artificial seawater and 0.1 mM EDTA; for subsequent experiments below 2°C, 5% glycerol was added to the final resuspension. Cell suspensions were subdivided into 1.5 ml Eppendorf tubes and sealed with parafilm, generating the cell reservoirs for the capillary tube assay. (B) Cell reservoirs and chemoattractants dissolved in motility medium, as well as chemoattractant-free motility medium (for controls), were allowed to equilibrate to treatment temperature for one hour. Chemoattractants and controls were then drawn into capillary tubes and capped with agar on one end. The open end of each tube was then inserted into its cell reservoir and allowed to incubate for the experimental period. (C) Contents of the capillary tube were expelled onto sterile parafilm and sub-aliquots were fixed for counting by epifluorescence microscopy.

**Table S1.** Treatment conditions, resulting data and statistical significance for capillary tube experiments.