

## ORIGINAL ARTICLE

# Diatom assemblages promote ice formation in large lakes

NA D'souza<sup>1,3</sup>, Y Kawarasaki<sup>2</sup>, JD Gantz<sup>2</sup>, RE Lee Jr.<sup>2</sup>, BFN Beall<sup>1</sup>, YM Shtarkman<sup>1</sup>, ZA Koçer<sup>1,4</sup>, SO Rogers<sup>1</sup>, H Wildschutte<sup>1</sup>, GS Bullerjahn<sup>1</sup> and RML McKay<sup>1</sup>

<sup>1</sup>Department of Biological Sciences, Bowling Green State University, Bowling Green, OH, USA and

<sup>2</sup>Department of Zoology, Miami University, Oxford, OH, USA

**We present evidence for the directed formation of ice by planktonic communities dominated by filamentous diatoms sampled from the ice-covered Laurentian Great Lakes. We hypothesize that ice formation promotes attachment of these non-motile phytoplankton to overlying ice, thereby maintaining a favorable position for the diatoms in the photic zone. However, it is unclear whether the diatoms themselves are responsible for ice nucleation. Scanning electron microscopy revealed associations of bacterial epiphytes with the dominant diatoms of the phytoplankton assemblage, and bacteria isolated from the phytoplankton showed elevated temperatures of crystallization ( $T_c$ ) as high as  $-3^\circ\text{C}$ . Ice nucleation-active bacteria were identified as belonging to the genus *Pseudomonas*, but we could not demonstrate that they were sufficiently abundant to incite the observed freezing. Regardless of the source of ice nucleation activity, the resulting production of frazil ice may provide a means for the diatoms to be recruited to the overlying lake ice, thereby increasing their fitness. Bacterial epiphytes are likewise expected to benefit from their association with the diatoms as recipients of organic carbon excreted by their hosts. This novel mechanism illuminates a previously undescribed stage of the life cycle of the meroplanktonic diatoms that bloom in Lake Erie and other Great Lakes during winter and offers a model relevant to aquatic ecosystems having seasonal ice cover around the world.**

*The ISME Journal* (2013) 7, 1632–1640; doi:10.1038/ismej.2013.49; published online 4 April 2013

**Subject Category:** Microbial ecology and functional diversity of natural habitats

**Keywords:** diatoms; ice nucleation; lake; *Pseudomonas*

## Introduction

Winter presents a logistical obstacle to our understanding of temperate and boreal lake ecosystems. Reflecting this, lake ecosystem models frequently underestimate, or even dismiss, winter parameters. Addressing the void in our understanding of the winter ecosystem in Lake Erie, recent coordination with Coast Guard icebreaking programs has facilitated winter surveys of the lake since 2007 (Oyserman *et al.*, 2012; Twiss *et al.*, 2012). Conducted during times of expansive ice cover, these surveys have documented phytoplankton blooms dominated by physiologically robust, filamentous centric diatoms (Saxton *et al.*, 2012; Twiss *et al.*, 2012).

The physical processes involved in the development of diatom blooms in Lake Erie during winter are unclear. With potentially expansive ice cover across mid-high latitude lakes, light attenuation due to the ice and accumulated snow should act as a selective pressure in shaping adaptive mechanisms of phytoplankton found below the ice. In an ice-covered lake, it is imperative that phytoplankton occupy a position near the surface, which is a challenge for diatoms given that ice cover reduces wind-driven mixing and diatoms are non-motile plankton with high sinking rates. In ice-covered lakes, convective mixing is proposed as a mechanism to keep plankton suspended in the photic zone (Bengtsson, 1996; Kelley, 1997). Likewise, incorporation into ice cover, possibly via scavenging by frazil ice (Garrison *et al.*, 1983), would offer a solution. Indeed, winter surveys of Lake Erie demonstrated the presence of viable diatoms in meltwater from lake ice (Twiss *et al.*, 2012), consistent with a recent report documenting abundant *Aulacoseira islandica* colonizing vertical channels in ice sampled from the St Lawrence River, downstream from Lake Erie (Frenette *et al.*, 2008).

Correspondence: RML McKay, Department of Biological Sciences, Bowling Green State University, Life Sciences Building, Bowling Green, OH 43403, USA.

E-mail: rmmckay@bgsu.edu

<sup>3</sup>Current Address: Lamont-Doherty Earth Observatory of Columbia University, Palisades, NY 10964, USA.

<sup>4</sup>Current Address: Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN 38105, USA.

Received 10 September 2012; revised 12 February 2013; accepted 16 February 2013; published online 4 April 2013

During a recent winter survey of Lake Erie, ice formation was observed in samples of diatom-dominated plankton suspended in filtered lake water (FLW) and left in an insulated cooler on the ship's deck overnight. Adjacent samples containing only filtered ( $<0.45\ \mu\text{m}$ ) lake water did not freeze. Biological ice nucleation associated with the diatom plankton offered a logical explanation for the discrepancy in whether samples froze.

Here, we document ice nucleation activity associated with filamentous diatoms sampled from the ice-covered Laurentian Great Lakes. Directed ice formation by diatoms promotes the formation of frazil ice, which provides buoyancy to the filaments and their subsequent attachment to overlying ice cover. This mechanism would increase fitness of diatoms by maintaining a favorable position in the photic zone, thereby facilitating their growth.

## Materials and methods

### *Study site and sampling*

Lake Erie was sampled on surveys aboard CCGS *GRIFFON* during February 2011–2012 and aboard R/V *LAKE GUARDIAN* during April 2010–2011. Sites in ice-covered western Lake Superior were sampled aboard USCGC *ALDER* in March 2011, whereas sampling in Lake Ontario was conducted aboard R/V *LAKE GUARDIAN* in April 2011. Physicochemical profiles of the water column were conducted as described in Supplementary Figure S1.

Plankton were collected by vertical net tows using a 154- $\mu\text{m}$  mesh size plankton net deployed to depths of 10–15 m in water freed of ice cover by the vessel. Turbulence associated with icebreaker motion resulted in mixing of the ice-covered surface waters. High rates of sedimentation by diatoms collected at these locations in excess of  $0.2\ \text{m h}^{-1}$  guided the decision to collect samples integrated through these depths to ensure recovery of sufficient biomass for our analyses. Chlorophyll (Chl) *a* biomass was measured by fluorometry following extraction in 90% (v/v) acetone at  $-20\ ^\circ\text{C}$  (Twiss *et al.*, 2012). Water samples were collected using a 10-l Niskin bottle on a metered winch and by use of a submersible pump deployed 1 m below the surface. This water was filtered through a 0.2- $\mu\text{m}$  filter and used for diluting phytoplankton collected by nets. During the 2012 survey, samples of ice were collected from Lake Erie. Individual pieces of ice ( $\sim 20\ \text{cm}^2$ ) were passed through three rinses of filtered ( $<0.2\ \mu\text{m}$ ) lake water and placed in a sealed plastic bag to melt. The resulting meltwater was used for ice nucleation assays. Surface sediment was collected at several central basin sites using a ponar. Biofilm material adherent to 25-mm-diameter polycarbonate filters (0.2  $\mu\text{m}$ ) placed on the sediment surface was collected and resuspended in FLW before assay for ice nucleation.

### *Determination of temperature of crystallization of net plankton*

Temperature of crystallization ( $T_c$ ), was determined using  $\sim 30\ \mu\text{l}$  of sample in an  $80\ \mu\text{l}$  capillary tube (FisherBrand Micro-Hematocrit, Pittsburgh, PA, USA) to which a thermocouple was attached. Chl *a* biomass of phytoplankton samples loaded into microcapillary tubes ranged from 0.5–3  $\mu\text{g chl a ml}^{-1}$ . All samples collected in 2012 were diluted to  $0.5\ \mu\text{g chl a ml}^{-1}$  before freezing assay. Dilution trials ( $n=5$ ) conducted in April 2010 and February 2012 demonstrated that samples diluted to at least  $0.1\ \mu\text{g chl a ml}^{-1}$  retained the same ice nucleation activity as an undiluted sample (Supplementary Figure S2). The capillary was placed in a foam-plugged glass test tube and suspended in a  $5\ ^\circ\text{C}$  cooling bath. A thermologger recorded the temperature at 30 s intervals. Following equilibration to  $5\ ^\circ\text{C}$  for 10 min, the temperature of the cooling bath was decreased at a controlled rate of  $0.3\ ^\circ\text{C min}^{-1}$ . The  $T_c$  was recorded as the highest temperature at which ice crystals began to form, as indicated by the release of the latent heat of crystallization.

One-factor analysis of variances were conducted on samples collected during each field season, from each set of cultures, and during a heat denaturation experiment. Homogeneity of variance was tested for each analysis of variance by the Levene test with  $\alpha=0.05$ , and normality was checked using normal Q–Q plots. A  $\log(-T_c)$  transformation was required for homogeneity of variances in the denaturation experiment. Tukey's HSD was used, with  $\alpha=0.05$ , to compare  $T_c$  of samples and cultures against controls. All statistics were performed using the stats and car packages in R (version 2.12.2, www.r-project.org).

### *Scanning electron microscopy*

Plankton were transferred to a glass vial and fixed by addition of glutaraldehyde to a final concentration of 2.5% (v/v) in 0.1 M sodium phosphate buffer (pH 7.2). Following two rinses with this buffer, samples were dehydrated through a graded series of acetone to 100%. Critical point drying was replaced by treatment with a solution of hexamethyl disilazane for 5 min and then air dried onto cover slips and coated with a 10-nm Au–Pd layer using a sputter coater (Hummer VI-A) before viewing with a Hitachi S2700 SEM (Hitachi High Technologies America, Inc., Dallas, TX, USA).

### *Isolation and characterization of heterotrophic bacteria*

Plankton material collected by vertical net tows was streaked on Nutrient Broth agar plates (0.8% w/v nutrient broth (Difco, Becton Dickinson and Company, Franklin Lakes, NJ, USA), 1.2% w/v Bacto Agar (Difco)) and incubated at  $4\ ^\circ\text{C}$  in the dark for 72 h. Colonies from the plate were selected based on differences in colony morphology, and repeatedly subcultured at  $4\ ^\circ\text{C}$  so as to ensure purity

of the final isolates. Isolates thus obtained were assayed for  $T_c$  as described above following standing culture growth in nutrient broth for 60–90 h at 4 °C. Enumeration of cell density was made by flow cytometry with densities ranging from 0.2–3.3 × 10<sup>6</sup> cells per ml used in the ice nucleation assay trials. Based on volumes loaded to microcapillary tubes for  $T_c$  assay, we estimate each capillary to have contained 0.6–9.9 × 10<sup>4</sup> cells.

Samples collected in 2012 were serially diluted with FLW before plating on cetrimide agar (Fluka Analytical, Sigma-Aldrich Corp., St Louis, MO, USA), a selective medium for isolating *Pseudomonas* spp. Plates were incubated at 20 °C for 2–5 days before enumeration.

#### Molecular characterization of bacterial strains

Bacterial SSU rDNA sequences were amplified by colony-PCR for all Lake Erie water sample isolates from 2010–2011 using the primers 8F (5'-AGAGTTT GATCMTGGCTCAG-3') and 1492R (5'-GGYTACCTT GTTACGACTT-3'). The resulting PCR products were purified using a Qiagen PCR purification kit (Qiagen, Germantown, MD, USA). Purified fragments were sequenced by the DNA sequencing facility at the University of Chicago Cancer Research Center using standard methods. Ribosomal SSU DNA sequence data for the environmental bacterial isolates have been submitted to the GenBank database under accession numbers: JN201533–JN201573.

Thirty-four putative *Pseudomonas* isolate sequences were selected and combined with gamma-proteobacterial metagenomic sequences (Supplementary Figure S3), as well as the most similar NCBI sequences and published sequences of identified *Pseudomonas* taxa (Anzai *et al.*, 2000), as determined from BLAST searches. A local multiple sequence alignment was performed with the MAFFT 6.833 command line version (Katoh *et al.*, 2009; Katoh and Toh, 2010). The alignment file was manually evaluated with JalView (Waterhouse *et al.*, 2009) and SeaView 4.3.2 (Gouy *et al.*, 2010). Sequences shorter than 150 nucleotides were excluded from the final alignment. The alignment file was converted to the Nexus format with SeaView and subjected to phylogenetic analysis using PAUP 4.0b10 (Swofford, 1998). A total of 973 nucleotide positions were used for phylogenetic analysis. Neighbor-joining phylogenetic analysis was performed with 1000 bootstrap iterations.

We used sequences of nine known *ina* genes to design primers in an attempt to amplify the genes in our isolates. Sequences of four *ina* genes (*inaZ*, *inaK*, *inaV* and *ice4*) identified in *Pseudomonas syringae* were aligned using CLUSTAL-X software (www.clustal.org). The aligned sequences were then screened to identify regions of ~20 bp that were conserved across all genes, but were not part of the repeat sequences characteristic of *ina* genes. Once identified, primers were designed for these sequences, and verified using the Primer-BLAST

tool from NCBI. Similarly, primers were designed for other known ice-nucleating genes in *P. fluorescens* (*inaW*), *Xanthomonas campestris* (*inaX*) and *Erwinia* spp. (*iceE*, *inaU* and *inaA*). Details of the sequences used and primers designed are listed in Supplementary Table S1.

We randomly selected 10 environmental isolates collected in 2011 that showed elevated  $T_c$  levels and assayed for the presence of *ina* genes. Ice-nucleating strains of *P. syringae* (ATCC 39254), *P. fluorescens* (CPBG 5 and RSG, isolated from the gut of Colorado potato beetle and *Rana sylvatica*, respectively) and *P. putida* (CPBG 8, CPBG 10, RSG) were used as positive controls. A non-ice-nucleating strain of *P. fragi* (ATCC 4973) was used as a negative control. Primers designed for the *ina* genes (Supplementary Table S1) were used in colony-PCR reactions with a PCR Master Mix (Promega Corporation, Madison, WI, USA) and a MJ Mini Thermal Cycler (Bio-Rad, Life Science Research, Hercules, CA, USA) under the following conditions: initial denaturation at 95 °C for 300 s, 45 cycles of 95 °C for 30 s (denaturation), 63 °C for 60 s (annealing), 68 °C for 90 s (extension) and a final extension at 68 °C for 300 s. PCR products were purified using a Qiagen PCR purification kit and sequenced at the University of Chicago Cancer Research Center. Sequences obtained were cross-referenced with sequences in the GenBank NCBI database to identify them.

#### Freezing column experiments

The approach of Garrison *et al.* (1989) with some modifications was used to investigate the incorporation of plankton into ice. To a 600-ml double-jacketed Pyrex beaker was added 450-ml FLW and 20 ml of plankton material collected from each of three central basin sites. Chl *a* biomass of fresh phytoplankton added to each column ranged from 70–90 µg chl *a* l<sup>-1</sup>. The double-jacketed beaker was connected to a 4 °C recirculating cooling bath containing propylene glycol. Following equilibration to 4 °C for 10 min, the temperature of the cooling bath was decreased at a rate of ~0.2 °C min<sup>-1</sup>. Upon initial formation of frazil ice, the temperature of ice formation was noted and further cooling was stopped. Ice was separated from the liquid phase, the volume of each recorded and chl *a* biomass was determined from each phase. Assays were conducted in triplicate.

Controls differed in that the column was seeded with plankton that had been autoclaved for 20 min. Additional controls consisted of purified water (> 18 MΩ cm) and filtered (< 0.2 µm) lake water.

## Results and Discussion

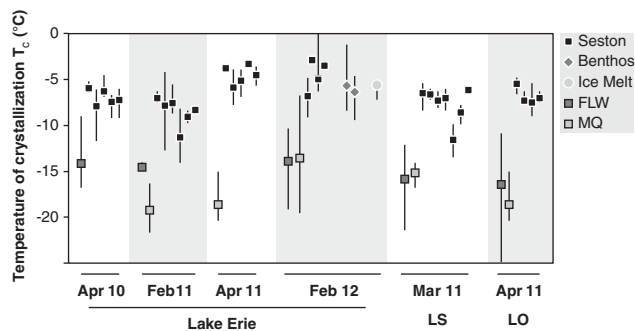
#### Ice nucleation in phytoplankton assemblages

We analyzed samples from winter and spring phytoplankton assemblages in Lake Erie between

2010 and 2012 for potential ice nucleation activity based on the  $T_c$  values of these samples. In samples subjected to a slow and controlled cooling process, the temperature at which ice formation is first detected is identified as the  $T_c$  of that sample. Mean  $T_c$  values for plankton from winter and spring assemblages were significantly higher than FLW or deionized water controls (Figure 1). Likewise, winter and spring assemblages from two other Great Lakes, Superior and Ontario, also showed elevated  $T_c$  values (Figure 1).

The ice nucleation activity was heat labile, consistent with it being derived from a biological protein source. The  $T_c$  of Lake Erie phytoplankton samples exposed to heat for 2 h decreased significantly compared with samples held at 4 °C (Supplementary Figure S4). Not all ice nucleation activity was lost as samples showed elevated  $T_c$  values compared with controls even after exposure to 45, 65 or 95 °C (Tukey's HSD test, symbols *bcd* on Supplementary Figure S4,  $P < 0.01$ ).

Ice nucleation activity was not restricted to samples from the water column but was also measured in samples from ice melt and surface sediment collected during February 2012 (Figure 1). Although Lake Erie was nearly ice-free during this survey, a field of pancake ice (Supplementary Figure S5a) was encountered near the junction between western and central basins. Chl *a* biomass was concentrated >100-fold in the ice (470  $\mu\text{g l}^{-1}$ ) compared with the underlying water. Likewise, sediment samples from central basin sites possessed a distinct brown-colored biofilm on the sediment surface (Supplementary Figure S5b) consisting of filamentous diatoms, which also possessed high ice-nucleating activity (Figure 1). Resting cells of



**Figure 1**  $T_c$  for phytoplankton samples collected during winter and spring 2010–2012 in the Laurentian Great Lakes. Each point represents the mean  $T_c$  ( $\pm$  range,  $n = 3$ –10) for plankton from a sampling location. Plankton samples from Lake Erie were significantly different than their controls (one-factor analysis of variance (ANOVA) for each sampling effort;  $F_{Apr10, 5, 30} = 23.6$ ,  $p_{Apr10} < 0.001$ ;  $F_{Feb11, 7, 16} = 11.0$ ,  $p_{Feb11} < 0.001$ ;  $F_{Apr11, 5, 12} = 34.8$ ,  $p_{Apr11} < 0.001$ ;  $F_{Feb12, 8, 79} = 32.7$ ,  $p_{Feb12} < 0.001$ ; *post hoc* comparison with control Tukey's HSD,  $\alpha = 0.05$ ). Likewise, plankton samples from Lakes Ontario and Superior were significantly different than their controls (one-factor ANOVA;  $F_{LO, 5, 19} = 15.5$ ,  $p_{LO} < 0.001$ ;  $F_{LS, 8, 25} = 16.5$ ,  $p_{LS} < 0.001$ ; *post hoc* comparison with control Tukey's HSD,  $\alpha = 0.05$ ). Controls consisted of purified water (MQ; > 18 M $\Omega$  cm) and FLW (< 0.45  $\mu\text{m}$ ).

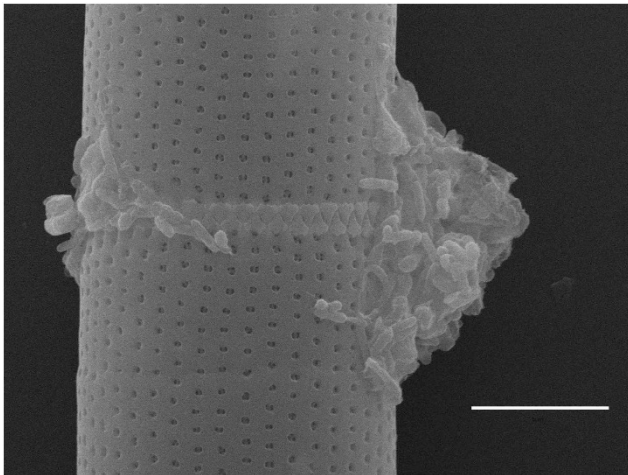
diatoms have been identified from surficial sediments in the Great Lakes with Lake Erie meroplankton dominated by *A. islandica* and *Stephanodiscus* spp. (Lashaway and Carrick, 2010).

Whereas sea ice supports diverse (Mock and Thomas, 2005) and productive (Arrigo *et al.*, 1997) assemblages of planktonic microbes in polar waters, only limited information is available on the ice cover habitat in freshwater ecosystems (Felip *et al.*, 1995; Priscu *et al.*, 1999; Bondarenko *et al.*, 2006; Frenette *et al.*, 2008). Diatoms are the most abundant phytoplankton in sea ice and have been implicated in ice-structuring activities that may promote their growth in ice (Raymond *et al.*, 1994; Krembs *et al.*, 2011). Extracellular polymeric substances produced by sea-ice microbes alter the structure of ice pores enhancing their habitability. Susceptibility of the activity to heat treatment as well as inhibition by glycosidase supports involvement of an ice-binding glycoprotein in the ice-structuring activity (Krembs *et al.*, 2011). These proteins are thought to adsorb to the face of ice crystals, inhibiting their growth and further acting to structure the ice surface to promote light scattering for the associated algal cells (Raymond *et al.*, 1994). Despite this ability to structure ice, the capacity to promote ice formation has not been specifically linked to sea-ice diatoms. Indeed, to our knowledge, ice nucleation activity associated with phytoplankton has been documented only twice before this report (Schnell, 1975; Schnell and Vali, 1975). In the former study, ice nucleation activity was not widespread with only phytoplankton collected from high latitude coastal waters of the North Atlantic Ocean testing positive. This was consistent with observations documenting bands of airborne ice nuclei at high latitudes between 40 and 55° (Bigg, 1973). Indeed, Schnell and Vali (1975) hypothesized that airborne ice nuclei might be biogenic in origin and derived from the ocean.

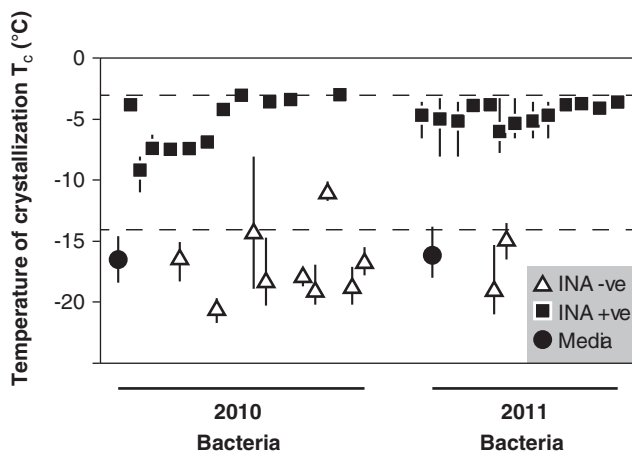
#### *Bacterial epiphytes of diatoms can promote ice nucleation*

Bacteria commonly colonize diatom cells (Amin *et al.*, 2012). Scanning electron microscopy analysis of diatoms growing in association with ice cover showed a close association of flagellated rod-shaped bacteria with extracellular polymeric substances found on filaments of *A. islandica* (Figure 2) and *S. binderanus* (D'souza, 2012), both abundant in the winter assemblage (Twiss *et al.*, 2012). Other diatom taxa were enumerated at low densities during winter and had few bacterial epiphytes (Supplementary Figure S6). Bacteria isolated from net plankton collected in 2010–2011 yielded 24 isolates exhibiting  $T_c$  values significantly higher than their growth medium (Figure 3), and similar to those from freshly collected diatom material (*cf.* Figure 1).

Among ice nucleation-active (INA) bacteria, ice nucleation activity resides on *ina* genes coding for



**Figure 2** Sample processed using a modified scanning electron microscopy processing technique shows colonization by flagellated, rod-shaped bacteria on the extracellular polymeric substance excreted on filaments of *A. islandica*. With the exception of *Stephanodiscus* spp., most other diatoms in the assemblage did not exhibit this association with bacteria (see Supplementary Figure. S6). Scale bar, 5  $\mu$ m.



**Figure 3**  $T_c$  analysis for bacteria isolated from Lake Erie net phytoplankton in 2010 and 2011. Each point represents the mean  $T_c$  ( $\pm$  range,  $n = 3$ ) for each cultured microorganism. INA bacterial isolates (■) had  $T_c$  significantly different from the  $T_c$  of the culturing media (●); one-factor analysis of variance for each set of cultures;  $F_{B2010,20,39} = 36.7$ ,  $p_{B2010} < 0.001$ ;  $F_{D2010,3,8} = 0.8$ ,  $p_{D2010} = 0.53$ ;  $F_{B2011,15,32} = 24.8$ ,  $p_{B2011} < 0.001$ ; INA-positive cultures determined *post hoc* by Tukey's HSD test,  $\alpha = 0.05$ ). The area delineated by the dashed lines shows the range of  $T_c$  values observed for freshly collected plankton from which the bacteria were isolated. Controls consisted of Nutrient Broth in which bacterial isolates were cultured. Some of the bacterial isolates tested negative for ice nucleation activity ( $\Delta$ ).

ice nucleation proteins localized on the outer membrane of the bacterium (Orser *et al.*, 1985). Unfortunately, our analysis was unable to definitively identify the INA component of the winter diatom community as evidence for known *ina*-like genes was not deduced from an ice metagenome

from Lake Erie, nor did primers designed based on known *ina* sequences (Supplementary Table S1) amplify appropriate gene products from INA bacterial isolates (Supplementary Table S2). These same primers did amplify the *ina* genes in several control strains, including INA strains of *P. syringae*, *P. fluorescens* and *P. putida* (Supplementary Table S2).

Bacteria are the most active ice nucleators in the environment (Christner *et al.*, 2008a), capable of catalyzing ice nucleation at temperatures as high as  $-2^\circ\text{C}$  (Lindow, 1983). INA bacteria were discovered in the 1970s and are all gamma-proteobacteria, including species in the genera *Erwinia*, *Pseudomonas* and *Xanthomonas* (Schnell and Vali, 1972; Maki *et al.*, 1974; Lindow *et al.*, 1978). A common life strategy among these strains is their association either as epiphytes on plants (Lindow, 1983) or commensals in gut microflora of ectotherms (Costanzo and Lee, 1996). Although INA bacteria have been isolated from lakes and streams (Morris *et al.*, 2007; Morris *et al.*, 2008), their presence in such non-host environmental reservoirs has been attributed to runoff and atmospheric deposition as rain or snow (Christner *et al.*, 2008a; Christner *et al.*, 2008b) consistent with their proposed role as biological ice nuclei in clouds (Christner *et al.*, 2008a). However, a more recent analysis of genetic and phenotypic diversity identified clades of *P. syringae* distinct to water, arguing against the characterization of freshwater habitats as simple passive reservoirs in the life cycles of these bacteria (Morris *et al.*, 2010).

Analysis of ribosomal RNA small subunit gene (rDNA SSU) sequences identified the INA bacterial isolates from our study as members of the genus *Pseudomonas* with high identity ( $>99\%$ ) to congeners *P. chlororaphis*, *P. coronafaciens*, *P. fluorescens*, *P. fragi*, *P. syringae* and *P. viridiflava* (Supplementary Figure S3). Metagenome samples acquired from lake ice contained sequences from pseudomonads closely related to the INA bacterial isolates (Supplementary Figure S3). Standard biochemical characterization identified the INA bacterial isolates from Lake Erie as phenotypically related to *P. fluorescens* (Supplementary Table S3). Ice nucleation activity is documented in *P. syringae*, *P. fluorescens* and *P. putida* (Lindow, 1983; Costanzo and Lee, 1996), whereas *P. fragi* is a known psychrophile, but for which ice nucleation activity has not been reported (Zdorovenko *et al.*, 2004).

Environmental pseudomonads were enumerated from Lake Erie in February 2012. Consistent with a  $>100$ -fold partitioning of chl *a* biomass in the ice compared with the underlying water, *Pseudomonas* spp. were preferentially partitioned to the ice phase with log mean  $6.125 \log\text{CFU l}^{-1}$  ( $n = 4$ ) enumerated from melt water derived from surface ice. This was  $>3$  orders of magnitude higher cell density (one-tailed *t*-test,  $p < 0.0001$ ) than *Pseudomonas* spp. enumerated in whole water (log mean =  $2.773 \log\text{CFU l}^{-1}$  ( $n = 11$ )). *Pseudomonas* spp. recovered

from ice may include both diatom epiphytes and free-living bacteria, and their partitioning to ice is consistent with the active formation of frazil ice by biological ice nucleation as well as passive adherence of planktonic cells to frazil ice ascending to the surface as described previously (Garrison *et al.*, 1983). By comparison, median abundance of *P. syringae* enumerated from the headwaters of rivers at 11 sites on three continents was 2.75 logCFU l<sup>-1</sup> (Morris *et al.*, 2010). Assuming that *P. syringae* comprises only a modest fraction of the total pseudomonads enumerated as part of the present study, the abundance reported here from lake water is comparatively low. Seasonality may have a role in the discrepancy as might the trophic status of the environment with rivers and coastal systems expected to support higher population densities of heterotrophs compared with open lake sites. Indeed, enumeration of *Pseudomonas* spp. made during spring 2012 showed an abundance of 4 to 5.5 logCFU l<sup>-1</sup> with the highest abundance reported from the mouth of the Maumee River in the lake's western basin.

Despite the high abundance of *Pseudomonas* spp. recovered from lake ice and their potential role in ice formation, it is important to note that not every cell in a population of INA bacteria can serve as a potential ice nucleator. Depending on temperature and growth conditions, the ice nucleation frequency can change by >1 million fold (Hirano *et al.*, 1985). Accounting for volumes used in  $T_c$  assays, we estimate each microcapillary containing phytoplankton collected in 2012 contained <100 CFU of *Pseudomonas* spp. Considering typical ice nucleation frequencies of INA pseudomonads, by most accounts, this is too few bacterial cells to account for the ice nucleation activity observed. However, we cannot discount the likely presence of viable but non-culturable pseudomonads in our study system as highlighted elsewhere (Ahern *et al.*, 2007) leaving the identity of 'who' is responsible for the ice nucleation activity unresolved for now. Thus, while some of the viable pseudomonads recovered from net phytoplankton are shown to possess ice nucleation activity in culture, we cannot with certainty attribute the observed elevated  $T_c$  values of phytoplankton collected from Lake Erie to these bacteria. Further, regardless of their role in promoting ice nucleation, *Pseudomonas* spp. bacteria associated with the diatoms are expected to benefit from their partitioning to the ice phase. It is long recognized that dissolved organic carbon excreted by algae is exploited by heterotrophic bacteria (Cole *et al.*, 1982) and provides up to 50% of the bacterial carbon requirement (Baines and Pace, 1991). In the case of epiphytic bacteria, all of the carbon required for growth can be provided by the photosynthetic host (Kirchman *et al.*, 1984). Thus, for *Pseudomonas* spp., overwintering in association with actively growing diatoms is expected to increase fitness of the bacteria via enhanced nutrient acquisition.

Whereas previous studies strongly implicate bacteria as the INA component of phytoplankton (Schnell and Vali, 1975; Fall and Schnell, 1985) and meltwater samples from sea ice (Parker *et al.*, 1985), we cannot eliminate the possibility that it is the diatoms, themselves, which are responsible for ice nucleation activity observed in plankton samples. Horizontal gene transfer has been proposed as a mechanism to account for the high similarity between ice nucleation protein sequences from diverse bacteria (Edwards *et al.*, 1994). Further, recent evidence supports a role for the horizontal transfer of ice-binding protein genes from bacteria to sea-ice diatoms (Raymond and Kim, 2012), consistent with findings from recently sequenced diatom genomes showing a high rate of gene transfer between bacteria and diatoms (Bowler *et al.*, 2008). Alternatively, perhaps diatoms possess an as yet unidentified mechanism to promote ice nucleation. Indeed, there are multiple reports of eukaryotes active in ice nucleation, including lichens (Kieft, 1988; Henderson-Begg *et al.*, 2009), fungi (Pouleur *et al.*, 1992), higher plants (Gross *et al.*, 1988; Brush *et al.*, 1994) and several species of Antarctic chlorophyte soil microalgae (Worland and Lukešová, 2000). In most cases, the eukaryotic ice nucleation activity exhibits differences to that of bacteria, including greater resistance to heat treatment (Kieft, 1988; Pouleur *et al.*, 1992) thus drawing some parallels with results presented here on the heat lability of ice nucleation activity associated with net phytoplankton (Supplementary Figure S4).

#### *Ice formation influenced by INA microbes*

With the reduced radiant flux that accompanies winter in temperate and high latitudes coupled with expansive ice cover on lakes in these regions, reduced light availability is expected to have an important role in shaping adaptive mechanisms of phytoplankton located below the ice at this time. A few centimeters of snow accumulated on ice can result in high attenuation of photosynthetically active radiation (PAR) (Bolsenga and Vanderploeg, 1992).

As non-motile phytoplankton, diatoms rely mainly on physical mixing to remain suspended in the photic zone. As ice cover presents a barrier to wind-aided mixing, convective mixing has been proposed to allow diatoms to remain suspended near the water-ice interface in Lake Baikal (Kelley, 1997) that, like Lake Erie, experiences ice-associated blooms of *Aulacoseira* (Bondarenko *et al.*, 2006).

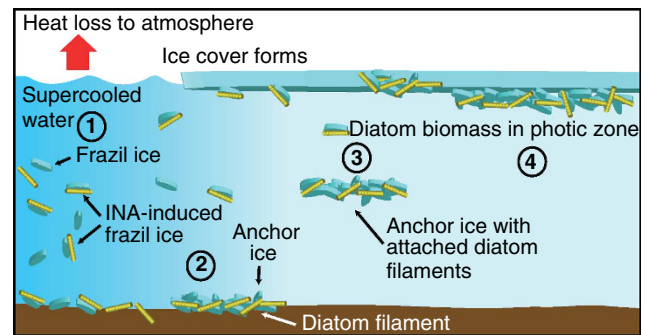
During cold nights in winter, supercooled (<0 °C) water can be mixed to the sediments. When seeded with ice crystals from the air above, these supercooled conditions promote the formation of frazil ice and ultimately anchor ice in rivers and lakes (Martin, 1981) including the Laurentian Great Lakes (Kempema *et al.*, 2001; Daly and Ettema, 2006). Much of the frazil and anchor ice formed in

supercooled water ascends to the surface and can become incorporated into ice cover. Scavenging of suspended particulate matter, including diatoms by frazil crystals and sediment-scouring anchor ice, may thus provide a means of recruitment to the photic zone for phytoplankton (Garrison *et al.*, 1983; Reimnitz *et al.*, 1993). However, rather than relying solely on passive recruitment, we propose that the phytoplankton assemblage actively promotes the formation of frazil ice, providing cells with a buoyant ice raft to ascend to the photic zone (Figure 4). Recently, a similar envirotactic role for an ice-binding protein produced by the psychrophilic bacterium *Marinomonas primoryensis* was proposed, whereby ice binding serves to maintain the bacteria close to the surface in meromictic Antarctic lakes where oxygen is more abundant (Guo *et al.*, 2012).

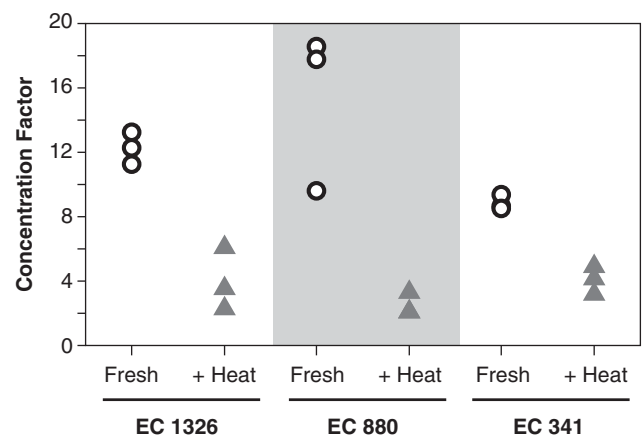
We tested our hypothesis using freezing columns seeded with plankton collected from Lake Erie. Phytoplankton biomass was preferentially partitioned to the ice phase in each assay with an average concentration factor (chl *a* in ice/water) of  $12.2 \pm 3.2$  (Figure 5). Plankton autoclaved before seeding the columns was also partitioned into the ice, although with a lower average concentration factor of  $3.6 \pm 0.9$  (one-tailed *t*-test,  $P < 0.01$ ) and similar to that reported from assays using sea-ice diatoms where partitioning was attributed to trapping and adherence by frazil ice (Garrison *et al.*, 1989). Likewise, here, the modest concentration of heat-treated plankton in ice may be attributed to passive physical mechanisms, whereas the higher concentration of fresh plankton in newly formed ice is consistent with the active formation of frazil ice by biological ice nucleation thereby promoting the association of plankton with ice. Further, when ice formed in columns seeded with fresh plankton, its formation was rapid, occurring within seconds (Supplementary Video S1). This, we attribute to the greater abundance of active nucleation sites available in samples containing fresh phytoplankton.

Our results with freezing columns clearly demonstrate the enhanced partitioning of phytoplankton biomass to the ice phase associated with phytoplankton that are effective ice nucleators and is consistent with our analysis of lake ice, which likewise showed both elevated phytoplankton biomass and culturable *Pseudomonas* spp. cells compared with the water column. We cannot identify with certainty 'who' is responsible for the ice nucleation activity. *Pseudomonad* bacteria isolated from the diatoms are effective ice nucleators, yet low abundance of culturable *Pseudomonas* spp. in  $T_c$  assays of phytoplankton prevents us from unequivocally attributing the activity to the bacterial epiphytes.

Regardless of the source of ice nucleation activity, we propose that association with the ice facilitates growth of diatoms during winter owing to a more



**Figure 4** Temporal schematic representation of ice formation influenced by INA microbes. (1) Open water with moderate winds during clear, cold winter nights results in the mixing of supercooled water through the water column to depths up to 30 m (Kempema *et al.*, 2001; Daly and Ettema, 2006). Frazil ice forms in supercooled water and on the sediment surface; INA-bacteria associated with diatom filaments likewise induce frazil ice formation. (2) Anchor ice forms on the sediment surface from aggregation of frazil ice, and entrains sediment and diatom filaments. (3) Anchor ice detaches from the sediment resulting in rafting of ice and ice-attached organisms to the surface or overlying ice cover (4).



**Figure 5** Phytoplankton were concentrated in ice during controlled freezing. Data show chl partitioned to ice conveyed as mean concentration factor (chl *a* in ice/water;  $n = 3$ ) for three central basin sites (Environment Canada (EC) 1326, EC 880 and EC 341) sampled during February 2012. Heat treatment by autoclaving reduced the partitioning of plankton to the ice fraction by 3- to 4-fold and resulted in lower ice yield.

favorable light climate in this microenvironment. Whereas transmission of PAR is compromised in the presence of ice cover, the decline need not be precipitous. Indeed, light transmittance in February 2011 on a clear day at a central basin location, which was covered with plate ice having a thickness  $> 20$  cm, was shown to attenuate PAR by  $\sim 60\%$  thus providing a similar level of light transmittance as reported as sufficient to support a massive diatom-dominated bloom in the ice-covered Chukchi Sea (Arrigo *et al.*, 2012). Whereas snow cover might compromise the ability of phototrophs to harvest light, an expansive, thick snowpack is

uncommon on large lakes such as Erie. Rather, snow falling on the ice surface of large lakes is likely to accumulate in discrete drifts with the resulting snow-free ice exhibiting high transmittance of PAR (Bolsenga and Vanderploeg, 1992) thereby facilitating the growth of cold-adapted diatoms partitioned to the ice and ice–water boundary zone.

## Conflict of Interest

The authors declare no conflict of interest.

## Acknowledgements

We thank D Smith, M Saxton, B Oyserman, N Levis, K Rapin, R Edgar, P Morris, M Twiss, S Wilhelm, R Smith, C Binding, S Watson, H Carrick, W Woityra and R Bourbonniere for assistance with field surveys and analyses. M Lee provided bacterial reference strains. B Boese, C Turcotte and L-J Ma and the 454 Life Sciences Early Access Program supported the metagenome analysis. Lake surveys were made possible through the support of the captains and crews of the CCGS *GRIFFON*, R/V *LAKE GUARDIAN*, USCGC *ALDER*, USCGC *NEAH BAY* and Technical Operations personnel from Environment Canada (B Lalonde and T Breedon) and EPA-GLNPO. We acknowledge the input from two anonymous reviewers whose insights greatly improved the manuscript. This publication is a result of work that was supported in part, by the Ohio Sea Grant College Program (project # R/ER-081 to RMLM, SOR and GSB) from the National Oceanic and Atmospheric Administration, U.S. Department of Commerce. Support is also provided by the Ohio Board of Regents, The Ohio State University, Ohio State University Extension, and participating universities. The Ohio Sea Grant College Program is administered by The Ohio State University. Support was also provided by Lake Erie Protection Fund grants 398-11 and 430-12 (RMLM), and by NSF grants IOS-0840772 (REL), ANT-0837559 (REL) and OCE-1230735 (RMLM and GSB).

## References

- Ahern HE, Walsh KA, Hill TCJ, Moffett BF. (2007). Fluorescent pseudomonads isolated from Hebridean cloud and rain water produce biosurfactants but do not cause ice nucleation. *Biogeosciences* **4**: 115–124.
- Amin SA, Parker MS, Armbrust EV. (2012). Interactions between diatoms and bacteria. *Microbiol Mol Biol Rev* **76**: 667–684.
- Anzai Y, Kim H, Park JY, Wakabayashi H, Oyaizu H. (2000). Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence. *Int J Syst Evol Microbiol* **50**: 1563–1589.
- Arrigo KR, Perovich DK, Pickart RS, Brown ZW, van Dijken GL, Lowry KE *et al.* (2012). Massive phytoplankton blooms under arctic sea ice. *Science* **336**: 1408.
- Arrigo KR, Worthen DL, Lizotte MP, Dixon P, Dieckmann G. (1997). Primary production in Antarctic sea ice. *Science* **276**: 394–397.
- Baines SB, Pace ML. (1991). The production of dissolved organic matter by phytoplankton and its importance to bacteria: patterns across marine and freshwater systems. *Limnol Oceanogr* **36**: 1078–1090.
- Bengtsson L. (1996). Mixing in ice-covered lakes. *Hydrobiologia* **322**: 91–97.
- Bigg EK. (1973). Ice nucleus measurements in remote areas. *J Atmos Sci* **30**: 1153–1157.
- Bolsenga SJ, Vanderploeg HA. (1992). Estimating photosynthetically available radiation into open and ice-covered freshwater lakes from surface characteristics; a high transmittance case study. *Hydrobiologia* **243/244**: 95–104.
- Bondarenko NA, Timoshkin OA, Ropstorf P, Melnik NG. (2006). The under-ice and bottom periods in the life cycle of *Aulacoseira baicalensis* (K. Meyer) Simonsen, a principal Lake Baikal alga. *Hydrobiologia* **568**: 107–109.
- Bowler C, Allen AE, Badger JH, Grimwood J, Jabbari K, Kuo A *et al.* (2008). The *Phaeodactylum* genome reveals the evolutionary history of diatom genomes. *Nature* **456**: 239–244.
- Brush RA, Griffith M, Mlynarz A. (1994). Characterization and quantification of intrinsic ice nucleators in winter rye (*Secale cereale*) leaves. *Plant Physiol* **104**: 725–735.
- Christner BC, Cai R, Morris CE, McCarter KS, Foreman CM, Skidmore ML *et al.* (2008b). Geographic, seasonal, and precipitation chemistry influence on the abundance and activity of biological ice nucleators in rain and snow. *Proc Natl Acad Sci USA* **105**: 18854–18859.
- Christner BC, Morris CE, Foreman CM, Cai R, Sands DC. (2008a). Ubiquity of biological ice nucleators in snowfall. *Science* **319**: 1214.
- Cole JJ, Liken GE, Strayer DL. (1982). Photosynthetically produced dissolved organic carbon: an important carbon source for planktonic bacteria. *Limnol Oceanogr* **27**: 1080–1090.
- Costanzo JP, Lee RE Jr. (1996). Mini-review: ice nucleation in freeze-tolerant vertebrates. *Cryo Lett* **17**: 111–118.
- Daly SF, Etema R. (2006). Frazil ice blockage of water intakes in the Great Lakes. *J Hydraul Eng-ASCE* **132**: 814–824.
- D'souza NA. (2012). *Psychrophilic diatoms in ice-covered Lake Erie*. Ph.D. dissertation. Bowling Green State University, (Online) <http://www.ohiolink.edu/etd/>.
- Edwards AR, Van Den Bussche RA, Wichman HA, Orser CS. (1994). Unusual pattern of bacterial ice nucleation gene evolution. *Mol Biol Evol* **11**: 911–920.
- Fall R, Schnell RC. (1985). Association of an ice-nucleation pseudomonad with cultures of the marine dinoflagellate, *Heterocapsa niei*. *J Mar Res* **43**: 257–265.
- Felip M, Sattler B, Psenner R, Catalan J. (1995). Highly active microbial communities in the ice and snow cover of high mountain lakes. *Appl Environ Microbiol* **61**: 2394–2401.
- Frenette J-J, Thibeault P, Lapierre J-F, Hamilton PB. (2008). Presence of algae in freshwater ice cover of fluvial Lac Sainte-Pierre (St. Lawrence River, Canada). *J Phycol* **44**: 284–291.
- Gaby WL, Hadley C. (1957). Practical laboratory test for the identification of *Pseudomonas aeruginosa*. *J Bacteriol* **74**: 356–358.
- Garrison DL, Ackley SF, Buck KR. (1983). A physical mechanism for establishing algal populations in frazil ice. *Nature* **306**: 363–365.
- Garrison DL, Close AR, Reimnitz E. (1989). Algae concentrated by frazil ice: evidence from laboratory experiments and field measurements. *Antarct Sci* **1**: 313–316.
- Gouy M, Guindon S, Gascuel O. (2010). SeaView version 4: a multiplatform graphical user interface for



- sequence alignment and phylogenetic tree building. *Mol Biol Evol* **27**: 221–224.
- Gross DC, Proebsting EL Jr, MacCrindle-Zimmerman H. (1988). Development, distribution, and characteristics of intrinsic, nonbacterial ice nuclei in *Prunus* wood. *Plant Physiol* **88**: 915–922.
- Guo S, Garnham CP, Whitney JC, Graham LA, Davies PL. (2012). Re-evaluation of a bacterial antifreeze protein as an adhesin with ice-binding activity. *PLoS ONE* **7**: e48805.
- Henderson-Begg SK, Hill T, Thyrhaug R, Khan M, Moffett BF. (2009). Terrestrial and air-borne non-bacterial ice nuclei. *Atmos Sci Lett* **10**: 215–219.
- Hirano SS, Baker LS, Upper CD. (1985). Ice nucleation temperature of individual leaves in relation to population sizes of ice nucleation active bacteria and frost injury. *Plant Physiol* **77**: 259–265.
- Katoh K, Asimenos G, Toh H. (2009). Multiple alignment of DNA sequences with MAFFT. In: Posada D (ed) *Bioinformatics for DNA Sequence Analysis, Methods in Molecular Biology* vol. 537. Humana Press: New York, pp 39–64.
- Katoh K, Toh H. (2010). Parallelization of the MAFFT multiple sequence alignment program. *Bioinformatics* **26**: 1899–1900.
- Kelley DE. (1997). Convection in ice-covered lakes: effects on algal suspension. *J Plankton Res* **19**: 1859–1880.
- Kempema EW, Reimnitz E, Barnes PW. (2001). Anchor-ice formation and ice rafting in southwestern Lake Michigan, U.S.A. *J Sed Res* **71**: 346–354.
- Kieft TL. (1988). Ice nucleation activity in lichens. *Appl Environ Microbiol* **54**: 1678–1681.
- Kirchman DL, Mazzella L, Alberte RS, Mitchell R. (1984). Epiphytic bacterial production on *Zostera marina*. *Mar Ecol Prog Ser* **15**: 117–123.
- Krembs C, Eicken H, Deming JW. (2011). Exopolymer alteration of physical properties of sea ice and implications for ice habitability and biogeochemistry in a warmer Arctic. *Proc Natl Acad Sci USA* **108**: 3653–3658.
- Lashaway AR, Carrick HJ. (2010). Effects of light, temperature and habitat quality on meroplanktonic diatom rejuvenation in Lake Erie: implications for seasonal hypoxia. *J Plankton Res* **32**: 479–490.
- Lindow SE, Arny DC, Upper CD. (1978). Distribution of ice nucleation-active bacteria on plants in nature. *Appl Environ Microbiol* **36**: 831–838.
- Lindow SE. (1983). The role of bacterial ice nucleation in frost injury to plants. *Annu Rev Phytopathol* **21**: 363–384.
- Maki LR, Galyan EL, Chang-Chien MM, Caldwell DR. (1974). Ice nucleation induced by *Pseudomonas syringae*. *Appl Environ Microbiol* **28**: 456–459.
- Martin S. (1981). Frazil ice in rivers and oceans. *Ann Rev Fluid Mech* **13**: 379–397.
- Mock T, Thomas DN. (2005). Recent advances in sea-ice microbiology. *Environ Microbiol* **7**: 605–619.
- Morris CE, Kinkel LL, Xiao K, Prior P, Sands DC. (2007). Surprising niche for the plant pathogen *Pseudomonas syringae*. *Infect Genet Evol* **7**: 84–92.
- Morris CE, Sands DC, Vanneste JL, Montarry J, Oakley B, Guilbaud C *et al*. (2010). Inferring the evolutionary history of the plant pathogen *Pseudomonas syringae* from its biogeography in headwaters of rivers in North America, Europe, and New Zealand. *Ambio* **1**: e00107–e00110.
- Morris CE, Sands DC, Vinatzer BA, Glaux C, Guilbaud C, Buffière A *et al*. (2008). The life history of the plant pathogen *Pseudomonas syringae* is linked to the water cycle. *ISME J* **2**: 321–334.
- Orser C, Staskawicz BJ, Panopoulos NJ, Dahlbeck D, Lindow SE. (1985). Cloning and expression of bacterial ice nucleation genes in *Escherichia coli*. *J Bacteriol* **164**: 359–366.
- Oyserman BO, Woityra WC, Bullerjahn GS, Beall BFN, McKay RML. (2012). Collecting winter data on U.S. Coast Guard icebreakers. *Eos Trans AGU* **93**: 105–106.
- Parker LV, Sullivan CW, Forest TW, Ackley SF. (1985). Ice nucleation activity of Antarctic marine microorganisms. *Antarct J US* **20**: 126–128.
- Pouleur S, Richard C, Martin J-G, Antoun H. (1992). Ice nucleation activity in *Fusarium acuminatum* and *Fusarium avenaceum*. *Appl Environ Microbiol* **58**: 2960–2964.
- Priscu JC, Fritsen CH, Adams EE, Giovannoni SJ, Paerl HW, McKay CP *et al*. (1999). Perennial Antarctic lake ice: an oasis for life in a polar desert. *Science* **280**: 2095–2098.
- Raymond JA, Kim HJ. (2012). Possible role of horizontal gene transfer in the colonization of sea ice by algae. *PLoS ONE* **7**: e35968.
- Raymond JA, Sullivan CW, DeVries AL. (1994). Release of an ice-active substance by Antarctic sea ice diatoms. *Polar Biol* **14**: 71–75.
- Reimnitz E, Clayton JR, Kempema EW, Payne JR, Weber WS. (1993). Interaction of rising frazil with suspended particles: tank experiments with applications to nature. *Cold Reg Sci Technol* **21**: 117–135.
- Saxton MA, D'souza NA, Bourbonniere RA, McKay RML, Wilhelm SW. (2012). Seasonal Si:C ratios in Lake Erie diatoms—evidence of an active winter diatom community. *J Gt Lakes Res* **38**: 206–211.
- Schnell RC, Vali G. (1972). Atmospheric ice nuclei from decomposing vegetation. *Nature* **236**: 163–165.
- Schnell RC, Vali G. (1975). Freezing nuclei in marine waters. *Tellus* **27**: 321–323.
- Schnell RC. (1975). Ice nuclei produced by laboratory cultured marine phytoplankton. *Geophys Res Lett* **2**: 500–502.
- Swofford D. (1998). *PAUP\*: Phylogenetic Analysis Using Parsimony*. Sinaur Associates Inc: Sunderland, MA.
- Twiss MR, McKay RML, Bourbonniere RA, Bullerjahn GS, Carrick HJ, Smith REH *et al*. (2012). Diatoms abound in ice-covered Lake Erie: investigation of offshore winter limnology in Lake Erie over the period 2007 to 2010. *J Gt Lakes Res* **38**: 18–30.
- Waterhouse AM, Procter JB, Martin DMA, Clamp M, Barton GJ. (2009). Jalview Version 2—a multiple sequence alignment editor and analysis workbench. *Bioinformatics* **25**: 1189–1191.
- Worland MR, Lukešová A. (2000). The effect of feeding on specific soil algae on the cold-hardiness of two Antarctic micro-arthropods (*Alaskozetes antarcticus* and *Cryptopygus antarcticus*). *Polar Biol* **23**: 766–774.
- Zdorovenko GM, Veremeichenko SN, Kiprianova EA. (2004). A comparative analysis of the ice nucleation activity of pseudomonad cells and lipopolysaccharides. *Microbiology* **73**: 425–430.

Supplementary Information accompanies this paper on The ISME Journal website (<http://www.nature.com/ismej>)